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REVIEW ARTICLE

LIVESTOCK METABOLOMICS – A OVERVIEW

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ABSTRACT

Metabolomics is the study of small, low molecular weight cellular metabolites that are the end products of metabolism. Metabolomics reflects the downstream of gene expression and very closer to the phenotype of animals than proteomics or genomics. In addition to gene expression, post-transcriptional and posttranslational modification regulate metabolic activities of living creatures. The potential advantage of metabolomic information add metabolomic data to SNP-based genomic prediction approaches integrated with conventional phenotypic data in genomic selection programs. This integration would be useful if the prediction accuracy was limited by the small number of phenotyped animals in the training populations. Metabolome include metabolite profiling, metabolic fingerprinting and metabonomics are the principle approaches for analyzing metabololites of biological system. The traits like disease resistance are unmeasurable require challenge study. The advancements in metabolomics research producing potential biomarkers but most of the identified biomarkers have failed to replace existing clinical tests. Potential biomarker should be confirmed and validated using hundreds of specimens and should be reproducible with high specificity and sensitivity. The metabolomics has contributed substantially to our understanding of different diseases and the development of metabolome analysis will helpful for the discovery of novel biomarkers for early detection of various diseases and improving production and reproduction traits of livestock and poultry.

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INTRODUCTION

Metabolomics is an comprehensive, qualitative and quantitative study of all the small molecules in an organism. The molecule size that are less than or equal to about 1500 daltons. But the study of metabolomics excludes polymers of amino acids and sugars. Because intermediary metabolites used to form the macromolecular structures and other small molecules participating in important metabolic functions and fulfilling critical roles such as signaling molecules or secondary metabolites. The term 'metabolome' was coined in 1998 used to describe the metabolite complement of living tissues (Oliver et.al. 1998). The metabolomics isa field of study is now firmly established as a functional genetics approach to understanding the molecular complexity of life (Wagner et.al. 2003). Mostly metabolomics is an extensive network of biochemical interactions but most of the metabolites not fully characterized. The three principal approaches for the analysis of the metabolome include metabolite profiling, metabolic fingerprinting, and metabonomics are the principle approaches for analyzing metabololites of biological system.

Metabolite profiling is an approach that identify and quantify metabolites, but it have methodological limitations and differences in analytical platforms. Most of the variation in this method is due to different extraction method used indifferent organism or tissue. Metabolic finger printing is an high-throughput approach which normally utilized in tissue comparison or discrimination analysis. Metabolic profiling includes sample preparation, separation and detection in comparison to metabolic profiling. Metabonomics is another part of metabolomics, which focuses on the metabolic response of organisms to pathophysiological stimuli or genetic modification. This approach is generally restricted to microbiological and other non-botanical studies. The metabolomics approach includes physiological assays, genomics, proteomics and transcriptomics studies.

Major application of metabolomics in veterinary field

- Drug discovery.
- Toxicology and pharmacokinetics study.
- Agricultural and plant development.

- Clinical biomarker for disease diagnosis.
- Metabolic biomarker used for selection of animals for milk meat egg production and improving reproduction of farm animals.
- Metabolomics also applied to animal breeding "next generation phenotyping" approaches that are needed to refine and improve trait description and improve prediction of the breeding values of the animals to cope with traditional and new objectives of the selection programs.
- Integration of metabolomics with livestock genomics for improving production and reproduction of livestock.
- Genome-wide association studies with metabotypes (mGWAS) links genomic variability with metabotype levels in relevant biofluids.
- Network reconstruction methodologies and complexity of metabolomics information and linking metabolomics with other omics data.
- Metabotypesis an novel applications in animal breeding by using new and conventional traits and related genetic architecture.

Importance of metabolomics in livestock production: Metabolites can be considered internal phenotypes or molecular phenotypes very close to the genetic makeup of the animals. The metabolic studies may help to understand the biological processes underlying genetic differences among animals which is an novel applications in animal breeding which links physiology and genetics. The advantages that metabolomics studies in livestock is easy to collect sample bio fluids or tissues that cannot be easily or routinely obtained in humans (e.g., milk in dairy species, muscle, or other tissues collected after slaughtering of meat species). The environmental factors like feeding, housing can be controlled more easily in animals than in humans but the standard operating protocols during the sampling and preparation steps of specimens for metabolomic analysis are more difficult to design in field studies (Zhang *et.al.,*2012).

Metabolomic approaches in livestock genetic studies have been designed to extract relevant metabotypes or biomarkers. Genetically derived factors in females and males may lead to metabolome differences between sexes. The bottom-up approaches try to describe metabolomes to predict external phenotypes and the variability of the level of metabolites controlled by genetics. The genome-wide association studies with metabotypes (mGWAS) are starting to directly link genomic variability with metabotype levels in a relevant biofluid or tissue. The Network reconstruction methodologies based on systems biology concept is very useful to the complexity of metabolomics information and linking metabolomics with other omics technology (Cox *et al.,* 2008).

The inborn errors of metabolism found in some farm animals due to disrupting mutations have been reported many other genetic variants might affect the level of many other metabolites in livestock which leads to minor modifications of metabolomic profiles. The metabolites as genetically influenced metabotypes (Suhre and Gieger, 2012). In humans, several metabolite-based genome- wide association studies (mGWAS). The markers present in genes encoding enzymes, transporter or other related proteins are associated with variability of the level of one or more metabolites in the biofluids (Beadle *et al.,*1941). The identification of direct link between the function of a gene and the metabolites very importance for biomarker study and the variation by the "level" in which this metabolomic information is placed after gene expression and protein production. These variants usually explain a high fraction (10–30%) of the observed genetic variance for the detected metabotype and are associated also with more complex diseases (but not as strongly as for the specific metabotypes), providing information to understand their etiology. Opportunities can be provided by mGWAS for the deorphanization of uncharacterized metabolites or metabolite features (in untargeted metabolomic analyses) as associated genes already characterized might contribute to disclose of their biochemical features. In case of livestock, population-based mGWAS have been reported by using targeted metabolomics on plasma in pigand using untargeted metabolomics in milk dairy cattle (Adamski *et al.,* 2012) but the biologically significant markers lower number in animals compared with humans. In case of animal metabalomic study easy to control environmental factors or to identify the potential sources of variability to be included in the metabolomic models. Incase pigs, the level of several circulating plasma nutrients were associated with specific genes and explaining a relevant fraction of the genetic variability of these metabotypes which is useful for nutrigenomics approaches. The metabotypes performed on Holstein milk shows genome-wide significant associations with eight metabolites and 21 chromosome-wide significant associations for 14 metabolites were also reported (Boehmer *et al.,* 2013).

Genes function might be indirectly associated with metabo type. The potential practical applications of the level of glycerophosphocholine as a biomarker for ketosis resistance in animals (Klein *et al.,*2012), the milk levels of phosphocholine, glycerophosphocholine, and the ratio of both metabolites were studied in a limited number of samples and finally identified variants in a gene (apolipoprotein receptor B, *APORB*). The milk metabolomic profiles or singlemetabolite levels in association studies have been performed using single markers or many markers associated study between the bovine *DGAT1* K232A polymorphism and milk lipid and serum metabolome compositions contributed to understanding the basic biological mechanisms affected by this mutation (Liu *et al.,* 2015). Genomic wide association studies for non-esterified fatty acid (NEFA), β-hydroxybutirate (BHBA) and glucose in bovine milk measured at different lactation periods. The gene enrichment approaches that substituted conventional single-marker analyses (Hiss *et al.,* 2009).

Application of metabolomics in animal breeding: The complexity of the biological systems requires unconventional approaches to describe the interactions among the different levels of biological information and their dynamics. The systems genetics is a part of the systems biology which includes important elements are the genetic variants within a population. Genetic networks might be constructed using already established information or from novel relationships inferred from the produced data using various algorithms. The system genetics is useful to understand the general architecture of production traits in which metabolomics data represent the intermediate level in a three-level model including polymorphism data, metabolomic level and the production trait level. Metabolomics measured in the host can capture metabolites produced by the rumen or intestine microbiota (e.g., Morgavi *et al.,* 2015). Due to phenotypic buffering (Fu *et al.,* 2009).The gene level differences are not transmitted to

protein or metabolites level which is close to the final phenotypes which is important in animal breeding. This three-level approach can potentially be implemented in practice to understand the biological architecture underlying complex traits. Samples like blood or milk samples easier for metabolomic analyses than to collect tissues in living animals for gene expression analyses. Initial attempt to indirectly include metabolomic information in genomic selection for the prediction of traditional milk traits in dairy cows were studied. Milk metabolite profiles used to select important SNPs regarding an investigated milk trait. The potential advantage of metabolomic information add metabolomic data to SNP-based genomic prediction approaches or even to integrate conventional phenotypic data in genomic selection programs. This integration would be useful if the prediction accuracy was limited by the small number of phenotyped animals in the training populations. The quantitative traits like disease resistance are unmeasurable require challenge study. Integration of genomic and metabolite information with predictive value is very important (Ehret *et al*., 2015).

Metabolic biomarker of seminal fluid: The early estimation of fertility in bulls helps in cost effective production of frozen semen for artificial insemination. In vivo assessments of bull fertility is the most accurate but is expensive, laborious and time-consuming method. Young bulls when rejected for their poor quality semen or fertility leads to a heavy loss to farm. So development of some of the *in vitro* method to predict the bull fertility will be very helpful in commercial farming conditions. The seminal plasma are secreted from accessory sex glands. The protein present in seminal plasma contribute to sperm motility, sperm membrane protection, protection from oxidative stress, sperm cap acitation, acrosome reaction and oocyte penetration. The semen contains several proteins, amino acids, enzymes, fructose and other carbohydrates, lipids, and major minerals and trace elements. The majority of seminal plasma constituents are derived from blood and some from seminal plasma-specific used as a seminal biomarker (Moura *et al.,* 2006). Blood contributes albumin, antitrypsin, b-lipoproteins, and orosomucoids and all these components help for osmotic regulation, maintenance of pH and transport of ions, lipids and hormones. Seminal plasma-specific proteins include androgen binding proteins, osteopontin, clusterin, spermadhes in as well as calmodulin- binding proteins, forward-motility proteins and heparin-binding proteins. The seminal plasma specific protein components regulate oviductal sperm reserves, capacitation, uterine immune modulation and sperm transport in the female genital tract as well as in gamete interaction and fusion (Topfer-Petersen *et al.,* 2005). In the seminal plasma, some of the fertility associated proteins are also identified from dairy bulls (Moura *et al.,* 2006) and crossbred bulls. The high amount of bovine seminal plasma protein, clusterin, albumin phospholipase A2 and osteopontin is found in the accessory sex gland fluid of high fertility bull semen (Moura *et. al.,* 2007). The concentrations of citrate, lactate, glycerylphosphorylcholine, and glycerylphosphory lethanolamine are reportedly higher in the seminal plasma of infertile bull than fertile bulls (Deepinder *et al.,* 2007). The presence of citric acid, aglutamyl transferase and acid phosphatase in seminal plasma and leptin in serum are also predictors of epididymal function, prostate function and sperm morphology (Kumar Ajeet *et al.,* 2015).

Functions of seminal plasma metabolites: High-fertility seminal plasma contain low levels of citrate and isoleucine and high levels of tryptamine, taurine, and leucine. Citrate is the main anion of seminal plasma, which chelates calcium ions and reduces the sperm capacitation and spontaneous acrosome reactions but low levels of citrate are favoured in bullsperm to undergo capacitation and the acrosome reaction for fertilization (Moura *et al.,* 2006). The citric acid has been associated with the gelification, coagulation, and liquefaction of semen in rats (Hart, 1970), monkeys (Hoskins and Patterson, 1967) and humans (Huggins and Neal, 1942). High citrate concentration in seminal plasma was measured in infertile men (Cooper *et.al.,* 1991). The increased citrate concentration in seminal plasma act as aprominent biomarker for prostate cancer. Tryptamine promotes the acrosomal reaction and regulates sperm motility in capacitated hamster sperm. Amino acid taurine is high in the seminal plasma of humans and cats (Buff *et. al.,* 2001). Which is enhancing enhance post-thaw motilityand sperm survival when used as an exogenous supplement (Chhillar *et al.,* 2012). The amino acids is oleucine and leucine are responsible for delaying calcium uptake by ejaculated sperm by altering active calcium transport across the sperm plasma membrane. The presence of these differential metabolites in seminal plasma likely control the fertility potential of sperm by regulating calcium availability which affects sperm motility, capacitation and acrosome reaction. Excessive production of reactive oxygen species (ROS) leads to oxidative stress. These markers include CH, -NH, -SH, C=C and OH which are found in male reproductive tracts affecting sperm quality and function. The citrate, tryptamine / taurine, isoleucine, leucine, aglutamyl transferase, acid phosphatase and oxidative stress metabolites in seminal plasma and is oleucine, as paragine, glycogen, citrulline in serum are some of the metabolites which can be used to detect early fertility in bulls. The detection of these metabolites will help the breeders to make reasonable decisions in choosing the superior quality bulls (Agarwal, 2005).

Follicular fluid biomarker: Follicular fluid is responsible for providing nutrient supply to oocyte and giving protection against proteolysis as well as aiding in the extrusion process during ovulation and acting as a buffer against adverse blood substances (Richards, *et al.,* 1998). Follicular fluid may provide an appropriate microenvironment for the oocyte that leads to proper embryonic development (Gosden *et al.,* 1998). The postpartum cows that there is a close correlation between the levels of certain metabolites in follicular fluid blood serum. The potential variations in serum concentrations of metabolites may affect follicular fluid which may lead to changes in the quality of granulosa and oocyte cells. Recently the follicular fluid has been used for conservation and maturation of oocytes with the purpose of performing *in vitro* fertilization in cattle due to the high protein content than foetal bovine serum (Sirard *et al.,* 2006). Follicular fluid is formed through the transudation of the fluid produced by the theca and granulosa cells in the follicular antrum. This phenomenon occurs during the growth phase of the follicles, which increases the pressure inside the follicular antrum, the follicular fluid composition and quantity can be changed during the development of the follicle. The steroids and glycoproteins of follicular fluid are synthesized by dominant follicular cells and other substances that are synthesized by ovarian somatic cells and these compounds contributes to the metabolism of cells and follicular oocyte. Follicular fluid of ovary contain variety of polyunsaturated fatty acids, with linoleic acid found in larger quantities in small follicles which is responsible for inhibition of meiosis in bovine oocytes. Studying the composition of

follicular fluid is of great importance for evaluation parameter of oocyte quality and fertility (Bender *et al.,* 2010). Analysis of follicular fluid can be useful in livestock breeding and improving milk and meat production. The liquid chromatography techniques are commonly used for untargeted metabolomics screenings and LC-MS based systems are a considerably demanding alternative in terms of time and costs to identify unknown compounds in complex samples (Nandi *et al.,* 2007). The direct-infusion mass spectrometry (DIMS) with mass-selective detection is capable of providing high specificity of chemical information, which includes molecular mass and characteristic information of the fragmented small molecules buthigh-resolution mass spectrometry (HRMS) is ideal for fast screening with minimal sample preparation and a high-throughput analytical process in shotgun lipidomic approach. The Cannabinoid receptors 1 and 2 have been described in human endometrium where CB1 signalizes for the transport of embryos through the oviduct as well as for embryo establishment in the womb. Recent contributions have shown that the endocannabinoid an and amide metabolites plays an important role during the fertilization process in humans and an and amide influence the estrous cycle in bovines (Liu *et al.,* 2015). The high concentrations of an and amide are related to the hormonal peak of estradiol during ovulation is responsible for the release of the ova. High levels of an and amide in humans are related to embryonic mortality and reduction in levels of progesterone. The *N*-acyl ethanol amines inhibit an and amide degradation through compete for fatty acid amide hydrolase, but both compounds are hydrolyzed by the same enzymatic reaction. The high levels of an and amide and lower level of progetsrone leads to abortion in some individual.

The high concentration of progesterone in follicular fluid will increase the fertility of cattle (Agarwal *et al.,* 2005). *N*-docosahexaenoyl phenylala nine and *N*-eicosanoyl ethanolamine as markers for the low oocyte number. Resveratrol 4′-glucoside, Lupinisoflavone N and Peonidin acetyl 3,5-diglucoside in the positive ion mode and 3,3′,4,5′-tetrahydroxy- trans-stilbene, 5,7-dihydroxy-6-methyl-8-prenylflavanone, xanthohumol, and prostaglandin M are negative. The oocytes and embryos are highly vulnerable to oxidative stress and other conditions when cultured *in vitro.* Resveratrol 4′-glucoside and 3,3′,4,5′-tetrahydroxy-trans-stilbene, or piceatannol, are compounds that belong to the class of stilbenes. Piceatannol is a metabolite of resveratrol that has greater antioxidant potential than its precursor due to the position of its hydroxyl groups act as a free radical scavenger. The resveratrol can improve the quality of bovine embryos when added during oocyte maturation. Resveratrol was consumed for a longer period will increase the number of oocytes in female mice. Cows were fed with green pastureleads to Resveratrol 4′-glucoside selected as a marker for the group of cows with high oocyte number production due to its high antioxidant potential and increase oocyte maturation process and the future development of new embryos in cattle. Isoflavones, anthocyanidins, and prenylflavonoids, Lupiniso flavone N, peonidin acetyl 3,5-diglucoside, 5,7-dihydroxy-6-methyl-8-prenylflavanone and xanthohumol Lupiniso flavone N are the is of lavone found in plants. Isoflavones are phenolic compounds have antioxidant character and supplementation of animal feeding with lupines grass increase the ovulation rate in sheep and also to improved reproductive efficiency in the case of ruminants (Lopez *et.al.,*2003). Peonidin acetyl 3,5-diglucoside belongs to an thocyanins and flavonoids have high antioxidant potential, phytoestrogenic and antioxidant properties and xanthohumol has anticancer, antidiabetic, antibacterial and anti-inflammatory activities. The presence xanthohumol in the follicular fluid may be traced back directly to the animal's feed. The flavonoids and its derivatives are ubiquitous to the metabolism of several plant species especially grasses such as *B. decumbens* and *B. Brizantha.* Most important biomarker for fertility is Prostaglandin M. PGE-M is a metabolite of PGE-2 which in turn is directly linked to ovulation as it increases its levels when there is an increase in luteinizing hormone. The PGE-2 is one of several signaling molecules which coordinate oocyte maturation and enhanced expression of proteases associated with follicle rupture and release of an optimally mature oocyte during ovulation. The presence of PGE-M as a marker of the high oocyte number group is indicative of a higher performance of PGE-2 in the animals of this group. The antioxidants level high in fertile female animals (Wise *et al.,* 1987).

Metabolic biomarker for mastitis resistance in dairy cow:

Mastitis is an economically important disease in dairy animals. The 13 different types of proteins are responsible for high and low mastitis resistance in cow. Most of the proteins were membrane-bound and were therefore less suitable as a biomarker so sample preparation and protein quantification with a high-throughput technique like ELISA is more difficult. Gamma–glutamyltranspeptidase1 (gGT1) and lactoferrin (LF). Gamma-GT1 is an enzyme involved in the Meister glutamyl cycle and is responsible for the transfer of glutathione across the cell membrane to maintain homeostasis (Liu *et al.,* 2010). gGT1 levels in blood reflect liver performance and intoxication. The gamma GT1 levels measured in urine can be an indicator for renal damage (Ferguson *et al.,* 2008). The association with disease and damage makes gGT1 in milk an interesting candidate marker for disease resistance. Lactoferrin is a glycoprotein produced by glandular epithelial cells and neutrophils which is present in all body fluids. Lactoferrin is a part of the innate immune system and has antimicrobial, antiviral, antifungal, anti-inflammatory and anti-oxidative properties among others by iron sequestering (Kanwar *et al.,* 2015) and also act as an acute phase protein. The cows suffered with mastitis showed similar lactoferrin levels inmilk. Low-resistant cows with lameness had significantly higher lactoferrin levels in milk compared to high-resistant cows without lameness. The two potential biomarkers, Gamma -glutamyl transpeptidase1 and lactofeerin were selected fromthe proteins that were significantly different between high and low-resistant cows because the soluble proteins are related to disease (Cheng *et al*., 2008). The elevated level of Gamma -glutamyl transpeptidase1 in blood is an indicator for cholestasis and liver failure. The enhanced Gamma -glutamyl trans peptidase1 activity in serum of Rathi cattle is a marker for stress and metabolic dysfunction (Kataria and Kataria, 2012).

Increased Gamma -glutamyl transpeptidase1 levels in urine were related with renal injury (Ferguson *et al.,* 2008). Gamma -glutamyl transpeptidase1 levels were used as an indicator for colostrum uptake in young calves and lambs. The Gamma -glutamyl transpeptidase1 levels in milk were below the detection limit of the available capture ELISAso relevance of Gamma -glutamyl transpeptidase1 levels in milk forbovine health could not be determined. Lactoferrin levels were shown to be significantly higher in low resistant cows which plays an important role in the induction of innate immunity by sequestering iron and thereby limiting the availability of free

iron which is essential for bacterial growth. So the higher Lactoferrin levels imply a better protection against disease. A higher somatic cell count is usually due to an influx of neutrophils in the udder to fight a bacterial infection. Similar to Lactoferrin the higher somatic cell count helps to control the infection and cows that had suffered from lameness in the past had higher Lactoferrin levels in milk. The elevated levels of the acute phase proteins like serum amyloid A, haptoglobin and fibrinogen in serum leads to lameness (Kujala *et al.,* 2010). Cows with higher serum non estrified fatty acid levels around parturition is an indicator for increased fat mobilization and a negative energy balance (NEB). Cows with more metabolic stress after calving had more health disorders like lameness, milk fever etc. The low-resistant cows had higher Lactoferrin levels in milk suffered at least two times from periparturient diseases and showed a significant association between Lactoferrin levels and lameness. The increase in CD36, BTN1A1, IDH1 and kappa-casein in milk of low-resistant cows, while others showed these proteins to be decreased in milk from cows with ongoing mastitis.

Biomarker for meat quality assessment: The breeding industry is increasingly using genetic markers to improve the genetic potential of livestock through genomic selection (Calus and Veerkamp, 2007). The quality of pork analyzed based on the biomarker. The biomarkers also take these environmental effects into consideration by detecting the expression of the genome which is the combination of genetic potential and environmental variation (Goodsaid and Frueh, 2007). The biomarkers are molecular components of biological processes regulating phenotypes of animals and meat quality. Different phenotypes were regulated by differences in the biological processes underlying the phenotype. The specific biomarkers are derived from improved knowledge of the biological mechanism underlying economically interesting and complex phenotypic traits such as meat quality. Biomarkers may be molecules of any type (e.g. RNA, proteins, metabolites) or may consist of a profile of several primary and secondary metabolites. Mostly the level of the biomarker is associated with quantitative aspects of the trait variation (Goodsaid and Frueh, 2007).

Development of specific biomarker: Biological Mutations at the genomic DNA affect the functions of proteins or affect the expression of the genes. The expression of the genes is also regulated by differences in methylation of the DNA. The activity of non-coding small RNA molecules such as miRNAs, activities of diverse transcription factors and the activity of the translation components of the cell (Li *et al., 2*013). The activity of the proteins is regulated by post-translational modifications and regulating the flux through biological mechanisms and differentially regulating metabolite concentrations in the cell. High-throughput omics techniques can measure the biological levels and genomics measures single nucleotide polymorphisms (SNPs) related to genetic variation leading to the development of specific genetic markers. The transcriptomics measures RNA and miRNA levels related to the activity of the genome and proteomics measures protein levels and posttranslational modifications related to the biological functionality of the genome and metabolomics measures metabolite levels related to the biological processes leading to the development of metabolic biomarkers. Bioinformatics is necessary to describe the underlying networks and pathways and the functional biological processes (Cox *et al.,* 2008). Systems biology integrates the knowledge of all levels in complex biological models. The biomarkers are developed on the knowledge of only one or two of the omics methods. The traits like meat quality characteristics are multi-gene complex traits and contrasting pheno types including also large datasets with gradual but great variability in meat quality parameters. The quality of phenotypes should be measured with conventional methods such as colour and pH at 1 and 24 h (relatedto many meat quality traits such as the water-holding capacity and the lightness of the meat) determined by muscle energy metabolism and buffering power, glycogen content at time of slaughter, drip loss, intramuscular fat content (IMF) (the last two both related to tenderness and juiciness), shear force, sensory tenderness, juiciness, and flavour by a trained panel. Combining the information from omics studies and phenotype information using bioinformatics and statistics shows the relationship between conventional and molecular method (Zang *et al.,* 2012).

Characteristics of good biomarkers: Biomarkers are the naturally occurring molecules which is used to detect particular pathological, physiological and disease condition of an organism.

The biomarker should have following character

- High predictive value for the trait.
- Easy and cheap to detect biomarker
- Easy to measure during the production phase using non-invasive techniques and cheap sampling methods of tissues such as blood or excreted body fluids, urine, faeces and milk
- The biomarker should remain specific for the trait and preferably constant over a certain period of time even when variability in the trait between animals is low.
- Automation of testing combined with information technology may speed up the industrial procedures and reduces the costs of production in industries for large scale testing with repeatability.

Metabolic biomarker for the detection of metabolic disorders of dairy cows: Metabolomics is the detection of low molecular weight metabolites from bio fluids or tissues which are more significance. It is used widely in many fields such as pharmacology, toxicology and diagnostics and its use and technological development have increased rapidly (Zhang *et al*., 2012). Metabolite changes that are observed in diseased individuals as an important primary indicator and important part of clinical practice. Many diseases are often discovered in an advanced stage because of the lack of specific symptoms and the diagnostic facilities. The more advanced stage of diseases causes more invasive diagnostic and treatment interventions. The early molecular diagnosis is vital importance in order to increase the survival rate. A good diagnostic method should have the characteristics of high sensitivity, specificity and functionality and meets the requirements of high throughput, portability and low cost for clinical application (Nicholson *et al*., 1999).

Milk fever: Milk fever is a complex metabolic disorder usually occur in heavy producing dairy cows in and around parturition. The biochemical characteristic of this condition is severe hypocalcemia (usually <1.5 mmol/L) which most likely explains the clinical signs associated with milk fever. Investigation of this disease has chiefly focused on blood

calcium, ionized calcium or total calcium contents in the transition period because of the close connection between blood calcium and milk fever. The blood phosphorus status has also been checked because of the prominent interaction between calcium and phosphorus at the time of lactation. Several other substances such as magnesium, alkaline phosphatase, hydroxyproline, osteocalcin, parathormone, calcitionin, and 1,25-dihydroxy-vitamin D which are associated with the regulation of calcium metabolism. Serum amyloid A protein (SAA), Calcitonin-gene related peptide (CGRP), Endopin 2B,Serpin peptidase inhibitors (SPI), Downregulated proteins - fibrinogen beta chain, IGg heavychain C-region (IGg -CH), and albumin are metabolites used for detection of milk fever(Collard *et al.* ,2000).

Fatty liver syndrome: The critical period between parturition and early lactation also involves major critical physiologic changes in heavy producing dairy cows. The excessive demand for nutrients due to the increased performance required for milk production results in a negative energy balance (Mullins *et al*., 2012). One major adjustment consists in the rapid mobilization of energy sources from tissue for depots in the form of non-esterified fatty acids. Adipose tissue is the major site of fatty acid synthesis in cattle. The liver plays a central role in coping with sudden increases in the energy requirement (Kreipe *et al.,* 2011). Fatty liver disease develops during this critical transition period of lactation. The hepatic uptake of non-esterified fatty acids liberated from the adipose tissue exceeds their elimination from the liver which causing their hepatic storage as triacylglycerols. The TAG and total lipids increased and glycogen, phospholipids, cholesterol, enzymes of gluconeogenesis, enzymes of β oxidation and enzymes of glycolysis may decreased during fatty liver syndrome (Bobe *et al*., 2004).

Ketosis: Ketosis is one of the most prevalent metabolic disorder of dairy cows during peak lactation period of heavy producer. Metabolic disturbances of ketosis involve in multi-biochemical pathways such as glycolysis, gluconeogenesis, amino acids metabolism, fatty acids metabolism and pentose phosphate pathway (Huxley *et.al.,*2013). The B-hydroxybuty rate (BHBA), blood glucose levels (Glc), total triglycerides (TG), nonesterified fatty acids (NEFA) including palmitic acid (PA), heptadecanoic acid (HA), stearic acid (SA), trans-9-octadecenoic acid (T-9-OA), myristic acid (MA), cis-9-hexadecenoic acid (C-9-HA), long chain unsaturated fatty acids, and saturated acids and aspartate aminotransferase (AST) are increased. Leucine (3-hydroxyisovaleric acid (3HIV)), 4-aminobutyric acid (GABA) – Lglutamic acid catabolism, melibiose from galactose metabolism, erythritol, a precursor of fructose 6-phosphate and l-serine (L-ser) are decreased (Zang *et al.,* 2012).

Abomasal Displacement: Abomasal displacement anterioventral displacement of theabomasum is an gastrointestinal disorder normally found in high producing dairy animals. Abomasal displacement mainly due to hypomotility of abomasum due to high concentrate feed and other metabolic disorder and downer cows. The amino acid Valine, 3 β-hydroxybutyrate, alanine, glutamine and glutamate are increased and succinate concentration decreased in blood.

Techniques used for detection of metabolites in diseased animals: Common metabolomic technologies include GC-MS, nuclear magnetic resonance (NMR) and other chromatography coupled mass spectrometry such as liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis–mass spectrometry (CE-MS), metabolites chip are widely used for analysis of primary and secondary metabolites (Nicholson *et al*., 2002). Among the analytical techniques, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are the most commonly used in metabolomic studies in livestock. Metabolome is vastly complex subject. It is very difficult to find and analyse all metabolic pathways that need to be mapped accounting every intermediate and flow through their various cycles on their way to biomass accumulation, excretion, exudation, etc. The complexity increases particularly when considering the cooperative nature of the different levels of organization of biological systems and the effect that the environment can produce on the metabolism of an organism. Analysis platforms such as chromatography-mass spectrometry.

The gas chromatography-mass spectrometry (GC/MS) is probably the most popular analytical platform used in metabolic analyses. Most of the biological extracts to be analyzed via GC/MS must first be chemically derived with agents that make the chemical constituents present in the sample more volatile. There is two-fold separation of sample components based on differences in volatility and size of molecules in gas chromotograhy. Usually the larger molecules take a longer time to move through the column than do small molecules and amongst molecules of similar size different molecular species display different volatile compounds. Mass spectroscopy systems coupled with nuclear magnetic resonance systems are the best platforms for identification of unknown chemical compounds but are prohibitively expensive for most scientific laboratories (Xie *et al.,* 2016).

Nano Metabolomics: The advances in nanotechnology provide an opportunities for their application to biological systems including metabolomics. The main characteristic of nanoparticles is that they have all three spatial dimensions confined to the 1–100-nm range. The nanoparticle can be categorized based on their atomic compositions (inorganic, organic and polycomponental), their shapes (nanospheres, nanocubes, nanorods and nanoshells) and their physical-chemical properties (optical, magnetic and electronic). Nanoparticles possess properties that qualitatively differ from their macroscopic counterparts due to their chemical properties, large surface-to-volume ratio and unique quantum effects. Currently application of nanotechnology to biological systems one of the emerging new subfields such as nano biotechnology and nanomedicine which focus among others on the development of new bio analytical methods, new therapeutics and new bioimaging techniques. The metabolite-nanoparticle interactions studies are not much explored. Because the metabolome directly reflects certain phenotypic traits and metabolites are often the initial, intermediate and end products of biological processes. Better characterization of metabolite-nanoparticle interactions at atomic or near-atomic resolution will useful nanoparticles based multitude approaches. The development of novel nanoparticle-based assays to facilitate the quantitative analysis of the metabolome of different types of biological samples and different stages are very important. Metabolite-nanomaterial interaction is an growing technology in metabolomics. The metabolites display a large structural and chemical diversity which presents unique challenges for their characterization. The quantitative binding affinities of the 20 natural amino acids to silica nanoparticle

(SNPs) surfaces were recently determined based on ^{13}C-NMR spin relaxation experiments (Scida *et al.,* 2011). The binding affinities across the different amino acids display a wide dynamic range which illuminates the various physical-chemical factors dictate their binding properties. The complex mixtures of metabolites requiring little or no prior physical separation and they are both suitable for high-throughput applications. Other analytical methods like optical spectroscopy, colorimetric assays, electrochemical analysis or immunosensors can provide valuable information that is complementary to NMR and MS (Xie *et al.,* 2016). The nanoparticle-assisted technologies have the potential for future advances and breakthrough the NMR- and MS-based metabolomics drawbacks. Nanoparticles can be effectively applied to these steps requiring minimal manipulation of the sample. Nanoparticle scan serve as efficient, low-cost and environmentally-friendly components which helps to control the composition of metabolic mixtures in solution. Nanoparticles can help simplify the spectrum and differentiate peaks based on spin relaxation or ionization properties and producing more informative spectra that help uncover the structures and other properties of metabolites (Scida *et al.,* 2011).

Conclusion

Metabolomics is an fast-growing, highly cross-disciplinary field of research and emerging "omic" science with the purpose of elaborating a comprehensive analysis of the metabolome with complete set of small molecules intermediates. Metabolome is a data-rich source of information concerning all the metabolites in a biofluid of an organism. Metabolomics has great potential for improving diagnosis and therapeutic treatment. Synergistic developments in biomedicine, biotechnology, analytical metabolomics is also well-positioned to provide some important advances in both live stock research and the livestock health, breeding and production. Wide variety of biofuids have received attention for metabolomics research such as metabolic profiling of milk, plasma, serum and urine will minimizing animal suffering. The structural determination of unknown metabolites with little or no mixture separation, absolute concentration measurements and synergistic uses of NMR and MS. New applications of nanotechnology are likely to emerge that will drive the development of analytical methods with better sensitivity, resolution, quantification and throughput. Many diseases induces characteristic changes in the metabolite profiles of fluids prior to development of clinical symptoms. These metabolites are often useful for selecting diagnostics biomarkers. Identifying biomarkers for the early detection of diseases will result in more efficient treatments, reduction in suffering of animals and lower mortality rates. Metabolomics is as strategies to analyze, understand and construct the metabolic pathways.

One of the major challenges inmetabolomics is validation of fingerprint molecules to identify specific pathways in metabolic aberrations and the identification and interpretation of metabolic biomarkers is also a challenging task. Metabolic pathway-based approaches have increasingly become a good technology in metabolomics allowing capture of complex interactions in biological systems with high-throughput evaluation of a large number of metabolites. The advancements in metabolomics research producing potential biomarkers but most of the identified biomarkers have failed to replace existing clinical tests. Potential biomarker should be confirmed and validated using hundreds of specimens and should be reproducible specific and sensitive. The ideal biomarker for disease diagnosis will be developed soon but we cannot apply the single perfect marker for detection of diseases and production and reproduction traits but combining different molecules will provide information compensating for the shortcoming of individual tests. The accumulated clinical research experience and continuing exploration of the metabolomics ensure that there will be no shortage of newly discovered candidate biomarker molecules for the future.

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