Metabolomics and Its Applications in Animal Nutrition (Postprint)

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Abstract

Metabolomics represents a comprehensive assessment of endogenous metabolites, enabling the separation, detection, characterization, and quantification of metabolites from biological samples and their associated metabolic pathways, thereby reflecting nutritional metabolic changes, nutritional status, and even the progression of certain diseases in organisms. However, research on metabolomics technology in animal nutrition started relatively late and is currently still in its initial stages; nevertheless, as metabolomics research platforms continue to be perfected, the application value of this technology in animal nutrition will undoubtedly become increasingly prominent. This article provides a review of the conceptual characteristics of metabolomics, analytical techniques, and its applications in animal nutrition.

Full Text

Metabolomics and Its Application in Animal Nutrition

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Abstract: Metabolomics is a comprehensive assessment of endogenous metabolites that enables the separation, detection, characterization, and quantification of metabolites and their associated metabolic pathways from biological samples, thereby reflecting nutritional metabolic changes, nutritional status, and even the progression of certain diseases in organisms. However, research on metabolomics technology in animal nutrition started relatively late and is still in its infancy. Nevertheless, with the continuous improvement of metabolomics research platforms, the application value of this technology in animal nutrition will undoubtedly become increasingly prominent. This paper provides a re-

view of the conceptual characteristics, analytical techniques, and applications of metabolomics in animal nutrition.

Key words: metabolomics; animal nutrition; research progress

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With the advent of the post-genomic era and continuous scientific and technological progress, animal nutrition has gradually expanded from traditional studies on nutrient digestion and absorption, nutrient efficacy, feed nutritional value, and animal nutrient requirements to encompass multiple aspects including nutrient regulatory mechanisms, macro-level nutritional information transfer mechanisms, self-regulatory functions of animal nutrition, and the mechanisms of environmental impacts on organismal nutritional metabolism [1]. Consequently, conventional animal nutrition analysis methods such as chemical analysis, digestion and metabolism trials, balance experiments, and feeding trial techniques can no longer meet the increasing technical demands of animal nutrition research [2-3]. Metabolomics technology can reveal metabolic patterns in biological reaction processes and metabolic pathways, thereby discovering potential biomarkers for disease diagnosis and nutritional assessment, which provides important new insights for animal nutrition research [4-5].

However, as one of the latest omics technologies, metabolomics is an interdisciplinary approach that requires the use of one or more sophisticated measurement instruments. It demands high sample processing and analytical technical standards, and standardized metabolite spectral libraries need further improvement while data analysis and mining are complex. These factors have, to some extent, constrained the application of metabolomics technology in animal nutrition.

This paper reviews the concept and characteristics of metabolomics, its analytical techniques, and applications in animal nutrition. It also introduces specific applications of metabolomics in animal nutrition research from four aspects: studying the effects of nutritional intervention on animal organisms, evaluating animal nutrient requirements, investigating endogenous metabolites and individual metabolic differences, and exploring animal disease mechanisms and diagnosis, aiming to provide reference for the widespread application of metabolomics technology in animal nutrition research.

1. Concept and Characteristics of Metabolomics

The metabolome refers to all metabolites of a particular organism or cell, while metabolomics is an emerging technology and discipline that conducts qualitative and quantitative analysis of small-molecule metabolites (molecular weight less than 1,000 u) produced by animal organisms before and after internal and external stimuli, along with their related metabolic pathways, to study the systematic metabolic profile and functional regulation of the whole animal and reveal the essential nature of organismal metabolism [6-7].

Metabolomics can be divided into targeted and untargeted metabolomics according to research objectives. Targeted metabolomics generally focuses on one or several related metabolic pathways and quantifies specific metabolites by comparing samples with standards [8], whereas untargeted metabolomics comprehensively detects the entire metabolome of an organism, seeks and analyzes as many metabolic pathways as possible, and identifies the chemical structures of differential metabolites [9]. Currently, most metabolomics studies employ untargeted strategies [10].

Although metabolomics can be considered an extension of genomics and proteomics, it offers numerous advantages over these two omics approaches. Genomics and proteomics study animal organisms at the gene and protein levels, respectively; however, the loss of a particular gene or protein may be compensated by others, resulting in no damage to the organism. Therefore, metabolomics technology, which targets small-molecule metabolites, can accurately interpret the life activities of organisms [11]. Moreover, the types and numbers of metabolites are far fewer than those of genes and proteins, and their molecular structures are relatively simple. Metabolomics research also amplifies subtle changes in gene and protein expression, and does not require the establishment of whole-genome sequencing databases or large-scale expressed sequence tag databases, making metabolomics detection technology relatively easier [12]. Additionally, metabolites are consistent across all biological systems, making the techniques employed in metabolomics research more universal and readily accepted.

2. Analytical Techniques of Metabolomics

The research process of metabolomics generally includes sample preparation, data acquisition, data preprocessing, pattern recognition analysis, and biological mechanism analysis [13]. The greatest advantage of metabolomics technology lies in its ability to qualitatively and quantitatively study the metabolic profiles of cell extracts, tissue extracts (drugs, viruses, bacteria), and various body fluids (blood, urine, saliva, cerebrospinal fluid, etc.) using different modern analytical techniques, combined with pattern recognition analysis to obtain characteristic variables from the profiles [14]. Subsequently, corresponding metabolites are identified according to metabolomics databases, thereby interpreting the life activities of animal organisms by understanding the metabolic pathways of these metabolites [15].

Currently, metabolomics analytical techniques comprise high-throughput detection technologies and data processing technologies. Commonly used metabolomics detection technologies include nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). Among these, NMR is the most common, and due to its advantages of minimal sample damage, unbiased detection, small sample requirement, simple pretreatment, and short measurement time, it is widely applied in the analysis of samples with complex compositions [16-17]. GC-MS technology is suitable for detecting metabolites

with relatively small molecular weights [18], offering the advantages of a relatively complete standard spectral library, high resolution, and good separation effects [19]; however, this technology struggles to analyze non-volatile metabolites in samples. LC-MS technology can effectively supplement or even replace GC-MS technology [20] and is increasingly widely applied in metabolomics, now extending to high-performance liquid chromatography-mass spectrometry (HPLC-MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Such technologies offer the advantages of good selectivity, high sensitivity, and wide dynamic range [21].

Additionally, metabolomics data processing technologies include basic data analysis focused on pattern recognition analysis and deeper-level personalized data analysis [22]. The purpose of pattern recognition analysis is to apply a series of chemometric and multivariate statistical analysis methods to obtain characteristic variables from profiles, typically including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA) [23-24]. Deep-level data mining analysis helps us identify key metabolic pathways, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differential metabolites and hierarchical clustering analysis [25-26]. Through the analysis of these metabolic and regulatory pathways, we can more comprehensively and systematically understand changes in biological processes caused by altered experimental conditions, as well as the mechanisms of trait or disease development and drug action.

3. Application of Metabolomics in Animal Nutrition Research

In animal nutrition, the physiological and biochemical mechanisms of nutrient metabolism constitute important research content; however, such research has historically been constrained by technological limitations. With the emergence and vigorous development of metabolomics technology, the bottlenecks in animal nutrition research have gradually been alleviated. The application of metabolomics in animal nutrition research mainly includes evaluating the effects of nutritional intervention on animal organisms, estimating animal nutrient requirements, studying endogenous metabolites and individual metabolic differences, and investigating animal disease mechanisms and diagnosis.

3.1 Evaluating the Effects of Nutritional Intervention on Animal Organisms

Metabolomics technology plays a significant role in studying the effects of nutritional intervention on animal organisms, primarily by investigating changes in endogenous metabolites caused by nutritional intervention, thereby reflecting alterations in metabolic processes and states within animals. Adjusting nutritional interventions to promote optimal health status in animals represents the

mainstream direction for future development in animal nutrition.

Glutamine plays a crucial role as an energy substance for intestinal cell metabolism in weaned piglets. Xiao Yingping [27] applied GC-MS metabolomics technology to study the effects of early weaning and dietary glutamine supplementation on serum metabolites in piglets, identifying metabolic pathways of carbohydrates, amino acids, and lipids under early weaning conditions. Through PCA pattern recognition analysis, it was found that dietary glutamine supplementation could increase the content of creatinine, D-xylose, 2-hydroxybutyric acid, trans-9-hexadecenoic acid, and α-L-galactofuranose in piglet serum, thereby improving metabolic processes in piglets. This demonstrates that metabolomics technology can provide a comprehensive understanding of the mechanism of glutamine action in piglet nutrition, offering important guidance for swine production.

Sun et al. [28] applied UPLC-MS metabolomics technology to detect plasma, fecal, and urine samples from pigs fed high-fat diets versus basal conventional diets. Through hierarchical clustering analysis of metabolomics data, they discovered that differential metabolites including bile acids, lipid metabolites, fatty acids, amino acids, phosphatidic acid, phosphatidylglycerol, glycerophospholipids, phosphatidylcholine, and tripeptides exhibited common alteration patterns after feeding different diets. They further speculated that the most significantly altered metabolites could serve as biomarkers for identifying metabolic disorders associated with improper feeding.

Nie Cunxi et al. [29] collected plasma metabolite profiles from chickens fed different cottonseed meal-derived fermented diets using LC-MS metabolomics technology. PLS-DA pattern recognition analysis revealed that, compared with the control group, the *Candida* fermentation group showed extremely significant increases in phosphatidylcholine, phosphatidylethanolamine, cholesterol esters, sphingomyelin, diglycerides, and triglycerides; the *Saccharomyces cerevisiae* fermentation group showed increases in phosphatidylcholine, cholesterol esters, sphingomyelin, diglycerides, and triglycerides; and the mixed fermentation group showed increases in phosphatidylcholine, phosphatidylethanolamine, and diglycerides. KEGG analysis of these differential metabolites identified them all as lipid metabolites, indicating that dietary supplementation with cottonseed meal-derived microbial fermented feed increased lipid metabolism in chicken plasma. Metabolomics technology provides a theoretical basis for the rational formulation of microbial fermented feed and healthy animal production, holding significant practical importance.

Wang Xiaoxue [30] employed NMR metabolomics technology to collect fingerprint profiles of small-molecule metabolites from urine samples of domestic cats fed diets with different protein contents. Combined PCA and PLS-DA pattern recognition analysis revealed differences in endogenous metabolite levels in cat urine before and after nutritional intervention with high- and low-protein diets, as well as among different protein content intervention groups. Further analysis using metabolic network databases such as KEGG identified significant changes

in the relative content of metabolites including taurine and urea. Therefore, this study confirms the impact of nutritional intervention on animal organisms from a metabolite perspective.

Lin Gang [31] conducted differential metabolomics analysis of biological fluids from intrauterine growth-restricted and normal fetal pigs, revealing significant differences in metabolites such as glucose, fructose, urea, ammonia, amino acids, and lipids. By culturing porcine embryonic trophoblast cells with glutamine and fructose within physiological concentration ranges, they confirmed that these compounds could act as bioactive factors to individually or synergistically promote pentose phosphate cycle activity in the placenta and improve embryonic and fetal development. This study demonstrates that glutamine or fructose intervention can alleviate issues such as impaired placental pentose phosphate pathway and differential nutrient supply from mother to fetus in intrauterine growth-restricted pigs, offering significant guidance for reducing morbidity and mortality in newborn pigs and their subsequent growth and development.

Huo Wenjie [32] fed goats high-grain diets with different corn addition levels (0, 25%, and 50%) and analyzed rumen metabolites using GC-MS metabolomics combined with PCA and PLS-DA pattern recognition methods. The study found that high-grain diets could significantly affect rumen fermentation and reduce rumen microbial diversity, successfully identifying 78 characteristic metabolites including endotoxins and biogenic amines. This research demonstrates that metabolomics technology can be used to study the effects of nutritional intervention on rumen microorganisms, providing experimental evidence and theoretical foundation for further investigation of rumen microbiological mechanisms.

3.2 Application in Evaluating Animal Nutrient Requirements

Both excessive and insufficient nutrient intake can disrupt normal metabolic balance in animals; therefore, evaluating animal nutrient requirements is an essential issue in animal nutrition research. The application of metabolomics technology to assess animal nutrient requirements involves analyzing metabolites associated with excessive or insufficient nutrient intake to determine appropriate requirements for macronutrients.

Shi Meng [33] utilized metabolomics technology to analyze metabolic pathways in sow blood under conditions of insufficient or excessive energy intake. Differential biomarkers were first analyzed using PCA and PLS-DA pattern recognition methods, and further identification of differential metabolites was conducted based on compound mass numbers using databases such as METLIN (http://metlin.scripps.edu), ChemSpider (http://www.chemspider.com/), and KEGG. The study found that insufficient energy intake caused excessively low glucose and cholesterol levels in sow blood, leading to inadequate luteinizing hormone secretion and ultimately delayed or absent estrus. Conversely, excessive energy intake resulted in disorders of bile acid metabolism and sphingo-

sine metabolism, with substantial reductions in phosphatidylinositol and phosphatidylglycerol content, affecting normal cell membrane function. The above research demonstrates that metabolomics technology can effectively evaluate appropriate net energy requirements for replacement gilts during both puberty initiation and breeding stages.

Metzler et al. [34] conducted LC-MS metabolomics analysis of serum from pigs fed high calcium levels. PLS-DA pattern recognition analysis indicated that hexose might serve as a marker for excessive calcium intake, suggesting that quantifying specific biomarkers can assist in evaluating animal nutrient requirements.

Noguchi et al. [35] performed metabolomics analysis of plasma from rats fed diets with different protein contents, identifying key metabolic pathways of amino acids under high protein intake conditions. They noted that high protein intake manifests as accumulation of high concentrations of amino acids or production of toxic metabolites in target tissues, thereby altering normal metabolic pathways and enabling determination of appropriate protein levels.

Zhou et al. [36] explored animal protein requirements using GC-MS-based metabolomics technology. After long-term feeding of low-protein diets to pigs, concentrations of isobutyrate and isovalerate in the pig cecum decreased, suggesting these two substances may provide evidence for determining protein requirements in pigs.

Ruan et al. [37] investigated the effects of tryptophan supplementation on rat serum metabolism using NMR metabolomics combined with PCA pattern recognition analysis. They found reduced urea and cholesterol levels in the serum of tryptophan-supplemented rats, inferring that urea and cholesterol content may provide evidence for determining tryptophan requirements in these animals. These experiments collectively demonstrate that metabolomics technology can assist in evaluating and scientifically describing animal nutrient requirements by constructing nutrient requirement assessment models based on metabolic markers.

3.3 Application in Studying Endogenous Metabolites and Individual Metabolic Differences

With the continuous improvement of people' s living standards, the objectives of animal nutrition research have expanded from simply pursuing economic indicators of animal production quantity to encompassing animal health and product quality. However, due to differences in genetics, animal life activities, gut microbial metabolism, diet, and rearing environment, the metabolic states or processes of different animals exhibit certain variations, posing significant health risks to humans who rely on animal products as an important food source. Differentiating biological individuals and the internal/external environments of animals through differences in endogenous metabolites represents a promising direction for future animal nutrition research.

Bovo et al. [38] used LC-MS metabolomics technology to detect and analyze differential biomarkers in plasma and serum of Italian Large White pigs and Duroc pigs, investigating key metabolic pathways between different breeds using PLS-DA pattern recognition analysis. This research can provide references for evaluating relevant biomarkers in animal breeding and nutrition studies.

Wang et al. [39] studied the metabolite composition of breast muscle in Pekin ducks and Linwu ducks based on NMR metabolomics technology combined with multiple pattern recognition analysis methods. They found that Linwu duck breast muscle had higher contents of serine, carnosine, and niacinamide, but lower contents of succinic acid, creatine, and inositol, indicating that different animal breeds may have different meat metabolite compositions.

Trabi et al. [40] analyzed plasma from heifers stored at -20 °C for 2 to 15 years using NMR metabolomics technology. The results showed that betaine content strongly correlated with plasma storage time in cattle and could be used to identify plasma from different storage durations.

Jung et al. [41] obtained differential metabolites including isoleucine, leucine, methionine, tyrosine, and valine from statistical analysis of 1H-NMR spectra of beef extracts from four countries, noting that these amino acids could serve as biomarkers for distinguishing the geographical origin of beef.

Regal et al. [42] conducted HPLC-MS metabolomics analysis of serum samples from cows under conditions of exogenous steroid hormone administration, identifying estradiol and progesterone as two differential metabolites. Metabolic pathway analysis revealed that significant changes in their content were closely related to exogenous hormone administration, suggesting that estradiol or progesterone could serve as potential markers for identifying cows illegally administered hormones.

Lu et al. [43] applied NMR metabolomics technology combined with multiple pattern recognition analysis methods to identify differential metabolites in the urine of mice exposed to industrial organophosphorus compounds versus those in normal living environments. They speculated that KEGG analysis could be used to identify metabolic pathways involving all differential metabolites, thereby estimating the pathogenesis of mice affected by industrial organophosphorus environments. This study also demonstrated that NMR metabolomics technology can identify mice exposed to organic pollutants.

Xu Meiyan et al. [44] employed GC-MS metabolomics technology with PCA pattern recognition methods to study the effects of dietary lutein and Sudan red supplementation in chickens. They found that palmitoleic acid, linoleic acid, oleic acid, and stearic acid contents in chicken liver increased with increasing dietary lutein levels, while malonic acid content in chicken meat and liver increased with increasing dietary Sudan red levels, and methyl galactopyranoside content decreased accordingly. They inferred that these substances could be considered potential biomarkers for endogenous metabolic changes induced by lutein and Sudan red in chickens, which is highly beneficial for traceability anal-

ysis of pigment residues in poultry products. These research results collectively indicate that metabolomics technology can differentiate abnormal animal individuals and identify sources of potential hazards, playing a crucial role in animal and human health.

3.4 Application in Animal Disease Mechanisms and Diagnosis

In animal nutrition research, animal health is the most fundamental and important requirement. Metabolomics technology can be used to study metabolic processes and specific biomarkers in animals under pathological conditions, as well as the ameliorative effects of certain nutrients on diseases, providing powerful modern technical means for animal disease diagnosis and treatment.

Broiler ascites syndrome is an important nutritional metabolic disease that threatens global broiler production. Shi Shourong [45] analyzed serum samples from broilers with ascites syndrome using UPLC-MS metabolomics technology, identifying lipid metabolism pathways under diseased conditions. Based on metabolic pathway database searches, dihydroxyacetone was found to be a potential metabolic marker for broiler ascites syndrome. This demonstrates that metabolomics technology can be used to deeply investigate the pathogenesis of broiler ascites syndrome, enabling effective measures to reduce its incidence, which is of great significance for promoting the healthy development of the broiler industry.

Sun Lingwei et al. [46] successfully identified 32 differential plasma metabolites between ketotic and healthy dairy cows based on GC-MS metabolomics technology combined with multiple pattern recognition methods. Additionally, 13 differential metabolites were found between plasma from clinical and subclinical ketotic cows. Further KEGG analysis of these differential metabolites revealed their involvement primarily in energy metabolism pathways including amino acid metabolism, lipid metabolism, and carbohydrate metabolism. This proves that metabolomics technology can effectively distinguish healthy, clinical ketotic, and subclinical ketotic dairy cows, providing new approaches for estimating and preventing dairy cow ketosis.

Bertram et al. [47] used NMR metabolomics technology to study the endogenous biochemical effects of rye-based, fiber-rich diets on hypercholesterolemic pigs. They found that plasma base content increased in hypercholesterolemic pigs fed high-fiber rye diets and identified markers for evaluating plasma base content, offering guidance for alleviating and treating hypercholesterolemia.

Hailemariam et al. [48] analyzed prepartum and postpartum dairy cows using LC-MS metabolomics technology, finding that carnitine and propionylcarnitine could serve as biomarkers for preventing periparturient diseases in dairy cows, providing important reference basis for improving herd productivity and reducing disease incidence.

Tian Zhong [49] established fingerprint profiles of small-molecule metabolites in

acute ischemic myocardium of diabetic and non-diabetic minipigs using UPLC-MS and NMR metabolomics technology. Statistical analysis of these fingerprints combined with clinical physiological and biochemical indicators and network databases identified the essential fatty acid arachidonic acid as a candidate molecule that could serve as a marker for diagnosis, treatment, and prognosis of diabetic myocardial ischemia.

Xiong et al. [50] employed GC-MS metabolomics technology to study the effects of polyphenols on broiler chicks under heat stress conditions. They found differences in glutathione peroxidase and ornithine decarboxylase activities, as well as epidermal growth factor and epidermal growth factor receptor contents in heat-stressed broiler chicks. KEGG analysis of differential metabolites revealed that polyphenols may affect the metabolic mechanisms of heat-stressed chicks through pathways involving energy metabolism, carbohydrate metabolism, amino acid metabolism, and glutathione metabolism. In an era of intensive and large-scale poultry production, utilizing metabolomics technology can identify effective measures for alleviating heat stress, which is of great significance for guiding production.

Compared with genomics and proteomics, metabolomics better reflects how animal organisms respond to environmental stimuli or changes, and metabolites are manifested in metabolic pathways. Therefore, correlation analysis between metabolomics and gastrointestinal microorganisms will become a commonly used method in animal nutrition research. However, given the current limitations of metabolomics analytical technology, a single detection technique cannot simultaneously acquire data for all metabolites. Moreover, data obtained from metabolomics research are often extremely complex, and there are currently no effective data processing technologies that can analyze and interpret all the information obtained. Therefore, in future research, constructing a comprehensive detection technology system and developing effective data processing technologies are necessary prerequisites for the widespread application of metabolomics in the field of animal nutrition.

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