Metabolomics in Dairy Cow Nutrition and Milk Quality and Safety: Research Progress (Post-print)

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Abstract

Metabolomics is a discipline that detects changes in low molecular weight (typically molecular weight less than 1 000 u) metabolites to investigate the composition and variation patterns of metabolites produced by organisms following pathological/physiological stimuli or genetic modifications. It is a new discipline developed in the post-genomic era and constitutes an important component of systems biology. Although metabolomics has been widely applied across various fields including physiology, pathology, pharmacology, animal nutrition, zoology, and botany, its application in research on dairy cow nutrition and milk quality and safety remains relatively limited. This article reviews the current applications of metabolomics in dairy cow nutrition, disease, heat stress, milk quality, and dairy product safety, beginning with its fundamental concepts, research frameworks, and methodologies.

Full Text

Research Progress on Metabolomics Application in Dairy Cow Nutrition and Milk Quality and Safety

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Abstract: Metabolomics is a discipline that detects changes in low-molecular-weight metabolites (typically with molecular weight less than 1,000 u) to study the composition and variation patterns of metabolites produced in organisms following pathological/physiological stimuli or genetic modifications. As an emerging discipline of the post-genomic era, metabolomics constitutes an important component of systems biology. While it has been widely applied in physiology, pathology, pharmacology, animal nutrition, zoology, botany, and other fields, its application in dairy cow nutrition and milk quality and safety research remains relatively limited. This review begins with the basic concepts, research approaches, and methodologies of metabolomics, and systematically summarizes current applications in dairy cow nutrition, disease, heat stress, milk quality, and dairy product safety.

Keywords: metabolomics; dairy cow; nutrition; milk; quality; safety

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Milk contains not only abundant proteins, carbohydrates, and lipids, but also numerous biologically active substances present in trace amounts yet serving important physiological functions, such as immunoglobulins, nucleotides, and oligosaccharides [1-2]. Many factors can influence milk yield and composition, including genetic factors, feed composition, seasonal variations, dairy processing procedures, and animal health status [2-5]. Therefore, by monitoring changes in various milk components, we can not only assess milk quality but also trace the physiological or pathological conditions of dairy cows [6-7]. Although conventional indicators based on biochemical or sensory parameters can monitor cow health or milk quality, these simple detection metrics inevitably yield limited results. Moreover, when parameters lack correlation, understanding the complete information about research subjects becomes more difficult. For living organisms, internal biochemical reactions are not only continuous but also interconnected among metabolic pathways, existing as metabolic networks within the body. Consequently, a holistic and systematic research philosophy is required to understand organismal metabolism. The emergence of systems biology has advanced life science research thinking, methods, and technology to a stage of holistic and systematic investigation [8]. With continuous innovation and progress in research concepts and techniques, metabolomics provides new opportunities for comprehensively understanding animal health and product quality and safety [8-9].

1 Basic Concepts, Classification, and Application Scope of Metabolomics

Metabolomics is a discipline that investigates numerous metabolites associated with time sequences produced by organisms following internal and external stimuli or genetic modifications [8,10]. The physiological parameters it measures can

directly reflect nutritional, stress, or disease states, and metabolic responses occur much faster than those in transcriptomics or proteomics. Therefore, metabolomics offers a more direct detection method, representing an important distinguishing feature from other omics approaches. Additionally, the number of metabolite types is far smaller than that of genes and proteins, and the instruments and methods used in research are more consistent, facilitating comparison among different studies. These advantages have enabled rapid development of metabolomics research in recent years and its widespread application in many fields, including nutrition, toxicology, disease diagnosis, and drug development [10].

1.2 Research Objectives, Instrument Categories, and Analytical Methods

The characteristics of metabolomics detection methods determine that the detected products are not only small in molecular weight but also diverse in type, requiring highly sensitive, precise, and high-throughput instruments for detection [8,10]. Currently, common metabolomics data acquisition primarily employs mass spectrometry (MS) and nuclear magnetic resonance (NMR) as core analytical technologies, supplemented by efficient separation equipment to form integrated metabolomics platforms. These include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS or LC-MS/MS), high-performance liquid chromatography-nuclear magnetic resonance (HPLC-NMR), and HPLC-NMR-MS technologies [8,10].

NMR technology is particularly suitable for analyzing high-abundance metabolites, requiring only simple sample preparation that essentially preserves sample structure and properties, thus offering excellent experimental reproducibility and high sensitivity. Moreover, NMR allows selection of experimental conditions within certain temperature and physiological buffer ranges, enabling analysis closer to physiological states and facilitating real-time and dynamic monitoring [8,10]. NMR detects isotopic atomic nuclei of elements, with the most common NMR spectroscopic analyses being proton NMR (^{1}H NMR), carbon NMR (^{13}C NMR), and phosphorus NMR (^{31}P NMR). These three spectroscopic-based methods can detect biological samples including biofluids, cell extracts, tissue fluids, and living tissues, with ^{1}H NMR eliciting responses from hydrogen-containing compounds in biological samples, enabling detection of most metabolites in biological samples [8,10].

GC-MS offers high sensitivity, making it suitable for analyzing volatile organic compounds or those that become volatile after derivatization. It also benefits from extensive searchable standard mass spectral libraries, such as the National Institute of Standards and Technology database, which facilitates accurate compound identification [8,10,11]. Current technology enables simultaneous determination of hundreds of chemically diverse metabolites, including organic acids, most amino acids, sugars, sugar alcohols, aromatic amines, and fatty acids [8,10,11].

LC-MS differs from GC-MS in that it distinguishes and identifies metabolites based on mass-to-charge ratio differences. Since LC-MS analysis does not require derivatization, the sample scope is not limited by compound properties, making it suitable for analyzing unstable, non-volatile, polar, and low-polarity components that are difficult to separate and detect by GC-MS. This feature makes LC-MS the most widely applicable technique for compound analysis. However, LC-MS lacks standard databases for metabolite identification, requiring online databases such as the Human Metabolome Database (HMDB) for compound identification [12].

Each technique has its advantages and inevitable limitations, but these can be compensated through combined application of multiple analytical technologies—an important trend in metabolomics research [8,10]. In HPLC-NMR and HPLC-NMR-MS studies, high-throughput HPLC separation technology efficiently separates metabolites and simplifies compositional complexity. NMR provides molecular weight and fragment ion information for metabolites, offering high selectivity and sensitivity for qualitative and quantitative analysis, while one- and two-dimensional NMR spectra can be used to determine molecular structures [13].

Metabolomics data analysis methods include unsupervised and supervised approaches. Unsupervised methods comprise principal components analysis (PCA), hierarchical cluster analysis (HCA), and self-organizing maps (SOMs). Supervised methods include discriminant analysis (DA), partial least squares (PLS), partial least squares-discriminant analysis (PLS-DA), orthogonal partial least squares-discriminant analysis (OPLS-DA), soft independent modeling of class analogy (SIMCA), and artificial neural network (ANN) [8,10].

1.3 Classification, Advantages, and Disadvantages

With advances in experimental techniques and data analysis methods, various metabolomics-based research approaches have been successfully applied in nutrition, toxicology, pathology, pharmacology, disease diagnosis, animal models, microbiology, plants, biomedicine, and environmental science [8,10]. Despite different research directions, metabolomics studies can be broadly classified into four categories: targeted metabolomics analysis, untargeted metabolomics analysis, metabolic fingerprinting, and metabolic profiling. Targeted metabolomics analysis can validate identified biomarkers but requires standards for qualitative and quantitative assistance, typically limiting quantification to a small number of compounds in large sample sets. Untargeted metabolomics primarily compares metabolites between control and experimental groups (all metabolites in an organism) to identify metabolic differences. Metabolic profiling requires researchers to predefine specific metabolic pathways or classes for deeper investigation of different metabolites along these pathways. Metabolic fingerprinting analyzes mass spectral peaks of metabolites in individuals to ultimately understand the structures of different compounds and establish a comprehensive analytical method for identifying characteristic features of these compounds

[8,10].

1.4 Advantages and Necessity of Milk and Dairy Products as Detection Subjects

Modern dairy cow nutrition research and milk quality safety detection require real-time, continuous, rapid, and non-invasive sampling and detection technologies. Traditional sample types such as blood, saliva, urine, or fecae borrowed from other research fields make timed or frequent sampling difficult, and sample quantities are often unstable or obtained in trace amounts, preventing continuous and precise testing. Milk and dairy products offer unique advantages over these samples. First, timed sampling is feasible, particularly in large farms equipped with dedicated milking equipment with relatively fixed milking schedules. Second, large sample quantities are obtainable, with single cows producing several to over ten kilograms per milking, providing ample research material. Third, since sampling occurs at fixed times, animals have adapted to the regular disturbance of milking, resulting in minimal stress responses during collection and ensuring samples contain minimal interfering factors. Fourth, multiple sample types are available. As one of humanity' s most important nutrients, raw milk and its derived products—including various types of milk powder, liquid milk, butter, cheese, whey, cream, and yogurt—can all serve as detection samples. These samples can be used not only to assess quality and safety but also to trace the nutritional status, breed, and species of the producing animals [14-16].

Milk is an important source of high-quality protein and lipids for humans. Additionally, vitamins, minerals, and immunoglobulins in milk play significant roles in human health [1]. Due to different processing methods, milk and dairy products contain varying nutrients, consequently exerting different effects on human health [2]. Therefore, research on milk quality and safety is of great significance. Metabolomics can detect small molecules in research subjects, making it a highly promising technique for identifying and quantifying metabolites in milk and dairy products [8,15-18]. Since different milk types have different nutritional and economic values, and composition varies significantly among milk from different livestock, biomarkers are needed in practical detection to effectively distinguish between milk from different species [14]. Milk quality and safety issues have become increasingly important public concerns. The presence of prohibited substances in milk and dairy products is also a key focus of detection, and targeted metabolomics can effectively address this task, such as metabolomics based on NMR analysis.

2 Applications of Metabolomics

2.1 Application in Dairy Product Processing Performance Detection

Raw milk is crucial to the dairy industry as it directly affects milk processability and the economic value of final products. Poor processability of raw milk inevitably results in low-quality dairy products [20-21]. Approximately 40% of

European raw milk production is used for cheese making, primarily through enzymatic coagulation. Therefore, rennet-induced coagulation properties are important indicators of processing performance [20]. Factors affecting milk coagulation characteristics include species, breed, seasonality, and composition [20], with milk coagulation inevitably involving many biochemical processes that are important for dairy processing technology. Previous research indicates that critical cheese-making steps typically include rennet coagulation and syneresis of the curd. Rennet coagulation is the process of enzymatic hydrolysis of casein producing para- -casein, hydrophilic caseinomacropeptide, and glycomacropeptide, which depends on the glycosylation degree of -casein molecules [20-21]. These biochemical processes involve many intermediate or terminal metabolites, but which metabolites change remains poorly understood. Some researchers have made beneficial explorations in this field. Sundekilde et al. [17] used NMR to analyze metabolic profiles of milk with different coagulation properties from different breeds, attempting to identify intrinsic relationships between milk metabolites and breed and technological characteristics. The analysis revealed that milk metabolite profiles were associated with cow breed and coagulation properties, with carnitine and lactose in milk components serving as indicators for breed classification. Changes in citrate, choline, carnitine, and lactose metabolites were related to milk coagulation properties. Analysis of coagulating and non-coagulating milk using LC-MS/MS revealed that oligosaccharides are also key factors affecting milk coagulation performance [21].

Another factor affecting dairy processing performance is raw milk heat stability. Milk thermal processing sometimes requires heating conditions exceeding 130 °C, causing biochemical changes in certain milk components. Heating may lead to protein coagulation, such as denaturation of whey proteins and casein (dephosphorylation, -casein hydrolysis) [22-23]. Metabolomics can also dynamically analyze and monitor changes in milk components. ^{31}P NMR can detect phosphorus-containing component metabolic changes in ultra-high-temperature (UHT) milk during storage [23].

Microbial contamination during dairy production, processing, and storage can cause spoilage. Severe microbiological changes can be detected through odor and color changes, but subtle biochemical processes in slightly spoiled products are often difficult to detect through conventional sensory and biochemical analysis. However, metabolomics analysis can help us understand metabolite changes during this process. For example, researchers inoculated milk with *Pseudomonas* and analyzed metabolite changes, identifying several metabolites indicative of spoilage. This study also demonstrated that exogenous microorganisms can indeed affect analytical results [24], making sample collection problematic in such studies as introduced microorganisms may interfere with final results.

2.2 Application in Milk Nutrition Research

The content and composition of nutrients in milk are of great concern to both producers and consumers [1]. NMR has become a powerful tool in food detec-

tion due to its minimal sample destruction and rapid detection characteristics. Comparative studies of human milk, cow milk, and infant formula revealed that cow milk contains significantly more metabolite types than human milk and formula, with metabolite content varying across different lactation periods [25-26]. Comparison of human and macaque milk metabolites found that human milk contains higher oligosaccharide and amino acid content, while macaque milk has higher glycerophosphocholine, hippuric acid, and trimethylamine-N-oxide [27].

Choline is an important component for building phospholipid layers of liver and brain cell membranes, and water-soluble acetylcholine is an important neurotransmitter serving crucial physiological functions in organisms. Comparison of choline content in human colostrum and bovine colostrum revealed lower total choline in human colostrum than in milk collected several days postpartum. It has been speculated that choline content in human breast milk may not meet infant growth and development needs, requiring supplementation from infant formula [28-29]. In addition to NMR detection, hydrophilic interaction liquid chromatography (HILIC LC-MS/MS) can also effectively detect choline and its metabolites, such as acetylcholine, betaine, glycerophosphocholine, and lysophosphatidylcholine. Research found that phosphocholine is the main form of choline in early lactation cow milk, with content similar to that in human milk, but decreasing exponentially thereafter. Conversely, phosphatidylcholine becomes the main form during mid and late lactation, with content increasing continuously throughout lactation [30].

Phospholipid particles participate in many biological functions and are key components of cell membranes. Milk phospholipids participate in forming the skeleton of milk fat globules and benefit animal brain development, making phospholipid content an important nutritional evaluation indicator. Milk contains not only different nutrients but also various elements. For example, ^{31}P NMR can compare phosphorus-containing component content differences in different milks [30]. However, existing milk nutrition research is mostly limited to single isotope spectral studies (such as ^{1}H, ^{13}C, and ^{31}P NMR), with very few metabolomics studies simultaneously targeting multiple isotope spectra. For instance, milk lipid metabolomics can use ^{1}H NMR, which is sensitive and provides rapid results, but ^{13}C NMR is more suitable for lipid qualitative and quantitative analysis as it provides more information [31-32]. Therefore, more research employing multiple isotope NMR and even combination with mass spectrometry is needed.

2.3 Application in Milk and Dairy Product Biomarkers

Cow milk accounts for the vast majority of world milk production, yet milk from minor dairy species such as buffalo, yak, camel, and mare is also valued in nutrition science. These milks can serve as functional foods for health promotion and disease prevention [33]. Due to their high nutritional and economic value, unscrupulous merchants often adulterate or counterfeit these specialty milks, which is difficult to detect through sensory or conventional testing meth-

ods, creating significant regulatory challenges. Therefore, rapid and accurate techniques are urgently needed to identify biomarkers for milk from different livestock species [34].

Milk metabolites represent the integrated expression of mammary epithelial cells, peripheral blood, and microbial genes, making milk metabolite profiles species-specific. NMR can help identify metabolite differences among different cow breeds (Danish, Holstein, and Jersey), with carnitine, choline, and citrate serving as potential biomarkers for breed identification [27-29]. GC-MS analysis indicates that valine and glycine can serve as specific metabolic markers to distinguish between cow and goat milk [35]. Combined LC-MS and NMR can effectively differentiate cow, goat, buffalo, yak, camel, and mare milk. Comparison revealed 68, 74, 54, 58, 77, and 91 differential metabolites between Holstein milk and Jersey, buffalo, yak, goat, camel, and mare milk, respectively. Furthermore, Holstein milk contained significantly higher lactic acid, acetylcholine, succinic acid, and pyruvic acid, but lower carnitine, uridine, and pyroglutamic acid compared to other animal milks [14].

Individuals with "lactose intolerance" and "milk allergy" can consume lactose-free dairy products or milk substitutes made from soy, oats, and cereals. ^{1}H NMR can detect quality of lactose-free beverages (lactose-containing milk, lactose-free milk, and soy/oat/cereal-based substitutes). Using nicotine as an internal standard enables quantitative lactose analysis for lactose-free beverage detection, making NMR suitable for rapid analysis of milk and milk substitutes [36].

Additionally, dairy products made from raw milk of different origins often embody different social, cultural, and economic values. With accelerated urbanization, improved transportation, and expanded human activity ranges, even local foods can be purchased far from their production areas, raising questions about authenticity and whether quality matches that of products sold at the origin. Rapid and reliable techniques are needed for identification and detection. Sacco et al. [37] combined NMR, high-performance ion chromatography (HPIC), inductively coupled plasma atomic emission spectroscopy (ICP-AES), isotope ratio mass spectrometry (IRMS), and chemometrics to differentiate metabolic component differences between milk from southern Italy and Central/Eastern Europe. Results showed significantly higher lactose content in Central/Eastern European milk. Integrated analysis of NMR and IRMS data revealed distinct metabolic differentiation features between milks from different origins. ^{1}H high-resolution magic angle spinning NMR (^{1}H HRMAS-NMR) can assess mozzarella cheese quality and trace whether it is made from Campania buffalo milk. Integrated metabolomic and microbiological analysis revealed high microbial diversity but low psychrotrophic bacterial diversity in buffalo milk mozzarella. Milk thermophilic bacteria (*Streptococcus thermophilus*) and higher levels of galactose and phenylalanine were identified, with orotic acid found to be the only milk metabolite associated with species differentiation [38].

3 Application of Metabolomics in Monitoring Dairy Cow (Metabolic) Diseases and Heat Stress

Negative energy balance metabolism occurs during early lactation, with insufficient feed intake being the most critical influencing factor. Previous studies have investigated metabolic characteristics of negative energy balance during early lactation from blood biochemical and physiological perspectives, but such research provides limited comprehensive understanding [18,26]. Metabolomics, however, can understand animal metabolic characteristics during this physiological period from a systems biology perspective.

Klein et al. [26] used NMR and MS to detect milk metabolites during early and late lactation to identify relationships between milk composition and cow metabolic status. Results indicated that acetone and -hydroxybutyrate were closely associated with mammary metabolic status during early lactation. NMR analysis revealed that besides -hydroxybutyrate and acetoacetate in milk as biomarkers for acute ketosis detection, the glycerophosphocholine to phosphocholine ratio in milk during the first month of lactation could serve as a potential marker for ketosis prognosis and health assessment, and these indicators could also be used in animal breeding to directionally select metabolically stable animals as parents [39-40]. Additionally, glucose, pyruvate, lactate, and alanine concentrations significantly decreased in plasma of cows with negative energy balance, consistent with -hydroxybutyrate content exceeding the reference limit for subclinical ketosis, indicating that feed could not meet glucose demand and ketone bodies were needed as energy sources. The consequent increase in plasma glycine reflected excessive protein reserve consumption and simultaneously predicted potential future vitamin B6 deficiency [41-42].

During peak lactation postpartum, feed intake cannot meet energy lost through milk secretion, inevitably mobilizing body fat to compensate for energy deficiency. Current research indicates that dairy cows primarily mobilize triglycerides from adipose tissue, with non-esterified fatty acids from further lipolysis serving as direct metabolic fuel to compensate for insufficient glucose metabolism. While the underlying mechanism of this alternative pathway has been studied, most research has focused on single metabolites, with the overall metabolic pathways and patterns remaining unclear [18,26]. Targeted metabolomics based on electrospray ionization LC-MS/MS (ESI-LC-MS/MS) can obtain more metabolite information, providing a more comprehensive metabolic pattern regarding excessive lipolysis in early lactation dairy cows [41]. Phosphatidylcholines involved in very low-density lipoprotein synthesis pathways in the liver are key metabolites in lipid metabolism. Lipid metabolomic analysis revealed characteristics of excessive fat breakdown, impaired insulin sensitivity, and acylcarnitine changes in high-lipolytic animals. A total of 37 key metabolites were identified across these metabolic pathways, involving sphingomyelins, phospholipids, lysophospholipids, and other lipid metabolites [41,43]. However, these studies are limited to dairy cows, and research on other minor dairy species is needed to obtain more information about metabolic

adjustments during negative energy balance in livestock [32,41].

Dairy cow mastitis involves not only health and welfare issues of infected cows but also drug residues in milk from treatment, posing a threat to milk quality and safety [43]. Therefore, identifying biomarkers for mastitis is extremely important. Research indicates that haptoglobin, serum amyloid A, and ATPase can serve as mastitis markers [43]. However, such research is limited to single or few metabolites. Mastitis involves many metabolic processes during its occurrence and development, with metabolites including various endogenous and exogenous chemical molecules such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, tea polyphenols, and alkaloids, making comprehensive systematic understanding difficult from limited information. Mass spectrometry and NMR technologies enable monitoring of numerous metabolites in metabolic processes [44]. Prostaglandins are important mediators in mastitis-related inflammatory processes. Monitoring metabolites in related metabolic pathways revealed increased arachidonic acid metabolites including thromboxane A2, prostaglandin E2, and prostacyclin in milk from infected animals. Increased concentrations of these metabolites indicate their important mediating role in the pathophysiological process of inflammatory responses induced by inflammatory factors released by *Escherichia coli* [45]. Hettinga et al. [46] used GC-MS to identify significant differences in volatile metabolites such as ethyl acetate and acetic acid between milk from mastitic cows and healthy individuals. This study also demonstrated that animal health status could be traced through milk metabolite profile detection. Subsequent research indicated these volatile metabolites were primarily induced by pathogenic bacteria and formed by blood esterases in cows. Additionally, detection of esterases in mastitic milk suggested increased leakage between blood and mammary gland barriers, which may explain why metabolites involved in esterase metabolism were detected in large quantities [47]. However, some studies have shown inconsistent results regarding connections between blood and milk metabolomic parameters in metabolically altered cows [39,43], requiring more research to elucidate differences and relationships among metabolomic characteristics of different body fluids under various physiological or pathological states.

Dairy cows often exhibit "heat stress" symptoms during hot summer weather, which damages animal health, reduces milk yield and quality, and causes enormous economic losses. However, traditional methods using temperature-humidity index and other indicators to determine heat stress status are simple but inaccurate. Furthermore, the physiological mechanism of heat stress remains unclear, and metabolic markers for heat stress physiological status are not well defined [16]. Plasma targeted metabolomics based on LC-MS identified 41 heat stress metabolic markers in lactating dairy cows, among which 13 metabolites—including trimethylamine, glucose, lactate, betaine, creatine, pyruvate, ethyl acetoacetate, acetone, -hydroxybutyrate, C16 sphingosine, lysophosphatidylcholine, phosphatidylcholine, and arachidonic acid—showed high sensitivity and specificity in diagnosing heat stress status, making them potential biomarkers. These potential diagnostic markers participate in car-

bohydrate, amino acid, lipid, and gut microbiota-derived metabolic pathways, indicating that heat stress affects various metabolic pathways in dairy cows [48]. Tian et al. [15-16] compared and integrated LC-MS and NMR data from milk samples under heat-stressed and non-heat-stressed conditions, identifying 53 biomarkers for heat stress diagnosis that participated in carbohydrate, amino acid, lipid, and gut microbial metabolic pathways. Comparison with existing results revealed significant correlations between lactate, pyruvate, creatine, acetone, -hydroxybutyrate, trimethylamine, oleic acid, linoleic acid phosphatidylcholine, and lecithin in milk and plasma before and after heat stress, suggesting that heat stress increases blood-milk barrier permeability. While progress has been made in heat stress milk metabolomics, these studies have only screened metabolite changes in different body fluids after heat stress variation. Further experiments are needed to verify whether these markers can indeed indicate metabolic changes in heat-stressed organisms. Additionally, research is needed to clarify similarities and differences in heat stress metabolic markers among different animal groups and detection samples. Finally, the reliability of these biomarkers in practical applications must be evaluated, and the physiological mechanisms of subsequent effects induced by biomarkers should be elucidated as much as possible.

4 Summary and Outlook

Metabolomics has been widely recognized as a discipline for less than twenty years, making it an emerging field. As a modern analytical technology featuring high throughput, high sensitivity, and high precision, it has been extensively applied in many fields, with its research philosophy achieving significant progress and success. However, many limitations remain. For example, metabolite identification based on NMR and LC-MS methods is still difficult due to the lack of standard reference databases. Sample pretreatment methods and loading matrices also vary, resulting in poor comparability among different studies. Second, due to the complex types, structures, and compositions of metabolites, single detection methods cannot achieve comprehensive detection. Most importantly, there is currently a lack of mature research philosophy derived from metabolomics methods, making it difficult to elucidate fundamental scientific questions using metabolomics.

Nevertheless, it is undeniable that systems biology research philosophy drives continuous progress and improvement in experimental methods and analytical techniques, enabling us to comprehensively understand life at different levels from subcellular to cellular, tissue, organ, and whole organism. Traditional animal nutrition research typically discusses issues based on a few indicators and their correlations, which cannot fully reflect organismal metabolic patterns and makes dynamic monitoring difficult. These traditional limitations are precisely the strengths of metabolomics, which can measure numerous small-molecule metabolites related to organisms, with metabolic profiles based on these data reflecting the complete picture of physiological or pathological states and pro-

viding more comprehensive information on animal metabolic regulatory mechanisms. Therefore, metabolomics is increasingly becoming an important systematic method in animal nutrition research. Moreover, with rapid economic and social development and improving living standards, animal product quality and safety have received increasing attention. Metabolomics can comprehensively analyze milk composition and constituents, offering advantages in both metabolic fingerprinting and metabolic profiling that other methods cannot match, thus showing good application prospects in milk quality and safety research and monitoring. In summary, the macro high-throughput and micro-detection features of metabolomics can help us more comprehensively understand and grasp information on dairy cow nutrition, health status, milk quality, processing performance, and safety. Continuous progress and improvement in metabolomics detection methods and data analysis, as well as integration and convergence with other omics methods, will provide greater intellectual support for the development of animal husbandry represented by dairy farming.

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Note: Figure translations are in progress. See original paper for figures.