Application of Multi-omics Technologies in Research on Rumen Metabolism and Lactation Physiology in Dairy Cows: A Postprint

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Date: 2018-12-20T00:00:00+00:00

Abstract

Compared with traditional research methods, omics technologies investigate biological questions from a more fundamental and comprehensive perspective, elucidating the molecular biological principles and mechanisms underlying substance metabolism. These technologies play a crucial role in life sciences and related fields, with their applications becoming increasingly widespread. To date, omics technologies have been extensively applied in research on dairy cow nutritional physiology, while integrated multi-omics approaches have also garnered growing attention. Therefore, this review primarily synthesizes the current research status of multi-omics technology integration in areas such as rumen metabolism and lactation physiology in dairy cows, providing theoretical support for dairy science research and healthy, efficient dairy farming.

Full Text

Multi-Omics Technology: Application in Rumen Metabolism and Lactation Physiology of Dairy Cows

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Abstract

Compared with traditional research methods, omics technologies investigate biological questions from a more fundamental and comprehensive perspective, analyzing the molecular biological principles and mechanisms underlying substance metabolism. These technologies play a crucial role in life sciences and are increasingly applied across various fields. To date, omics technologies have been widely utilized in dairy cow nutrition and physiology research, with multi-omics integration garnering growing attention. This review focuses on the current status of multi-omics applications in rumen metabolism and lactation physiology of dairy cows, providing theoretical support for dairy science research and healthy, efficient dairy farming.

Keywords: omics technology; rumen metabolism; lactation physiology; milk; application

With advances in biotechnology, various detection techniques and statistical methods in bioinformatics have emerged and matured, including the birth and application of omics technologies. Characterized by high throughput, non-targeted approaches, and big data, omics technologies provide systematic and comprehensive analysis of molecular regulatory pathways at the organism, system, organ, tissue, cellular, and subcellular levels, promoting development in related applied research fields. Each omics technology enables thorough understanding of biological information at its respective level, while integrated multi-omics analysis better facilitates systems biology development. In ruminant nutrition research, improving feed efficiency in dairy cows has long been a primary objective, as highly efficient cows not only reduce production costs but also enhance milk quality. Studies have reported that genomics can help identify cows with superior feed efficiency. Additionally, Wang et al. investigated microRNA distribution across four tissues (rumen, small intestine, liver, and mammary gland) in dairy cows, revealing that rumen miR-21-3p and liver miR-2285f regulate lactation processes and affect milk performance, while also distinguishing miRNA profiles between high- and low-quality forages and their impacts on lactation. Thus, omics technologies present new opportunities for dairy nutrition physiology research and industry development, offering possibilities to elucidate mechanisms by which nutrition, environment, and disease affect dairy production. Rumen metabolism and mammary lactation physiology are critical determinants of efficient, high-quality, and healthy dairy production. This review summarizes current applications of omics technologies in rumen metabolism, lactation physiology, and milk quality, aiming to provide insights and references for future dairy nutrition physiology research and theoretical support for industry advancement.

1 Overview of Omics Technologies

Currently, the most widely applied omics technologies include genomics, transcriptomics, proteomics, metabolomics, and specialized omics such as lipidomics, immunomics, and glycomics. Genomics focuses on molecular-level organization and function, employing methods like biomarkers and DNA microarray technology. Transcriptomics, an emerging discipline examining gene transcription profiles and regulatory mechanisms at the whole-cell level, primarily utilizes microarray-based hybridization and sequencing-based technologies, including expressed sequence tags, serial analysis of gene expression, massively parallel signature sequencing, and RNA sequencing. Proteomics explores protein function, mechanisms, regulation, and protein-protein interactions at the protein level, addressing issues such as mRNA expression levels and timing, post-translational modifications, and interactions between proteins and other biomolecules. Metabolomics investigates endogenous small-molecule metabolites (molecular weight $< 1{,}000$ u) in cells, tissues, and body fluids to discover their biological properties and variation patterns. In the post-genomics era, nutritional metabolomics has emerged as a new interdisciplinary branch combining nutrition science and metabolomics, quantitatively studying dynamic metabolic responses and changes under nutritional regulation and their health impacts at the molecular level. Current applications in dairy nutrition research primarily focus on blood, rumen fluid, mammary gland, liver, and urine. Most omics applications in dairy nutrition physiology identify differential genes, proteins, or metabolites to analyze key metabolic pathways, thereby investigating nutritional metabolism mechanisms at the molecular level.

2 Rumen Metabolism

Recent years have witnessed significant progress in applying omics technologies to rumen epithelium and microbiota. Transcriptomic studies of rumen epithelium have revealed cellular and molecular adaptation mechanisms of rumen papillae during the periparturient period in dairy cows. Wang et al. employed RNA sequencing to demonstrate that different physical properties and qualities of roughage regulate transcription levels of key rumen epithelial genes such as BAG3, HLA-DQA1, and UGT2B17, subsequently affecting papillae morphology. In rumen microbiota research, metagenomics has been applied to functional microorganisms, successfully screening urease-positive and lipase-positive clones. Using urease structural gene ureC and 16S rDNA specific primers to amplify urease clone plasmids, phylogenetic trees were constructed after sequencing, revealing the diversity of rumen functional microorganisms. Tian Yan comprehensively explained two rate-limiting processes in the complex metabolism converting plant cell wall polysaccharides to milk fat precursors (butyrate and acetate) through dairy cow rumen metatranscriptomics: degradation of plant cell wall polysaccharides to monosaccharides, and generation of milk fat precursors acetate and butyrate from acetyl-CoA. This provides scientific basis for regulating milk fat quality and new prospects for developing renewable cellulose and hemicellulose resources.

Livestock methane emissions represent a globally concerning issue, with methane from rumen methanogenic archaea comprising a significant proportion of greenhouse gas emissions. Sequencing methanogen genomes has identified biochemical pathways for methanogen growth and metabolism, offering targets for reducing rumen methane emissions through pathway modulation. Metagenomic analysis of rumen fluid identified 3,970 microbial genes, with 20 genes showing significant correlation with methane emissions, including mcrA and fmdB.

Metabolomics analyses based on mass spectrometry and nuclear magnetic resonance have shown that adding plant secondary compounds to ruminant diets affects methane production. Protozoa produce abundant hydrogen, the primary substrate for rumen methanogenesis, and their removal (defaunation) reduces in vivo methane emissions by 11%. In vitro studies revealed that catechin addition to rumen fluid acts as a hydrogen sink, significantly reducing methane production, with 1 mol catechin decreasing 1.2 mol methane emission. This study used metabolomics to elucidate catechin degradation and action mechanisms in the rumen. Additionally, saponins such as tea saponin and plant essential oils like lauric acid show promise as rumen fermentation modulators and methane inhibitors.

Regarding dietary effects on rumen metabolism, Ametaj et al. first applied metabolomics to study impacts of increasing dietary concentrate proportions, finding significantly elevated concentrations of N-nitrosodimethylamine, dimethylamine, lysine, leucine, phenylacetylglycine, niacin, glycerol, fumaric acid, butyric acid, and valine in rumen fluid under high-concentrate conditions. In cows fed high-concentrate diets, rumen protein digestion is dominated by several highly expressed proteins including actin, α-tubulin, and β-tubulin, identified as structural proteins via LC-MS/MS. Metabolomics research demonstrates that different roughage types significantly affect rumen microbial metabolomes, particularly organic acids, amines, and amino acids. Studies on differential rumen metabolites under varying roughage quality conditions revealed that most metabolites decrease in rumen fluid but increase in urine when feeding low-quality diets, with glycine, serine, and threonine metabolism, tyrosine metabolism, and phenylalanine metabolism showing the most significant differences. Comparative studies between NMR and LC-MS platforms for identifying metabolite types and relative concentrations in rumen fluid found significant detection differences for phenylalanine, ornithine, lysine, leucine, arginine, valine, and phenylacetylglycine across platforms, highlighting the importance and necessity of combined metabolomics platforms for studying rumen metabolite changes. Furthermore, sequencing and metabolomics approaches investigating major urea-decomposing bacterial groups and ruminant urea utilization mechanisms revealed that urea supplementation significantly increased ureC gene proportions. Pseudomonas, Haemophilus, Neisseria, Streptococcus, Actinomyces, Bacillus, and Succinivibrio are enriched with urease-positive bacteria, which helps understand metabolic pathways affected by urea nitrogen and may guide efforts to improve rumen urea utilization efficiency.

3 Lactation Physiology and Milk Quality

Milk yield is closely related to lactation physiology, which has been extensively studied. Phosphoproteomic analysis of dairy cow mammary epithelial cell nuclei established transgenic cloned cow milk fat globule membrane protein expression profiles, successfully identifying 1,225 milk proteins and 966 milk fat globule membrane proteins. Transcriptomics revealed that methionine and lysine nutrients regulate mTOR signaling in mammary epithelial cells through MAPK1 and eEF1B, affecting Stat5 gene expression and subsequently regulating milk protein gene expression. Transcriptomic studies on milk somatic cells demonstrated high correlation with mammary tissue transcriptomes, suggesting milk somatic cells could serve as biopsy alternatives for nutrigenomic studies. The mammary gland primarily absorbs glucose to provide energy and substrates for development and lactation, with lactose synthesis largely dependent on prolactin and its receptors. Zhang Hang conducted high-throughput transcriptome sequencing on mammary tissue and epithelial cells, elucidating mechanisms by which different dietary patterns and long-chain fatty acid supplementation affect milk fat synthesis, improving the milk fat metabolism model and providing theoretical basis for milk quality improvement. In human nutrition research, arteriovenous blood metabolomics changes reflect specific tissue organ metabolic physiological changes. A comprehensive MS-based metabolomics analysis of arteriovenous plasma samples from healthy individuals identified changes in lactate, alanine, glutamine, and glutamate during skeletal muscle activity, providing a more comprehensive assessment of blood circulation metabolic changes that offers guidance for dairy cow arteriovenous blood metabolomics research. Wang Bing' s arteriovenous blood differential metabolomics study revealed that besides conventional amino acids, glucose, and fatty acids, small molecules like hydrocinnamic acid, prostaglandin A, and talose also play crucial roles in dairy cow lactation. Therefore, arteriovenous differential metabolomics provides a novel approach for studying lactation physiology, warranting further research to reveal mammary lactation patterns.

Heat stress and mastitis harm the mammary gland, significantly reducing milk yield and quality while affecting cow health and reproduction, thereby impacting dairy industry development. However, physiological mechanisms of heat stress in lactating cows remain unclear. LC-MS and ^{1}H NMR metabolomics of milk from heat-stressed and normal cows identified significant concentration changes in lactate, pyruvate, creatine, acetone, β-hydroxybutyrate, trimethylamine, oleic acid, and linoleic acid, with these 10 biomarkers reflecting blood metabolomic changes induced by heat stress. Regarding mastitis, omics studies have identified potential biomarkers. Metabolomics research on milk during mastitis revealed that decreased lactose concentration may correlate with downregulated α-lactalbumin. Studies on subclinical mastitis using metabolomics identified valine, serine, tyrosine, and phenylalanine as significantly differential metabolites that could serve as predictive biomarkers. Comparison of colostrum protein profiles from cows with and without dry periods showed significantly lower immunoglobulin G, A, and M concentrations in colostrum from cows without dry periods, indicating suppressed immune function and compromised health. Blood-based omics research has also advanced mastitis treatment. Proteomic analysis of plasma protein changes revealed upregulated expression of serum amyloid A, $ $1-acid glycoprotein, and haptoglobin during parturition with subclinical mastitis, identifying these differential proteins as stress-responsive regulators during calving and lactation, providing references for studying postpartum subclinical mastitis.

As living standards improve, milk yield and quality receive increasing attention. Genome-wide association studies identified C4 binding protein α (C4BPA) as a milk fat-related gene, with its expression level in mammary tissue critically regulating milk fat percentage. Jiang Ping et al. found C4BPA expressed across different mammary tissues with significantly different expression between high- and low-milk-fat cows. Milk fat globule membrane proteins, important milk protein components, enable assessment of transgenic cloned milk protein safety and reflect mammary health status. These genes can be transferred into cows to enhance disease resistance and milk quality while breeding high-quality dairy populations. Two-dimensional electrophoresis exploring dietary effects on milk proteomics revealed that soybean meal promotes milk protein synthesis and secretion. Different mammalian milks contain specific differential proteins: camel milk contains unique whey acidic protein and quinone oxidoreductase; goat milk contains unique biglycan; buffalo milk contains unique clusterin; and cow milk contains unique primary amine oxidase, enabling species differentiation via proteomics. Metabolomics studies on milk metabolites found goat milk uniquely contains valine and glycine, while cow milk uniquely contains talose and malic acid, allowing species differentiation using these distinct metabolites. Proteomics can quantify added goat and sheep milk in food products, maintaining food safety. Comparative iTRAQ proteomics of human and bovine colostrum and mature milk fat globule membrane proteins identified 232 differential proteins. Recent NMR metabolomics research demonstrates effective investigation of small milk compounds. Thus, omics-based identification of small or large molecules in different species' milk helps discriminate dairy properties, reduce fraud, maintain food safety, and advance dairy nutrition science.

4 Prospects for Multi-Omics Integration

Although genomics, transcriptomics, proteomics, and metabolomics employ different technologies, methods, and research approaches to explore biological mysteries at distinct levels, they are intimately interconnected. Transcriptomics extends genomics, proteomics compensates for genomic limitations, and metabolomics reflects the final outcome of genome-transcriptome-proteome interactions under internal and external environmental influences. Genomic data can improve genetic prediction accuracy, while transcriptomics, proteomics, and metabolomics help identify coding and tag single nucleotide polymorphisms, demonstrating that multi-omics data resources in genetics can improve cattle breeds and promote healthy growth. Bacterial genomics can screen proteins synthesized by microorganisms in fermented foods, while proteomics can study protein heterogeneity in foods and characterize milk proteins and lactic acid bacteria protein synthesis. Their combination will better facilitate food protein research. Many researchers have applied integrated multi-omics analysis to comprehensively understand food functions, though integrated data analysis requires substantial work and high-level data processing skills. An integrated multi-omics (genomics, proteomics, metabolomics) approach revealed that dietary vitamin E supplementation affects rumen microbiome by increasing bacterial and protozoal populations. Transcriptomic and proteomic analysis of cows fed different diets (rice straw, alfalfa hay) identified 554 differentially expressed transcripts (423 upregulated, 131 downregulated) and 517 differentially expressed proteins (231 upregulated, 286 downregulated) in mammary tissue, indicating suppressed milk protein synthesis in high-energy diet-fed cows. Combined proteomics and metabolomics analysis showed that artificial nutrition-induced eating difficulties in neonates associate with specific saliva composition, providing references for calf nutrition physiology research. Research shows that overlapping results from multi-omics integration can mutually validate molecular-level change mechanisms, while discrepancies reflect differential biological mechanisms across levels, such as proteomics-transcriptomics differences indicating post-translational modifications and biological regulator effects. As bioinformatics technology advances, multi-omics research will apply to more fields and make greater contributions.

5 Conclusion

Omics technologies provide effective methods for studying various regulatory changes in dairy cows, forming the foundation of modern systems biology alongside bioinformatics. Future rumen metabolism research should combine metagenomics, metabolomics, and microbial pure culture techniques to specifically investigate metabolic and functional mechanisms of individual bacterial species or genera. Lactation physiology research requires more multi-omics integration to reveal physiological metabolic activities across different lactation stages, particularly between dry and peak lactation periods. This can be achieved through combined analysis of animal genomic background information, arteriovenous blood metabolomics, mammary epithelial cell proteomics, and mammary tissue omics across multiple samples, angles, and omics levels to identify biological thresholds for theoretical milk production capacity. As omics technologies mature and intersect with animal nutrition knowledge, they will better reveal how nutrition, environment, and other factors affect dairy cow growth, development, reproduction, production, and health, providing researchers and farmers with substantial effective data and knowledge to promote dairy nutrition and physiology development and advance the dairy industry.

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