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Metabolomics in ruminant food: bridging nutritional quality and safety evaluation

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ABSTRACT

Ruminant-derived foods, predominantly milk and meat, are globally recognized as staples of a high-quality diet. Despite their widespread popularity, there is a notable deficiency in comprehensive standards addressing the nutritional values and safety of these products. This gap significantly limits both the supply of and demand for premium quality milk and meat. This review endeavors to highlight the benefits of utilizing metabolomics for the evaluation of quality and safety in milk and meat products from ruminants. It identifies critical metabolites, genetic signals, and metabolic pathways related to the synthesis of ruminant-derived milk and meat, proposing their potential as nutritional or regulatory targets and biomarkers. These biomarkers are instrumental in predicting and assessing the quality and safety of dairy and meat products, offering guidance for quality-based pricing and food safety inspections in the market. This review offers and critical overview of current metabolomics-based platforms and tools for interpreting the quality and safety of ruminant foods. The core metabolic biomarkers and biological biosynthetic processes of milk and meat enhance our understanding of the interplay between conventional food production from animals and new synthetic biological technologies.

Keywords: Food quality and safety; Metabolomics; Adulteration; Biomarkers; Artificial meat

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1. Introduction

Understanding the landscape of animal-based food components is essential to inform potential nutritional material flow and assess protection on human health and food security [1]. the production of these substances through biological transformation is a critical aspect of One Health, particularly regarding their production processes in animals and their consumption by humans [2]. Milk or meat food component analysis of proteins, fats, carbohydrates, solids and/or ash are widely assessed by traditional identifying methods or standard. In addition, milk and meat processing, storage, origin, breed, feed regime, gender, age, and other factors have a strong effect on the metabolome of the milk and meat of ruminants. Many factors (nutrition, feed sources, genders, management, weather, processing, handling conditions, adulteration, and related concerns) impact the integrity and security of products[3]. Potential biomarkers and metabolic mechanisms associated with the meat and milk synthesis need more research, which could lay a strong foundation of the food quality standards and then improve the consumers' options and producers' initiative.

Metabolomics, also known as metabonomics, focuses on studying small molecules technology and compounds identification and quantification with the high-throughput techniques (<1500 Daltons) [4; 5], encompass a variety of both internal and external chemical compounds, including fatty acids (FA), peptides, amino acids (AA), carbohydrates, nucleic acids, vitamins, organic acids, alkaloids, polyphenols, minerals and just about any other chemical that can be utilized, produced, or consumed by specific cells or organisms. Metabolomics has contributed significantly to livestock research and industry. This includes breakthroughs in animal health, breeding, and production, showcasing its pivotal role in advancing agricultural practices. Till now, many reviews are focusing on the metabolomics technology summary [6; 7; 8] or

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metabolome database development related to livestock or food, such as a recent comprehensive livestock metabolome database (LMDB, available at http://www.lmdb.ca) was released for targeted metabolomic studies [9] and a bovine and bovine rumen fluid metabolome [10; 11]. Lipids, encompassing a range of FA and lipid-soluble bioactive compounds, are of paramount importance in enhancing the sensory qualities of foods, such as flavor and texture, while also extending shelf life. These effects are pivotal in food processing and shape consumer preferences[12; 13; 14]. Lipids found in bovine milk and meat possess a multitude of biological functions, having significant impacts on human health and the physical properties of food products. The emergence of metabolomics technology underscores the complex interplay between diet and health, highlighting the critical role of these lipids in both nutritional science and food technology [15; 16], which bring in a development of recognizing the milk and food quality through the metabolome. The application of metabolomics with selected markers is potentially useful in evaluating the genuineness of unidentified food specimens[17]. Ruminant food metabolomics plays a key role in food chemistry, food quality, and the identification of biomarkers linked to economically valuable traits. Thus, to build database of the ruminant foods of milk and meat metabolome (for 5 of the most common ruminant species namely dairy cow, beef cattle, camel, sheep & goats) is necessary for producers (food industry and animal farmers), consumer preference, and researchers.

Correlation analyses between metabolites and compositional traits of ruminant foods (milk or meat) provide insights into the underlying biological mechanisms and aid in developing fingerprints and biomarkers for identifying food properties [8; 18]. The core metabolic biomarkers and biological biosynthetic processes of milk and

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meat enhance our understanding of the interplay between conventional food production from animals and new synthetic biological technologies. In this review, we summarized the recent progress and applications of metabolomics in determining ruminant food products quality to acquire a detailed overview of the metabolite profile and its fluctuations, as well the biomarkers and indicators to reflect the food origin, adulterate, trait, quality, flavor, taste, safety, etc. The milk or meat metabolome not only directly represents food quality and safety but also serves as an indicator of the animal's metabolic properties and health status [19; 20]. Thus, this review aimed to clarify: 1) What are the preferred metabolomics technologies in ruminant foodomics? 2) What are the most obvious weaknesses and advantages in ruminant metabolomics relative to other fields of metabolomics research? 3) What are the known or measured metabolites and biomarkers for the 7 major ruminant food (cow, sheep & goat milk, camel milk, yak milk, beef, sheep & goat meat, yak meat). 4) What is the relationship between ruminant food metabolome and animal science, as well the connection between ruminant food metabolome and human health and nutrition; and 5) What role does metabolomics play in the production of ruminant food alternatives?

2. Metabolomics Techniques

2.1 Techniques and data acquisition

A variety of metabolomics technologies are available, such as nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), capillary electrophoresis–mass

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spectrometry (CE-MS), high-performance liquid chromatography with ultraviolet detection (HPLC-UV), and inductively coupled plasma mass spectrometry (ICP-MS), each with distinct advantages and disadvantages. Various analytical platforms, including NMR, HPLC-UV, LC-MS, GC-MS, ICP-MS, and CE-MS, are commonly used in metabolomics studies (Table 1). To date, one of the most exhaustive metabolomic analyses conducted involved the utilization of 5 distinct platforms in a study focusing on the metabolome of bovine ruminal fluid [10]. However, for the food-metabolomics study, the GC-MS, LC-MS, NMR based platform are the frequenters.

GC-MS is a cornerstone of metabolomic research, valued for its efficiency, reproducibility, reliability, selectivity, and robustness. It is distinguished by its exceptional sensitivity and highly consistent fragmentation patterns, making it highly effective for the precise analysis of complex metabolite profiles [21; 22]. Specifically, GC-MS based on volatile organic compounds (VOCs) is always used to detect the volatile metabolites to indicate the meat flavor, which can also be treated as volatilomics [23]. With the development of new techniques, nowadays, GC×GC-TOF-MS has gained widespread application in the analysis of complex food matrices, thanks to its enhanced resolution and sensitivity [24]. Ion Mobility Spectrometry (IMS) is a potent analytical tool, and when combined with Gas Chromatography (GC-IMS), it serves as a rapid approach for profiling VOCs in food [24]. Headspace solid-phase microextraction paired with gas chromatography-mass spectrometry (HS-SPME/GC-MS) emerges as a promising approach for distinguishing between different meat species [23]. To enhance the volatility and thermal stability of these analytes, strategies such as methoximation and trimethylsilylation are frequently employed in large-scale metabolomics research, facilitating their analysis via GC-MS [25; 26]. The most

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advantage of the GC-MS is its high sensitivity in identifying the volatile metabolites that are crucial factors for food flavor evaluation.

LC-MS has become a predominant tool in identifying food metabolites, owing to its high-resolution molecular mass determination and detailed fragmentation patterns observed in MS/MS spectra, thereby facilitating the analysis of complex mixtures with unparalleled precision [27]. In LC-MS-based metabolomics studies, samples can be directly analyzed with minimal preparation, often requiring only filtration.

HPLC-UV is an analytical technique combining high-pressure liquid chromatography with ultraviolet light detection [28]. In metabolomics, HPLC-UV is utilized to separate, identify, and quantify metabolites in complex biological samples based on their absorption of UV light [28]. This method is especially useful for analyzing compounds with known UV absorbance characteristics, making it valuable for targeted metabolite analysis and contributing to the comprehensive profiling of metabolomes in dairy and meat products [29; 30; 31].

NMR spectroscopy is a high-performance tool for the analysis of metabolome and organic compounds, which has been successfully utilized in milk and meat [32; 33]. NMR stands out in the realm of metabolomics for offering a wider spectrum of profiling data, coupled with the benefits of straightforward sample preparation and relatively swift analysis. NMR spectroscopy offers a distinct advantage over LC–MS and GC–MS: a direct and quantitative correlation exists between molar concentration and the intensity of NMR resonances [34]. On the other hand, compared with other MS based analytical techniques, NMR requires simple sample pretreatment, which is time saving and NMR analysis is environmentally friendlier due to its reduced consumption of organic solvents. However, low resolution and sensitivity hinder its utilization in identifying novel compounds and in foodomics. The 1H-NMR

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metabolomic approach has been successfully applied to study the potential biomarkers of different diet [35], geographical origin [36], and in detection of the adulteration in Chicken, Chevon, Beef and Donkey meat [7]. Recently, three quantitative NMR metabolomics analysis methods (ultrafiltration, solvent precipitation with either acetonitrile/acetone/methanol or chloroform/methanol) with excellent protein removal, high concentrations of metabolites and high reproducibility are recommended to be used for lamb meat metabolome analysis [37].

The choice of technique depends on the study's goals: GC-MS is preferred for analyzing volatile compounds, LC-MS for complex mixtures with high precision, NMR for broader profiling with simpler sample preparation, and HPLC-UV for targeted analysis of UV-absorbing compounds (Table 2). Combining these techniques can improve metabolome coverage and enhance the identification of metabolites, providing a more complete understanding of the biochemical processes under study. The integration of GC-MS, LC-MS, NMR, and HPLC-UV allows researchers to capitalize on the strengths of each method, thereby increasing the breadth and depth of metabolite detection and characterization, ultimately yielding a more comprehensive metabolic profile.

Nowadays, relying solely on a single platform poses significant challenges in identifying compounds, and the accuracy of data prediction is also limited. Combining GC–MS and LC–MS techniques is common in metabolomics studies to broaden the scope of detected metabolites. This integration aims to improve the accuracy, precision, and comprehensiveness of identifying phenotype-related metabolites [22]. These three methods collectively identified 353 metabolites, with only 65 detected by the GC–MS component. This underscores how employing diverse instruments in metabolomic profiling can augment the number of identified metabolites [38]. A

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comprehensive milk metabolome profiling was conducted, employing chemical isotope labeling and LC-MS techniques. Specifically, dansylation labeling was utilized to target the amine/phenol submetabolome, allowing for a focused analysis of these compounds, illustrating the complexity of the milk metabolome [39].

2.2 Targeted and untargeted approaches

In metabolomic approaches utilizing GC–MS and LC–MS platforms, it's crucial to delineate between targeted and untargeted methods. Targeted approaches focus on identifying and quantifying a specific set of known metabolites, typically ranging from tens to hundreds. These may include common marker compounds pivotal in clinical or technological analyses [40; 41]. Conversely, untargeted approaches aim to gather extensive information by annotating metabolites and examining both known and unknown metabolic alterations [41; 42].

In untargeted metabolomics studies, two commonly utilized data acquisition methods exist. The first relies on full scan MS-only acquisitions to provide accurate mass measurements for individual molecules (raw mass features), facilitating multivariate statistical calculations. Subsequently, data-dependent acquisition is employed for identification purposes, generating fragmentation patterns for the metabolites with the highest signal intensity. [42]. Another untargeted metabolomics strategy involves data independent acquisition, which integrates full scan MS-only acquisition with MS/MS fragmentation for all precursor ions, either concurrently or within specific mass ranges [42; 43]. A previous study systematically evaluated the advantages and disadvantages of targeted and non-targeted metabolomics approach [41]. It was due to the lack of standard pure compounds these days; most metabolites cannot be detected using targeted metabolomics. However, unlike targeted metabolomics, non-targeted approaches present the opportunity to discover new

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biomarkers, albeit with potentially lower robust accuracy due to the risk of false identification of metabolites or bias/signal drift induced by matrix effects.

Targeted metabolomics focuses only on a pre-selected set of known metabolites, typically ranging from tens to hundreds. This limited scope can be a drawback in food safety monitoring, where unexpected contaminants or novel metabolites may need to be detected. For example, in cases of food adulteration or contamination with unknown toxins, targeted metabolomics may miss important compounds simply because they are not part of the target list [(44)]. Targeted approaches require standard pure compounds for calibration and identification, which may not always be available, especially for newly emerging contaminants. In food safety, this poses a significant challenge, as many potential contaminants or toxins may lack commercially available standards [(45)]. The inability of targeted metabolomics to identify unknown or emerging contaminants limits its effectiveness in detecting unexpected food safety issues, such as novel pesticide residues or chemical contaminants that may appear due to changes in farming or food processing practices.

Untargeted metabolomics has lower accuracy compared to targeted approaches because of the risk of false identification [(46)]. In food safety monitoring, this lack of robustness can lead to misidentification or the inclusion of false positives, which can complicate or hinder regulatory decision-making [(47)]. Untargeted metabolomics generates large datasets, and interpreting these data can be challenging and time-consuming. Food safety monitoring often requires rapid response, but the complexity of data analysis in untargeted studies can delay actionable outcomes. In untargeted approaches, matrix effects and signal drift can significantly affect the results, making it difficult to achieve consistent quantification across different sample matrices [(48)]. This inconsistency is problematic in food safety monitoring, where reliable,

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quantitative information is essential for determining the level of risk posed by a contaminant. Unlike targeted metabolomics, untargeted approaches are less effective at accurate quantification of detected metabolites, particularly when the concentrations of specific contaminants are low. For food safety monitoring purposes, precise quantification is crucial for determining whether contaminant levels exceed regulatory thresholds.

Targeted metabolomics is always used for the detection of pre-selected set of known metabolites, typically ranging from tens to hundreds. However, in food safety monitoring, the unexpected contaminants or novel metabolites may need to be detected [(44)] as many potential contaminants or toxins may lack commercially available standards [(45)]. In the meantime, untargeted metabolomics has much more metabolites than can be detected but some of them with lower accuracy compared to targeted approaches because of the risk of false identification [(46)]. In untargeted approaches, matrix effects and signal drift can significantly affect the results, making it difficult to achieve consistent quantification across different sample matrices [(48)]. Thus, these two methods can be combined to mitigate the drawbacks.

2.3 Metabolome database

Nowadays, Human Metabolome Database (HMDB) (http://www.hmdb.ca/) [(49)] and Bovine Metabolome Database (BMDB) [(11)] represent two of the most comprehensive databases to work on metabolomics in ruminants biology. PubChem compounds of NCBI and KEGG COMPOUND also provide a reference metabolite in further details. An online database (http://www.lmdb.ca) includes data on the analytical platform(s), experimental conditions, field of research, and animal breed used in acquiring the metabolomic data (Table 3). To enhance consistency, concentrations of all metabolites with quantitative data were converted into a

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standardized unit, such as μM. Currently, five levels of confidence in identification have been established with the highest confidence of validated identification (Level 1), a putative identification (Level 2), preliminary identifications (Level 3), molecular formula candidates (Level 4), and de-convoluted experimental m/z features (Level 5) [50]. Moreover, a review of livestock metabolomic studies encompassing cattle, sheep, goats, horses, and pigs detected and/or quantified a total of 1070 metabolites [9].

3 Ruminant milk and dairy product

3.1 Classification and function of milk

Milk, an important biofluid of animals, is often called the "perfect food", rich in key nutrients such as proteins [51]. Milk stands as one of the most extensively consumed beverages globally, with 927 million tons produced in 2023 [52]. Comprising primarily water (85−87%), bovine milk also contains fats (3.8−5.5%), proteins (2.9−3.5%), and carbohydrates (5%) at the macronutrient level. Additionally, it harbors various bioactive compounds such as vitamins, minerals, biogenic amines, organic acids, nucleotides, oligosaccharides, and immunoglobulins at the micronutrient level [53].

Global milk production and commercial milk products is dominated by five ruminant species with 81% of total milk production coming from cows, followed by buffaloes with 15%, 4% for goat, sheep and camel milk combined [52]. Dairy cow milk is the most prevalent choice among consumers, whereas sheep, goat, and camel milk are significantly rarer in the market [7; 18; 53; 54]. Thus, we can refer to these as ruminant milk products. Bovine milk, a biofluid rich in nutrients and chemically intricate, encompasses a multitude of diverse components. The hydrolytic AA content was found to be greater in bovine colostrum compared to human colostrum, suggesting a need for further investigation into AA metabolomics and its implications for infant

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formula development [55]. A Web-accessible database called the Milk Composition Database (MCDB, http://www.mcdb.ca/) was constructed based on 2355 identified metabolites in bovine milk [53].

3.2 Factors affect milk metabolome

Milk and milk products are globally consumed and renowned for their nutritional richness. Consequently, safeguarding their nutritional quality and ensuring product safety have emerged as paramount concerns in ruminant food research. Metabolites serve as indicators of milk and milk products' quality, encompassing aspects such as nutritional value, authenticity, and safety [3]. Till now, several metabolites such as choline, citrate, valine, hippuric acid, 2-butanone, lactate and some FA have been used as robust biomarkers for milk quality, traceability and safety studies [56]. Milk metabolome studied is not only a sign of milk quality but also a metabolic indicator of animal performance [57; 58]. It was commonly known that milk composition varies with the cattle breed (i.e., Holstein, Jersey, Brown Swiss, etc.), stage of lactation, level of parity, number of viable pregnancies, and processing after milk collection [59; 60]. The fluctuations in milk metabolites are influenced by various factors such as dietary nutrition, genetics, dairy animal species, lactation stage, as well as external factors like season, geographic origin, disease, and processing and storage conditions (Fig. 1) [61; 62]. Following metabolomic analyses, potential ruminant milk biomarkers indicative of these factors was summarized (Table 4).

3.2.1 Feed factor

To enhance the health attributes of dairy products, dietary feed regulation has been made to increase their contents of unsaturated FA (UFA), especially that of omega 3 polyunsaturated fatty acids (n3-PUFA). Additionally, rumen protection techniques are applied to ensure that polyunsaturated FA are not degraded by rumen

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microbes, thereby validating the effects of dietary supplementation [63]. Untargeted metabolomics was used to investigate that both feed-derived (such as phenolic metabolites) and animal-derived compounds (such as FA) are potential biomarkers associated with dairy cows fed different feeding regimens [64]. Validation of these biomarkers involves using standardized analytical methods such as ultra-performance liquid chromatography–high-resolution mass spectrometry (UHPLC-HRMS) to ensure reproducibility and accuracy [64]. Alternatively, grazing has been found to increase the content of n3-PUFA in goat milk. Controlled feeding comparisons and the application of consistent analytical platforms such as LC-MS have been used to validate these observations, ensuring the robustness of biomarkers like n3-PUFA [65]. Milk sourced from animals grazing on pasture is commonly [66], albeit not always, considered healthier. Biomarker validation here involves repeated studies to confirm the effect of grazing on milk fatty acid content, with standardization achieved by using consistent sampling and analysis methods, such as GC-MS and LC-MS. In addition, many plants or plant extracts were shown to potentially change the milk flavor or taste by upregulate or downregulate milk specific metabolites, such as essential oils [67], purple perilla leaf [68], berry extracts [69], and bamboo leaf extract [70]. For instance, using LC–MS/MS, supplementation of *perilla frutescens* leaf could potentially modify the milk metabolome with elevated levels of oleanolic acid, nucleotides, PE-NMe (18:1(9Z)/18:1(9Z)) and DG (18:0/20:4(5Z,8Z,11Z,14Z)/0:0), influencing pathways like pyrimidine metabolism and the biosynthesis of UFA in dairy cows [68]. The validation of these biomarkers is performed through controlled supplementation trials, followed by standardized metabolomic analysis using LC–MS/MS to ensure reproducibility. Organic bovine milk exhibits a significantly higher concentration of beneficial FA, including conjugated (9-cis,11-trans)18:2 linoleic acid

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(CLA), α-linolenic acid, linoleic acid, and total UFA, alongside a reduction in caproleic acid levels. The validation process for these biomarkers includes multivariate analysis, such as PCA, PLS-DA, and ROC analysis, applied to NMR metabolomics data, which allows for consistent differentiation between organic and conventional milk profiles [71]. These distinctions were revealed through the application of 1H-NMR and 1D TOCSY NMR techniques, highlighting the impact of agricultural practices on the nutritional composition of milk.

3.2.2 Animal species and varieties

The dairy animals (cows, buffaloes, sheep, goats, camel) have their respective share (81% from cow, 15% from buffaloes and the rest of 4% from goat, sheep and camel) in overall milk yield of the world and an increase has been seen over years [72]. Bovine milk products are the main predominantly probiotic carrier in dairy foods [73]. The consumption of milk and dairy products obtained from sheep and goat is expected to increase by 26% and 53% respectively until 2030 [72; 74]. By integrating NMR with chemometrics, researchers identified ten metabolites—carnitine, N-acetylcarbohydrates, acetate, choline, ethanolamine, citrate, creatine, lecithin, D-lactose, and D-sucrose—as reliable markers for detecting milk adulteration. This approach enhances the ability to safeguard the integrity and authenticity of milk product [75]. Yak milk has a richer composition than Holstein milk, with higher levels of fat, protein, solids-not-fat, and calcium. Metabolomics has been applied to explore these differences, demonstrating yak milk's potential for specialized dairy products like cheese [76]. Traditional fermented yak milk, produced by Tibetan herders in Gannan, contains a diverse microbial community, including *Streptococcus salivarius*, *Lactobacillus helveticus*, and *Kluyveromyces marxianus*. Metabolomics helps link these microbes to flavor compounds, with O2PLS analysis identifying key bacterial

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and fungal genera contributing to flavor. This insight supports the development of traditional fermented yak milk products with enhanced flavor profiles [77]. Yak milk from the Tibetan Plateau is known for its high nutritional value, containing high protein, fat, lactose, and bioactive components than cow milk. Metabolomics has identified essential AA, unsaturated FA, and bioactive peptides, which contribute to antioxidant and immune-boosting effects. These qualities make yak milk ideal for functional dairy products that promote health benefits such as anti-fatigue and hypoxia resistance, particularly useful in high-altitude regions [78]. Recent metabolomic research has highlighted the unique nutritional profiles of different animal species and varieties of milk. Yak colostrum contains high levels of inositol, glycine, and carnitine, along with a favorable essential AA to total AA ratio, contributing to its superior nutritional value [79]. Yak milk also has elevated levels of creatine, lipoprotein lipase, and specific bioactive proteins that reflect its adaptation to high-altitude environments, making it particularly rich in health-promoting compounds [80]. Buffalo colostrum is rich in primary bile acids and bioactive peptides, enhancing its medicinal properties, such as antihypertensive, antioxidant, and anti-inflammatory effects, while supporting the survival of probiotic bacteria in fermented dairy products [79; 80]. In comparison, cow milk is characterized by higher concentrations of iminostilbene and osteopontin, which support bone health and immune function, though it has lower concentrations of functional components compared to yak and buffalo milk [80] These insights emphasize the distinct health benefits of each milk type, offering potential for developing specialized dairy products that cater to various health needs, including infant nutrition and functional foods.

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The production of various milk and dairy products from goat (*Capra hircus*) and sheep (*Ovis aries*) milk is on the rise, though their global market share remains significantly smaller in comparison to cow and buffalo milk. In certain regions, the issue of a "goaty" or "mutton" taste in milk products may arise, affecting consumer preference and acceptance [81; 82]. However, goat and sheep milk and related dairy products have gained increasing attention from both consumers and the industry due to their superior digestibility and higher concentrations of bioactive substances compared to cow or human milk. Studies on goat milk, particularly involving infant formula, were based on populations that included 62 infants in a randomized controlled trial, 200 infants in a 12-month study, and 79 infants in another trial, demonstrating the nutritional adequacy of goat milk formula compared to cow milk formula [83]. Sheep milk studies included various experimental models, including in vivo trials using Wistar rats to evaluate the health benefits of fermented sheep milk products, highlighting its anticancer, gastrointestinal health, and other health-promoting properties [83; 84]. Research has identified that valine and glycine are unique to goat milk, whereas talose and malic acid are distinctive markers of cow milk [85]. For instance, the goat milk not only has more digestible proteins and fats, but contains higher contents of short- and medium-chain fatty acids (MCFA), UFA, n-6 FA, n3 FA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than cow milk, which results in increasing particular interest by consumers, especially to infants and elder people [86]. In the comparison between sheep and goat milk, through a GC-MS-based metabolomics approach, it was discovered that arabitol, citric acid, α-ketoglutaric acid, glyceric acid, myo-inositol, and glycine are predominantly found in sheep's milk. Conversely, goat's milk exhibited elevated levels of mannose-6-phosphate, isomaltulose, valine, pyroglutamic acid, leucine, and fucose, highlighting the distinct

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metabolic profiles between these two types of milk [18], but there is still a lack of whole picture regarding the goat and sheep milk metabolite profiles as the animal varieties [18]. Camel milk is celebrated for its nutritional richness, containing all essential nutrients alongside compounds that may possess anticarcinogenic, antihypertensive, antioxidant, hypoallergenic, and cholesterol-lowering properties, making it a unique and beneficial addition to the diet [87]. Camel milk is a specific local food used in some countries and regions of Southeast Asian, Middle East, and African continent, showing with immunomodulatory effects and good for human health, and is easily digested and well tolerated by lactose intolerance people [54; 88]. The bioaccessible fraction of fermented camel milk has been shown to possess enhanced biological functionality compared to that of fermented bovine milk, highlighting its superior nutritional and health-promoting qualities [89].

The lipidome and FA composition in human, bovine and caprine milk were analyzed and compared using UHPLC-QTOF-MS and GC–MS. Human milk is rich in triglycerides (TG) containing linoleic acid (C18:2), sphingomyelin (SM) containing ultra-long-chain Fas and phospholipids (PLs) containing arachidonic acid (ARA)/DHA/ dihomo-γ-linolenic acid (DGLA). Caprine milk is rich in PLs, including hexosylceramide (Hexcer), Hex2Cer, SM, ceramide (Cer) and phosphatidylcholine (PC). Bovine milk is rich in PC and CL. The detailed examination of the lipid profiles of Chinese human, bovine, and caprine milk contributed valuable insights that could assist in formulating infant nutrition that is more precisely tailored to meet the dietary needs of Chinese babies [90]. These findings have significant real-world applications beyond infant formula production. Understanding the unique lipid and fatty acid profiles in these different milk types allows for better nutritional interventions for specific populations, such as elderly individuals or those with particular dietary

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requirements [91]. By leveraging the specific bioactive components found in caprine and bovine milk, it is possible to develop specialized nutritional products that address deficiencies in essential FA or phospholipids, enhance cognitive function, or support immune health [92]. The identification of FA like DHA and ARA, which are crucial for neural and visual development, is particularly useful for designing targeted nutritional supplements and functional foods [93]. Furthermore, these insights can be applied to improve the nutritional quality of dairy products aimed at supporting growth and development in young children, as well as enhancing the health benefits of dairy-based functional foods for adults.

3.2.3 Geographical origin

The NMR metabolomic technique has been validated as an effective method for determining the origin of authentic products, demonstrating its applicability across both narrowly defined geographic regions, such as the Mugello valley, and broader areas encompassing large-scale distribution networks [34; 50]. Using an untargeted approach that combines UPLC-Q-Exactive Orbitrap MS with multivariate statistical analysis, researchers identified 38 lipid molecules as potential indicators for determining the geographical origins of goat milk, and 19 lipid molecules for discerning its lactation stages, showcasing the method's precision in tracing the provenance and physiological status of goat milk [94].

3.2.4 Commercial processing

Metabolomics is useful to evaluate changes caused by food processing and can be seen as a crucial tool to support academia and industry on revealing the transformation of raw animal materials into ready-to-eat products [95]. The manufacturing and processing of milk commercially is for pasteurized beverage, Ultra-high-temperature (UHT), evaporated milk, ice cream, butter, milk powder and

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cheese, whey protein concentrate, paneer, ghee, traditional milk products, even soaps, lotions, and sweets, besides the popular cheeses and yoghurt [81]. Fermentation and thermal processing enhance the flavor and texture of dairy foods, making them more appealing [96]. Hydroxyglutaric acid is a biomarker and unique metabolite of pasteurized goat or cow milk [85]. In recent years, there are 60 marker metabolites from three categories of peptides, lipids, and nucleic acids that were detected for distinguishing between UHT and reconstituted milk by UPLC-Q-TOF-MS [97]. Applying the same technique, researchers identified 7 oxylipids—9-hydroxydecanoic acid, 12-hydroxydodecanoic acid, 2-hydroxymyristic acid, 3-hydroxytetradecanoic acid, 5-hydroxyeicosatetraenoic acid, 3-hydroxyhexadecanoic acid, and 10-hydroxyoctadecanoic acid—as effective markers for differentiating UHT milk from raw and pasteurized varieties [98]. Furthermore, L-carnitine, succinate, and acetate were pinpointed as biomarkers to differentiate UHT and reconstituted milk, based on comparisons with standard NMR-spectra databases [99]. Post-pasteurization, the percentage of sphingomyelin in milk saw an increase, whereas fermentation into yogurt did not affect its levels [65]. Following the fermentation of brown goat milk, there was a notable increase in the contents of organic acids, peptides, medium- and long-chain FA, and heterocyclic compounds through a comprehensive approach that integrated lipidomics and metabolomics [100].

Cheese, a fermented dairy delight, hosts a variety of microbial communities that evolve over time and differ based on the cheese variety and the specific starter and adjunct cultures used in its production. The milk of all ruminant species can be used to make cheese, but to improve efficiency, cheese-making procedures need to be optimized to take into account the large differences in their coagulation, curd-firming, and syneresis properties [72]. The deployment of metatranscriptomics, metaproteomics,

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and metabolomics—collectively referred to as "cheesomics"—utilizes a multi-omics approach to enhance our comprehension of cheese's microbial makeup and predict cheese characteristics such as flavor, quality, texture, and safety, as well as uncovering bioactive metabolites that may impact human health [(6)]. However, untargeted metabolomic approach is the most commonly used method to detect the cheese quality. For instance, the chemical fingerprints distinguishing Protected Designation of Origin (PDO) Grana Padano cheeses from non-PDO "Grana-type" cheeses have been established using UHPLC/QTOF-MS. This analysis revealed that lipids (FA and their derivatives, phospholipids, and monoacylglycerols), AA, and oligopeptides, along with plant-derived compounds, emerged as the markers with the highest potential for discrimination [(101)]. Utilizing a synergistic metabolomics and peptidomics strategy, researchers identified potential markers to detect counterfeit cheeses, particularly those with an excessive rind content (> 18%). The compounds 2-hydroxyadenine and argininic acid, along with 5-hydroxyindole acetaldehyde, were pinpointed as the most effective indicators of rind inclusion. Furthermore, the medium-chain aldehyde 4-hydroperoxy-2-nonenal emerged as a common marker indicative of both the cheese's ripening duration and abnormal rind inclusion [(102)]. During the Mongolian cheese storage, it was found that the bitter AA, bitter peptide (Phe-Ile), and organic acids (sinapic acid, butyric acid) increased accompanied with the increased contents of short-chain FA, 2-undecanone and ethyl esters, which increased the cheese unpleasant smell and decreased the overall acceptability [(103)]. Metabolomic analysis of the commercial processing of milk highlights several significant findings regarding fermentation and co-fermentation, as well as cheese production. co-fermentation with *Bifidobacterium adolescentis* and other probiotics improved the stability of probiotic fermented beverages (PFBs) compared to single-

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strain fermentation, enhancing levels of health-promoting metabolites such as gamma-aminobutyric acid (GABA) and L-malic acid even after 30 days of storage [104]. In cheese production, various fermentation methods, including the use of probiotics like *Lactobacillus plantarum* and *Lactobacillus helveticus*, have been shown to enhance the production of AA, FA, and other bioactive compounds, improving both the nutritional quality and sensory properties of cheese. These probiotics also influence key metabolic pathways related to flavor and bioactivity, leading to health-promoting effects such as anti-inflammatory benefits, thereby providing opportunities for the development of functional dairy products [105].Thus, metabolomics is useful for assessing the cheese quality through the changing of small molecule compounds.

3.2.5 Dairy cow physiological condition

Diseases such as mastitis, lameness, and rumen acidosis are very common in dairy cows, beef cattle, sheep, and goat, exerting huge economic loss. Heat stress and mastitis represent significant financial challenges within the dairy industry. The metabolomics approach is helpful for better understanding the pathobiology of these disease [106; 107; 108]. The analysis of whole raw milk from Holstein dairy cows affected by lameness, utilizing direct inject/LC-MS and NMR techniques, has proven beneficial for identifying and potentially mitigating lameness-associated pathological processes [106]. Significant variations of metabolome were found between healthy and mastitis cows by a novel metabolomics technique based on LC-MS. Milk arginine and Leu-Leu were increased in both the clinical and subclinical mastitis groups compared to healthy cows, indicating these metabolites were the potential biomarkers [108]. Furthermore, the enrichment of the tricarboxylic acid cycle and the biosynthesis pathways of phenylalanine, tyrosine, and tryptophan were identified to elucidate the mechanisms behind the variation in the metabolome of mastitic milk, employing an

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untargeted 1H-NMR approach [107]. NMR-based metabolomics analysis showed an increase in lactate, butyrate, isoleucine, acetate, and β-hydroxybutyrate levels, while levels of inosine and fumarate decreased in milk exhibiting high somatic cell counts [109].

Milk choline, phosphocholine, N-acetylcarbohydrates, lactate, and β-hydroxybutyrate have been identified as potential markers of inflammation, exhibiting varying patterns dependent on the ambient temperature [110]. An integrative metabolomics investigation employing LC-MS and 1H NMR spectroscopy identified several biomarkers in milk—lactate, pyruvate, creatine, acetone, β-hydroxybutyrate, trimethylamine, oleic acid, linoleic acid, lysophosphatidylcholine 16:0, and phosphatidylcholine—that effectively indicate heat stress [111]. Changes in concentrations of individual milk metabolites (volatile metabolites, and nonvolatile metabolites) can be related to the ruminal CH4 production pathway (van Gastelen et al., 2018). Furthermore, multi-omics studies represent an important gap revealing in livestock research [112; 113; 114]. The combination of metabolomics and proteomics was always used in dairy milk characteristics [19; 115].

4 Ruminant meat metabolomes

Meat quality is generally focused on the phenotypes, including nutrient contents, meat color, tender, juiciness, etc., which may be dependent on the subjective preference by consumers and have limited information for the food characteristics. However, the meat metabolome could show the basic fingerprint of food and help consumers and health professionals make informed decisions (Fig. 2). Metabolomics, supported by bioinformatics, identifies biomarkers in muscle and meat that enhance animal production and meat quality, benefiting both producers and consumers [14]. The breed of the animal, its diet, and even the specific part of the meat influence meat

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quality and its metabolites. Consequently, potential biomarkers in ruminant meat indicative of these variables have been systematically compiled (Table 5).

The compounds such as N'-formylkynurenine, kynurenine, and kynurenic acid (all part of tryptophan metabolism) and the oxidative stress indicator dityrosine and 3-dehydroxycarnitine are mechanistically connected to pathways associated with red meat, distinguishing them from those linked to white meat [116]. Ruminant meat is typically red meat, high in saturated FA (SFA), and red meats from ruminants exhibit a higher SFA to UFA ratio compared to meat products derived from monogastric animals and fish [117]. However, ruminant meat, especially for beef and mutton, is high in CLA. The cis-9, trans-11 isomer stands out as the primary dietary form of CLA present in products from ruminants. This particular isomer is generated through partial biohydrogenation of linoleic acid in the rumen or via endogenous synthesis within the tissues themselves [118]. Boosting the CLA content in meat could enhance its nutritional and therapeutic benefits. To elevate CLA intake in the human diet, options include either consuming more ruminant-derived foods or increasing the CLA levels in milk and meat products [119]. On the negative outcome side, due to the rumen biohydrogenation, the beef and mutton are always showing lower UFA, especially of PUFA compared to the pork, chicken, and fishes. Nowadays, the enhancement of PUFA, especially for n3-PUFA in beef and mutton are mainly through supplementing of n3-PUFA enriched feed sources but with relative lower transformation rate, or using rumen protected methods to release the n3-PUFA in gut [120]. Till now, differences in meat between ruminant animals and monogastric animals have been conducted by GC-MS based metabolomics, such as the comparison between beef and pork [121]. However, this study did not use fresh muscle samples. Another limitation is that the animal species and sample number are not enough to show the meat

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metabolome differences using same analyzed metabolomics method. Future research could address these limitations by using fresh muscle samples to ensure that the metabolomic profiles accurately reflect the native biochemical state of the meat. Moreover, increasing the number of animal species and sample sizes would provide a more comprehensive understanding of metabolome variations. Standardizing the metabolomics protocols across different species would also enhance comparability and reproducibility, leading to more robust conclusions about ruminant and monogastric meat differences.

Furthermore, the mixing of high-price meat species with low-quality/-price meat can be seen in food market or catering industry [7]. The ruminant meat is commonly in higher price than other livestock meat, thus, metabolomics can be used in adulteration of beef or mutton. NMR-based metabolomics has emerged as an effective technique for identifying unique signatures (potential biomarkers) to distinguish meats from different sources. This method holds promise for quality control applications, offering a way to differentiate between meat types—an important factor for ensuring food safety and addressing public health concerns [7].

4.1 Beef

Beef, a staple food in numerous countries, faces increasing scrutiny due to misinformation regarding beef products. Concerns include false claims about origin, species, and production methods, highlighting the need for accurate information and transparency in the beef industry [122; 123]. Metabolomics has been utilized to explore potential biomarkers associated with meat quality traits, concentrating on factors like the genetic background of the animal, sensory characteristics, feeding systems, and formulations. This research also encompasses processes such as postmortem storage and hygiene management, aiming to comprehensively understand how these elements

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influence meat quality [7]. The volatile compounds like 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol, 2-propylpyrazine, 1-furan-2-ylpropan-2-one, 1H-pyrrole, 2-methylthiophene, and 2-(furan-2-ylmethyldisulfanylmethyl)furan are potential key contributors to beef-related attributes and flavor in glutathione-Maillard reaction products [124]. Both UPLC-Orbitrap-MS and GC–MS analytical platforms were used and the geographical origins and feeding regimes could be differentiated by the potential biomarkers including AA, several sugar metabolites, and a number of PCs and Pes, which offers a method to identify the geographical origin of beef at any point along the supply chain and could be used to develop a verifiable traceability system [123]. Various AA and succinate emerge as potential biomarkers for discriminating the geographical origin of beef sourced from four countries: Australia, Korea, New Zealand, and the United States [36]. Utilizing NMR-based metabolomics with High Resolution Magic Angle Spinning (HR-MAS) enables the classification of meat samples based on their storage time [125]. This study represents the first metabolomic investigation of Japanese Black cattle using GC/MS analysis, comparing metabolites across different muscle and intramuscular fat (intermuscular fat) in marbled beef. MCFA implicated in triacylglycerol synthesis were exclusively detected in fat tissue. Additionally, decanoic acid, uric acid, elaidic acid, and 3-phosphoglyceric acid emerged as potential biomarkers for IMF, indicative of marbling levels. Notably, decanoic acid and glutamine were identified as potential biomarkers associated with oily flavor, wagyu beef aroma, and overall sensory evaluations [121]. Differences in enzymatic activities (lactate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase), pyruvate contents, and the number of metabolites associated with the tricarboxylic acid cycle were observed between *longissimus lumborum* and psoas major muscles

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from Chinese Jinjiang yellow cattle [126]. Yak (*Bos grunniens*), a special beef cattle, is mainly located in the Himalayan highlands region [127], the longissimus thoracis from locally farmed Jiulong yaks had higher concentrations of carnosine and formate and lower concentrations of mannose, inosine, threonine, alanine, valine, isoleucine, tyrosine, phenylalanine and leucine compared to biceps femoris by an untargeted NMR metabolomic approach [33]. Beef from grass-fed animals is characterized by tenderness, lower total fat content, a higher omega-3/omega-6 ratio, and superior protein content compared to grain-fed counterparts [128; 129]. Metabolomics has been extensively used to understand the biochemical changes in beef during processing and storage, with particular emphasis on color stability and tenderness. The metabolomic investigation of fresh beef, lamb, and venison using NMR spectroscopy highlighted the metabolite changes affecting color stability in meat under retail display conditions. It was found that the stability of fresh beef color was greater than that of lamb and venison, with metabolites such as leucine, isoleucine, valine, succinate, inosine monophosphate, and choline playing key roles in these differences [130]. In another study, LC-MS-based metabolomics revealed that superchilling treatment of beef accelerated the degradation of μ-calpain and caspase 3, leading to improved tenderness. The metabolomic profiles of superchilled beef were distinguished from other treatments, indicating changes in pathways such as arginine and proline metabolism, which are associated with tenderness [131]. Additionally, metabolomics has been employed to investigate the effects of chilling regimes on the metabolome of beef, showing that superchilling improved the tenderness of beef by affecting key metabolic pathways, including ATP and its degradation products, which were influenced during the early post-mortem period [132].

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On the other hand, the occurrence of dark cutting meat has a notable impact on meat quality attributes and concentrations of *post-mortem* glycolytic metabolites in Angus Í Nellore crossbreed cattle, which was associated with mitochondrial activity and energetic metabolic pathways [(133)]. Significant differences in lactic acid, alanine, methionine, fumaric acid, inosine, inosine monophosphate, creatine, betaine, carnosine, and hypoxanthine were observed based on aging type (dry and wet aging) in the beef metabolome [(134)].

4.2 Lamb/mutton meat

Meat flavor, one of the most important sensory characteristics and main attributes that determines consumers' decisions to purchase a meat, is attributed to some volatile compounds [(135)]. In mutton, either from sheep or goat, a specific mutton dodur exists due to the volatile medium and short chain FA such as 4-methyloctanoic acid, 4-ethyloctanoic acid and 4-methylnonanoic acid [(136)]. It was revealed that significant variations in FA, aldehydes, ketones, lactones, alkaloids, flavonoids, phenolics, and drug residues among three types of goat meat: Lubei white goats, Boer goats, and Jining grey goats. This underscores how untargeted LC-MS can elucidate the subtle differences in flavors and sensory attributes among these varieties [(137)]. Among the lamb breeds studied, (E)-2-hexenal was exclusively detected in Tan lambs, whereas (E)-2-nonenal and (E, E)-2,4-nonadienal were only present in Dorper lambs. Hu lambs exhibited the fewest volatile compounds. Analysis demonstrated that Dorper lambs had a higher proportion of PUFA, AA, and volatile compounds compared to Tan and Hu lambs. However, specific PUFA derivatives in Dorper lambs were found to have a negative influence on the odor profile [(138)]. Using lipidomics and targeted metabolomics, hypoxanthine metabolites and volatile compounds of 1-octen-3-ol and hexanal were significantly increased by castration, which might be beneficial in lamb

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quality [139]. NMR spectroscopy was employed to delve into the confinement odour phenomenon in lamb meat, establishing correlations between this odour and specific meat and drip metabolites. These included tyramine, formate, alanine, carnosine, urea, proline, aspartate, glutathione, and nicotinate, which are substrates or products of glucose fermentation and AA catabolism [140]. The study on Mongolian sheep highlighted the dynamic changes in metabolites during early postmortem chilled aging, with significant alterations in AA, fatty acyls, and glycerophospholipids, which are crucial for the flavor and quality of meat. Metabolites like AA and small peptides accumulated significantly, enhancing flavor through pathways like amino acid metabolism and protein digestion [141].

From the view of lipids composition, low IMF leads to the decrease of flavor precursors in lamb [142]. Lipids such as PC, phosphatidylethanolamine (PE), and TG and their structures with SFA and UFA are critical to the thermal oxidative capacity of glycerol chain-based lipids [143]. A validated UPLC-Q-Orbitrap HRMS combined lipid screening strategy method based lipidomics was used for identification of Tan sheep meat products with different thermal processing methods. For atherosclerosis patients, the boiled cooking method proved to be preferable due to greater losses of sphingomyelin compared to ceramide in meat. Conversely, the steamed approach resulted in fewer losses of phosphatidylcholine and lysophosphatidylcholine, indicating that Tan sheep meat prepared in this manner might be more suitable for elderly and infant populations [144].

Volatile biomarkers, including alkanes, ketones, terpenes, and 2,3-octanedione, were found in ruminant tissues could distinguish exclusive pasture diets from exclusive concentrate diets [145]. The feed regimes affect the metabolites found in *longissimus dorsi* muscle of sheep, showing that total cholesterol, esterified

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cholesterol, choline, glycerophosphocholine, and glycerophospholipids were significantly lower in calcium soap of palm FA and prilled fat plus lecithin diets, while glycerol and sphingomyelin were significantly higher in calcium soap of palm FA and prilled fat plus lecithin diets [146]. An untargeted metabolomic and lipidomic method based on UPLC-Q-TOF/MS provided a basis for differentiation of meat from pasture-fed and concentrate-fed sheep/goats [147]. It was also found that the contents of 1- octen-3-ol and 2,3-octanedionone in mutton from pasture-fed animals were significantly higher [147]. Using both untargeted and targeted metabolomics, the main increased N-acetyl-L-aspartic acid, N-acetylaspartylglutamate, acetylcarnitine, and L-carnitine, but decreased carnosine and creatinine were the main newly found grazing feeding regime associated metabolites, which might contribute to the improved lamb meat quality under artificial pasture grazing [148]. Incorporating *Perilla frutescens* seeds into Tan lamb diets enhances n3-PUFA levels and flavor, marked by increased volatile compounds like acetaldehyde and 1,2,4-trimethyl-benzene. This inclusion boosts beneficial lipids and metabolites such as PG(18:1/18:1), PG(18:2/18:2), and 5′-inosinic acid, while reducing lysophosphatidylcholine, guanidinosuccinic acid, 3-hydroxydecanoic acid, and 2-methylbutyroylcarnitine [13]. It has been summarized previously that volatile alcohols, like 1-octen-3-ol, and ketones, such as 2-heptanone and 3-hydroxy-2-butanone, contribute to the distinctive aroma of lamb. Studies indicate that compounds like 1-octen-3-ol, which imparts a "mushroom-like" note, are often appreciated by consumers for enhancing the umami aspects of cooked meat, leading to increased consumer preference. The aldehyde content, including hexanal, nonanal, and heptanal, contributes significantly to the characteristic mutton odor. Compounds like (E)-2-nonenal and (E)-2-octenal impart fresh, fatty, and slightly green aroma notes, which are desirable for a well-rounded lamb flavor, and the 4-

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methyloctanoic acid and 4-ethyloctanoic acid, which are responsible for the specific "mutton" flavor [135]. Volatile compounds like phenylacetaldehyde and methional have been noted to provide antioxidant properties, which help in reducing oxidative stress. These antioxidants are significant for maintaining the overall nutritional quality of lamb meat and may contribute to the health benefits of reducing the risks associated with high-fat diets. Castration in lambs has been found to alter the concentration of volatile compounds, like hexanal and 1-octen-3-ol, reducing off-odors and enhancing the flavor profile, which might positively impact consumer preferences while also increasing the levels of beneficial lipids [149]. Such dietary adjustments suggest significant metabolomic advantages and potential for healthier lamb meat production, as analyzed by HD-mix LC-MS/MS for lipid and metabolite profiling.

5 Metabolomics for ruminant food alternatives

5.1 Synthetic/artificial milk

With the gradual increase in world milk consumption and concerns about the safe production of ruminant products under modern intensive farming conditions, some problems caused by efficient intensive farming, such as animal health, environmental pollution, animal welfare, etc. are closely related to “One Health” concept contradicts [150]. In this context, the new technology of “Lab-grown food” “replace an extremely inefficient, input-intensive and waste-producing traditional agricultural sector with precise, targeted and easy-to-process systems” production system by using small land and drastically reduces water and nutrient requirements [151]. To produce ruminant food, the most direct way to think of is to use controllable artificial conditions and the technical basis of laboratory cultivation to synthesize meat, eggs, and milk. Synthetic milk has emerged as a new potential alternative to cow’s milk, unlike plant-based oat, nut, and soy milks, which are designed to replicate

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its taste, appearance, and mouthfeel. Synthetic milk may have a smaller carbon footprint than dairy production, cause less pollution, and apparently eliminate animal welfare concerns [152]. Here we need enough information to understand the composition of naturally produced or real milk to make synthetic milk closer to natural milk. Metabolomics as a powerful tool can play a crucial role in understanding the real composition of milk for lab-grown milk production. The application of metabolomics has strong advantages in understanding the chemical characteristics and dynamics of dairy products, as well as potential biomarkers for differentiating the consumption of different dairy products and identifying milk quality, traceability and safety [9; 56]. Applications of NMR-based metabolomics in milk research include linking milk metabolite analysis to nutritional aspects and technical quality of milk. Identification of novel metabolites through metabolomics as biomarkers or bioactive compounds [153]. The application of metabolomics can provide a detailed understanding of milk composition, reveal changes in metabolite levels related to lactation, breed, diet, and other factors, and provide more valuable information to produce synthetic milk.

5.2 Ruminant meat alternatives

A new wave of plant-based or cell-based meat alternatives, designed to replicate the taste and nutritional profile of red meat, has garnered significant consumer interest, research focus, and media attention. Plant-based diets exclude or substantially limit the consumption of meat and animal products and are of growing interest to many due to their sustainability and health benefits [148; 154]. Nowadays, the plant-based meat is an artificial processed meat that are in evolution with the developed understanding and upgrading knowledge of meat metabolites and structure [155]. The clearer

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revealing of the meat metabolome contributes to that the artificial meat is more nearly taste and flavor close to the real animal feeding meat (Fig. 3) [155; 156; 157].

The metabolomics analysis found that metabolite abundances between the plant-based meat alternative and grass-fed ground beef differed by 90%. Nutrients such as DHA, niacinamide (vitamin B3), glucosamine, hydroxyproline, and antioxidants including allantoin, anserine, cysteamine, spermine, and squalene was exclusively present in beef. Conversely, ascorbate (vitamin C), phytosterols, and several phenolic antioxidants such as loganin, sulfurol, syringic acid, tyrosol, and vanillic acid were only found in the plant-based meat alternative [157]. For the future study of ruminant meat, metabolomics in targeting animal blood and meat can be used as a non-invasive technology to prediction of meat quality [14].

5.3 Spatial metabolomics and future artificial meat

The development of plant-based and cell-cultured meat has accelerated in recent years due to the advancements in metabolomics, 3D printing, and cell culture technologies. Specifically, 3D printing techniques play a crucial role in the production of ruminant meat alternatives [154; 158]. To bridge the gap between traditional meat and artificial meat, spatial metabolomics provides detailed information about the localization and chemistry of small molecules in individual cells [159], tissues [160], and the host–microbe interface [161]. For instance, spatial metabolomics has been used to map phospholipid distributions in the tissues of marine bivalves [160], revealing insights that can also be applied to understand the deposition of intramuscular fat and specific lipids in beef and mutton. Furthermore, imaging MS allows spatial metabolomics to identify the localization of limited content compounds in plants [162]. Thus, the use of spatial metabolomics offers a potential bridge between traditional and synthetic food production by providing a detailed map of the food metabolites.

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6 Ruminant food metabolites on human health

As is well known, ruminant has a unique rumen that contributes to a large number greenhouse gas production, which brings increasing attention due to its threat to the world climate change and human being living safety [163]. Ruminant animal-based foods not only have high social and climate costs but have strong effects on human health. For instance, the CLA that is a special ruminant FA has beneficial effects on human health [164; 165]. In our previous study, we found that indoxyl sulfate in lamb meat was increased by feeding high energy diet but flavory AA such as L-glutamine, L-serine, L-glutamate, and oleic acid were decreased [166]. The L-glutamine, L-serine, L-glutamate and oleic acid were good taste or health beneficial [167] but sulfate compounds were potentially harmful for human health and easily result in human liver and kidney diseases [168]. One Health and Global Health are based on the idea that human health and animal health are interdependent as well as being linked to the health of the ecosystems of which they are part. Thus, ruminant production is a key procedure in One Health that is the collaborative effort of multiple health science professions to attain optimal health for people [169].

Furthermore, antibiotics are a class of secondary metabolites mainly produced by microorganisms. The abuse of antibiotics in livestock would easily result in antimicrobial resistance, and the antibiotic residues in animal derived food would accelerate the spread of resistant bacteria within and between these sectors and around the globe [169]. The application of different analytical techniques for metabolomics have been successfully used in environmental, food or health sciences with various advantages and drawbacks [170]. Living systems encompass microorganisms, plants, animals, and humans, with food systems directly impacting nutrition and human health, which is from farm to human [171]. The ruminant food metabolomics are

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helping to interpret and connect the front-end animal production to back-end human life and open a window toward a better understanding of the complex interactions between food and human health [172]. However, very limited research did the traceability and metabolites flow from planting to animal food, and then to human health.

The emergence of metabolomics enables the discovery of biomarkers that enhance the deposition of functional substances during nutritional interventions, thereby improving meat quality [17]. Simultaneously, it facilitates the detection and regulation of harmful compounds, ensuring the safety of food products [19; 20]. By constructing a comprehensive metabolite spectrum, metabolomics surpasses traditional methods in capturing the complexity of food quality and safety. Traditional approaches to evaluating food quality and safety often focus on individual components, such as protein, fat, or specific contaminants. While effective, these methods fail to address the intricate interplay of metabolites that collectively define food quality and safety [173]. In contrast, metabolomics integrates a broad array of metabolites, including amino acids, fatty acids, organic acids, vitamins, and bioactive compounds. This holistic approach provides a more nuanced and accurate representation of food quality and safety [174]. By aligning with the goals of modern food science, metabolomics ensures the production of high-quality, safe, and trustworthy food products for consumers. However, as metabolomics databases for ruminant-derived foods continue to expand, this field holds the potential for even greater precision and comprehensiveness in identifying and regulating food quality and safety.

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7. Conclusions

Metabolomics has been optimally and effectively utilized in the field of ruminant food science and nutrition research, facilitating the identification of crucial metabolites including AA, n3-PUFA, and various organic acids. These metabolites serve either as precursors or as critical determinants in assessing food quality, flavor, and taste, which in turn influence consumer preferences (Fig. 4). The development of instrumentation would make metabolite detection and quantification more sensitive, accurate, robust, automated, and comprehensive. While promising advances need to improve and replenish the relevant metabolome. The most difficulties for metabolomics analysis are potentially the new metabolites or compounds identification, and the internal relationship among these metabolites in the food generation. For many of the small molecules, such as AA and FA, are part of the macronutrients such as protein and fat. Many more reference spectral or chromatographic databases on food components need to be developed and the name of metabolites should be uniform with chemical name and trivial name. A specific database related to the nutritional and ruminant food relevant compounds can be routinely identified or quantified.

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Competing Interests

The authors declare none.

Authorship and Contributorship

B.Y.Z: Investigation, Writing – original draft. **J.K.W**: Writing – review & editing.

B.W: Conceptualization, Investigation, Supervision, Writing – review & editing.

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Table 1. Metabolomics techniques used in metabolomics studies.

Technique	Application	Advantages	Disadvantages	Specific Uses
GC–MS	Detecting volatile organic compounds (VOCs)	High efficiency, reproducibility, reliable, selective, strong sensitivity, highly repeatable fragmentation	Requires complex sample processing for non-volatiles	Indicating meat flavor through volatilomics; studying complex food matrices
LC–MS	Food metabolites identification	High resolution of molecular mass, direct analysis without extensive pretreatment	requires significant sample preparation, is subject to ionization and matrix effects, and demands expert data analysis and regular maintenance	Analyzing a large number of metabolites; identifying lipids and bioactive compounds
NMR Spectroscopy	Analysis of metabolome and organic compounds	Broad profiling information, simple sample pretreatment, rapid detection, high repeatability, non-destructive	Low resolution and sensitivity	Studying potential biomarkers of diet, geographical origin, detecting adulteration in meat
HPLC-UV	Separating, identifying, and quantifying metabolites based on UV light absorption	Useful for analyzing compounds with known UV absorbance, contributes to comprehensive profiling	Limited to UV-absorbing compounds	Targeted metabolite analysis in dairy and meat products
GC × GC-ToF-MS	Studying various complex food matrices	Enhanced resolution and sensitivity, ability to analyze complex mixtures, structured chromatograms, and faster	more complex and expensive than single-dimensional GC-MS, requires expert operation and interpretation	Detailed profiling of volatile and semi-volatile compounds, differentiating chemically similar species, environmental analysis, food

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		analysis times		flavor and fragrance chemistry
IMS	Already noted as profiling VOCs of food	Rapid analysis times, operates at atmospheric pressure, can be coupled with other mass spectrometry techniques for enhanced selectivity	Limited resolution compared to other mass spectrometry techniques, sensitivity to moisture and temperature	Fast method to profile VOCs
HS-SPME/GC–MS	Meat species discrimination	Allows for the concentration and analysis of volatile compounds from complex matrices without solvent use, making it environmentally friendly and sensitive	Tedious sample processing and derivatization required	Analyzing volatile flavor compounds in foods and beverages, environmental monitoring, forensic applications
Combined GC–MS and LC–MS	Enhancing metabolite detection and accurate identification	Complementary strengths of both techniques	Requires access to multiple analytical platforms	Increasing the number of identified metabolites, enhancing phenotype-related metabolite identification

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Table 2. The choice of analytical platform for ruminant foods

Ruminant Food Type	Preferred Platform	Reasons
Milk	NMR	Non-destructive, minimal prep, broad profiling. Suitable for overall quality assessment.
	LC-MS	High precision for non-volatile metabolites like amino acids and lipids.
	HPLC-UV	Cost-effective for specific UV-absorbing compounds (e.g., vitamins).
Meat	GC-MS	High sensitivity for volatile flavor compounds, important for aroma evaluation.
	LC-MS	Comprehensive analysis of non-volatile compounds, ideal for nutritional profiling.
	NMR	General metabolic profiling, useful for comparisons. Limited by low sensitivity.
Fermented Milk(cheese)	GC-MS	Ideal for detecting volatile fermentation by-products contributing to flavor.
	LC-MS	Suitable for a wide range of fermentation metabolites (organic acids, vitamins).
	NMR	Non-destructive, good for overall profiling and comparison. Limited in detecting low-abundance compounds.

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Table 3. Metabolome database used in metabolomics studies.

Database	Focus Area	Features
Human Metabolome Database (HMDB)	Human metabolomics	Comprehensive data on human metabolites, including structure, function, and concentrations.
Bovine Metabolome Database (BMDB)	Ruminant biology, specifically cattle	Detailed information on bovine metabolites for research in ruminant biology.
PubChem Compounds of NCBI	General reference for chemical compounds	Extensive database of chemical molecules and their biological activities.
KEGG COMPOUND	Biochemical compounds involved in metabolic pathways	Offers detailed biochemical pathways and molecular interaction networks.
Livestock Metabolome Database (LMDB)	Livestock metabolomics	Includes data on analytical platforms, experimental conditions, and animal breeds in metabolomic studies.

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Table 4. Summary of screened potential biomarkers for ruminant milk are shown when available.

Item	Target	Metabolites	Pathways	Platform	Source
Breed					
goat, cow, soy	raw milk	rich in short- and medium-chain fatty acids (MCFA), USFA, ω-6 FA, ω-3 FA, EPA and DHA of goat milk rich in Cer, TG and DG of cow milk rich in phospholipids of soymilk		UPLC-Q-Exactive Orbitrap Mass Spectrometry based lipidomics	(86)
yak	Raw milk	Rich in protein, fat, lactose, and bioactive components such as essential amino acids, CLA, EPA, DHA		UPLC-Q-Exactive Orbitrap Mass Spectrometry based lipidomics	(78)
yak	Raw milk	High levels of fat, protein, solids-not-fat (SNF), calcium, larger casein micelles		Dynamic Light Scattering (DLS), Optical Microrheology Analysis	(76)
yak	fermented milk	17 amino acids, 52 volatile compounds (including ketones, esters, aldehydes, alcohols, alkenes, fatty acids, and others), higher levels of lactic acid, minerals, and vitamins B and C	Glycolysis, proteolysis, lipolysis, KEGG pathways	Gas Chromatograph with Ion Mobility Spectrometry (GC-IMS), Liquid chromatography mass spectrometry (LC-MS)	(77)

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sheep, goat	raw milk	Sheep's milk exhibited higher abundance of arabitol, citric acid, α-ketoglutaric acid, glyceric acid, myo-inositol, and glycine. Conversely, goat's milk displayed elevated levels of mannose-6-phosphate, isomaltulose, valine, pyroglutamic acid, leucine, and fucose	Gas Chromatograph mass spectrometry (GC–MS)	(18)
goat, cow	raw milk	Valine and glycine were found exclusively in goat milk, while talose and malic acid were unique to cow milk	Gas Chromatograph mass spectrometry (GC–MS)	(85)
milk adulteration	soy milk, goat milk, bovine milk	D-lactose, D-sucrose, choline, citrate, lecithin, ethanolamine, N-acetylcarbohydrates, acetate, creatine, and carnitine	nuclear magnetic resonance (NMR)	(75)
camel, human, bovine	raw milk	Human milk is rich in TGs containing LA, SM containing ultra-long-chain FAs and PLs containing ARA/DHA/DGLA Caprine milk is rich in PLs, including HexCer, Hex2Cer, SM, Cer and PC. Bovine milk is rich in PC and CL.	UHPLC-Q-TOF-MS based lipidomics	(90)
camel vs. bovine	fermented milk	The bioaccessible fraction of fermented camel milk displayed enhanced biological functionality in comparison to fermented bovine milk	ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight (UPLC-QTOF)	(89)

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Italian buffalo vs. cow mozzarella	cheese	Italian buffalo mozzarella cheese were higher in threonine, serine, valine, and lower in orotic acid and urea		Gas Chromatograph mass spectrometry (GC–MS)	(175)
Feed					
different feeding regimens	cow milk	feed-derived (such as phenolic metabolites) but also animal-derived compounds (such as fatty acids) are the potential biomarkers		ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS)	(64)
grazing vs. confined	goat milk	Grazing increased omega 3 fatty acid and phospholipid		gas chromatograph + HPLC combined with an evaporative light-scattering detector	(65)
essential oils from caraway	cow milk	increased creatinine, choline, omega citrate, decreased N-acetyl hexosamine, glutamate, carnitine, and hippurate		nuclear magnetic resonance (NMR) spectrometry	(67)
purple perilla leaf	cow milk	more PE-NMe (18:1(9Z)/18:1(9Z)) and DG (18:0/20:4(5Z,8Z,11Z,14Z)/0:0), oleanolic acid, and nucleotides	pyrimidine metabolism and biosynthesis of unsaturated fatty acids	ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS)	(68)
berry extracts	UHT cow milk	reduced Maillard reaction product N ε-(carboxymethyl)-L-lysine during UHT processing		gas chromatography-mass spectrometry (GC–MS)	(69)
bamboo leaf extract	cow milk	upregulated glycerophospholipids and fatty acyls, and downregulated	sphingolipid signaling,	liquid chromatography-mass spectrometry (LC-MS)	(70)

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		moracetin, sphinganine, and lactulose	glycerophospholipid metabolism, sphingolipid metabolism, and necroptosis		
organic vs. conventional	bovine milk	increased content of caproleic acid, α-linolenic acid, linoleic acid, conjugated (9-cis,11-trans)18:2 linoleic acid (CLA), total unsaturated fatty acids (UFA), allylic protons, and decreased content for unsaturated fatty acids		1H-NMR and 1D TOCSY NMR methods	(71)
Geographical origin	goat milk	38 and 19 lipid molecules		UPLC-Q-Exactive Orbitrap MS	(94)
Processing					
pasteurized vs. UHT	goat and cow milk	Hydroxyglutaric acid		gas chromatography-mass spectrometry (GC–MS)	(85)
UHT vs. reconstituted milk	commercial milk purchased	60 marker metabolites from three categories of peptides, lipids, and nucleic acids		ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS)	(97)

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	from supermarkets			
UHT milk vs. raw milk vs. pasteurized milk		2-hydroxymyristic acid, 3-hydroxytetradecanoic acid, 3-hydroxyhexadecanoic acid, 5-hydroxyeicosatetraenoic acid, 7 oxylipids (9-hydroxydecanoic acid, 12-hydroxydodecanoic acid, and 10-hydroxyoctadecanoic)	ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS)	(98)
UHT and reconstituted milk	bovine milk	L-carnitine, succinate, and acetate	nuclear magnetic resonance (NMR)	(99)
yoghurt	brown goat milk	Organic acid, peptide and medium- and long-chain fatty acid contents increased	UPLC-Quadrupole-Orbitrap HRMS based lipidomics	(100)
rind inclusion	cheese	2-hydroxyadenine and argininic acid and 5-hydroxyindole acetaldehyde	Untargeted metabolomics analysis based on UHPLC-Orbitrap-HRMS and Peptidomics profiling by UHPLC-QTOF-HRMS	(102)
both ripening time and anomalous rind inclusion	cheese	medium-chain aldehyde 4-hydroperoxy-2-nonenal	Untargeted metabolomics analysis based on UHPLC-Orbitrap-HRMS and Peptidomics profiling by UHPLC-QTOF-HRMS	(102)

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Table 5. Summary of screened potential biomarkers for ruminant meat are shown when available.

Item	Target/ Treat	Metabolites	Pathways	Platform	Source
Ruminant meat	Ruminant compared to monogastric animals and fishes	higher in the ratio of saturated fatty acids (SFA) than unsaturated fatty acids (UFA); high in conjugated linoleic acid (CLA)			(120; 121; 122)
Beef					
Beef stock	glutathione-Maillard reaction products	1H-pyrrole, 2-methylthiophene, 2-propylpyrazine, 2-(furan-2-ylmethyldisulfanylmethyl) furan, 2-methylfuran-3-thiol, 3-sulfanylpentan-2-one, furan-2-ylmethanethiol, and 1-furan-2-ylpropan-2-one		gas chromatography–time-of-flight mass spectrometry (GC–TOF/MS)	(125)
Beef	geographical origins and feeding regimes	amino acids, several sugar metabolites, and several PCs and PEs		UPLC-Orbitrap-MS and gas chromatography-mass spectrometry (GC–MS)	(124)
Beef	geographical origin of beef from four countries: Australi	various amino acids and succinate		nuclear magnetic resonance (NMR)	(36)

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Beef, strip loin	a, Korea, New Zealand, and the United States aging type (dry and wet aging)	Lactic acid, alanine, methionine, fumaric acid, inosine, inosine monophosphate, creatine, betaine, carnosine, and hypoxanthine		1H-NMR spectroscopy	(135)
Marbled beef of Japanese Black cattle	Tissues types (muscle, intramuscular fat, and intermuscular fat)	Medium-chain fatty acids were uniquely detected in fat tissue, but decanoic acid, uric acid, elaidic acid, and 3-phosphoglyceric acid are potential biomarkers for intramuscular fat to assess marbling levels		chromatography-mass spectrometry (GC–MS)	(122)
Chinese Jinjiang yellow cattle	longissimuss lumborum vs. psoas major	pyruvate, and numbers of metabolites involved in tricarboxylic acid cycle	tricarboxylic acid cycle	UPLC-MS/MS	(127)
Farmed Jiulong yaks	the longissimus thoracis vs. biceps femoris	higher concentrations of carnosine and formate and lower concentrations of mannose, inosine, threonine, IMP, alanine, valine, isoleucine, tyrosine, phenylalanine and leucine		nuclear magnetic resonance (NMR)	(33)
Lamb/ mutton					
Hu sheep	castration	hypoxanthine metabolites and volatile compounds of 1-octen-3-ol and hexanal		liquid chromatography–mass spectrometry (LC-MS) and gas chromatography-	(140)

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				mass spectrometry (GC–MS)	
Sunit sheep	paster	higher 1- octen-3-ol and 2,3-octanedionone		UPLC-Q-TOF/MS	(148)
Tan sheep	artificial pasture grazing	increased N-acetyl-L-aspartic acid, N-acetylaspartylglutamate, acetylcarnitine, and L-carnitine, but decreased carnosine and creatinine	linoleic acid metabolism	liquid chromatography–mass spectrometry (LC-MS)	(150)
Dorper sheep	calcium soap of palm fatty acids and prilled fat plus lecithin diets	decreased total cholesterol, esterified cholesterol, choline, glycerophosphocholine and glycerophospholipids		nuclear magnetic resonance (NMR)	(147)
Dorper sheep	calcium soap of palm fatty acids and prilled fat plus lecithin diets	higher glycerol and sphingomyelin		nuclear magnetic resonance (NMR)	(147)
Tan sheep	boiled approach	more losses of sphingomyelin, less losses of phosphatidylcholine and lysophosphatidylcholine		UPLC-Q-Orbitrap HRMS	(145)

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Fig. 1. Overview of ruminant milk and dairy product classification and factors influencing the milk metabolome. (Created in BioRender. Zhang, B. (2024) BioRender.com/s41o643)

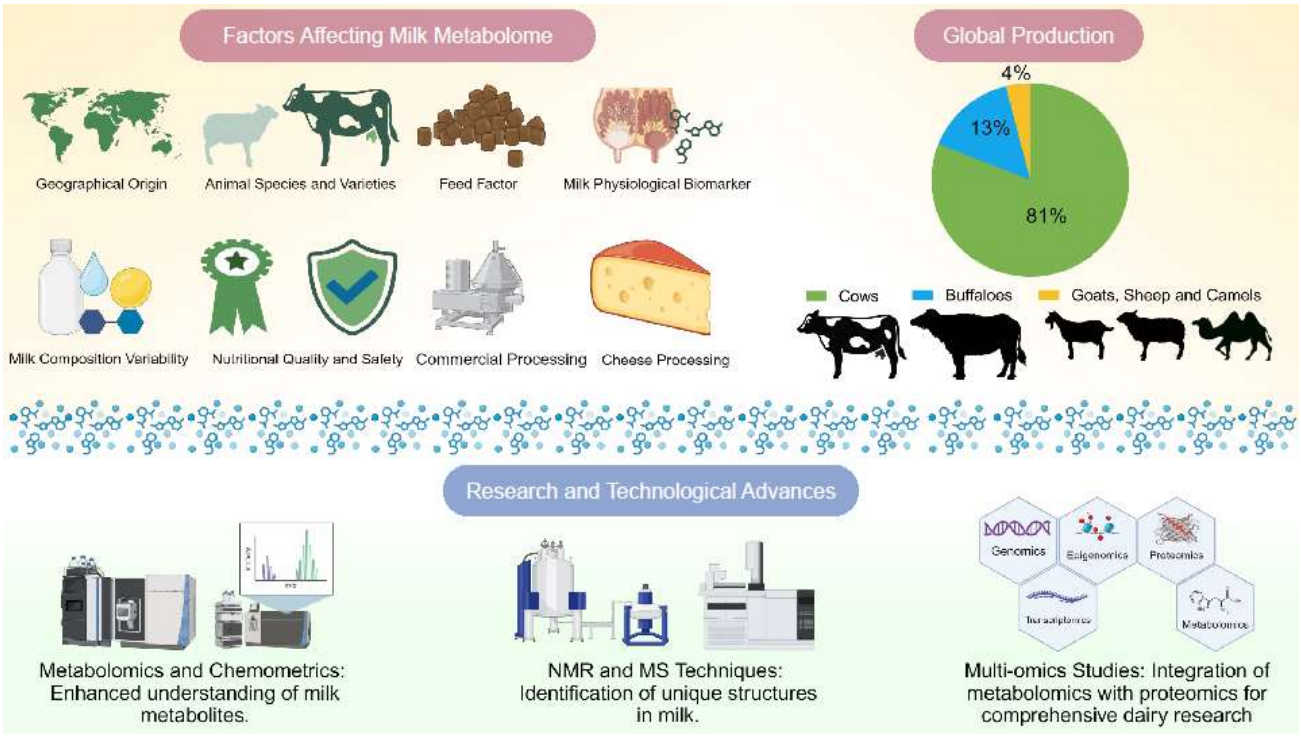

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Fig. 2. Metabolomic insights into ruminant meat quality, nutritional traits, and authenticity assessment. (Created in BioRender. Zhang, B. (2024) BioRender.com/m65h597)

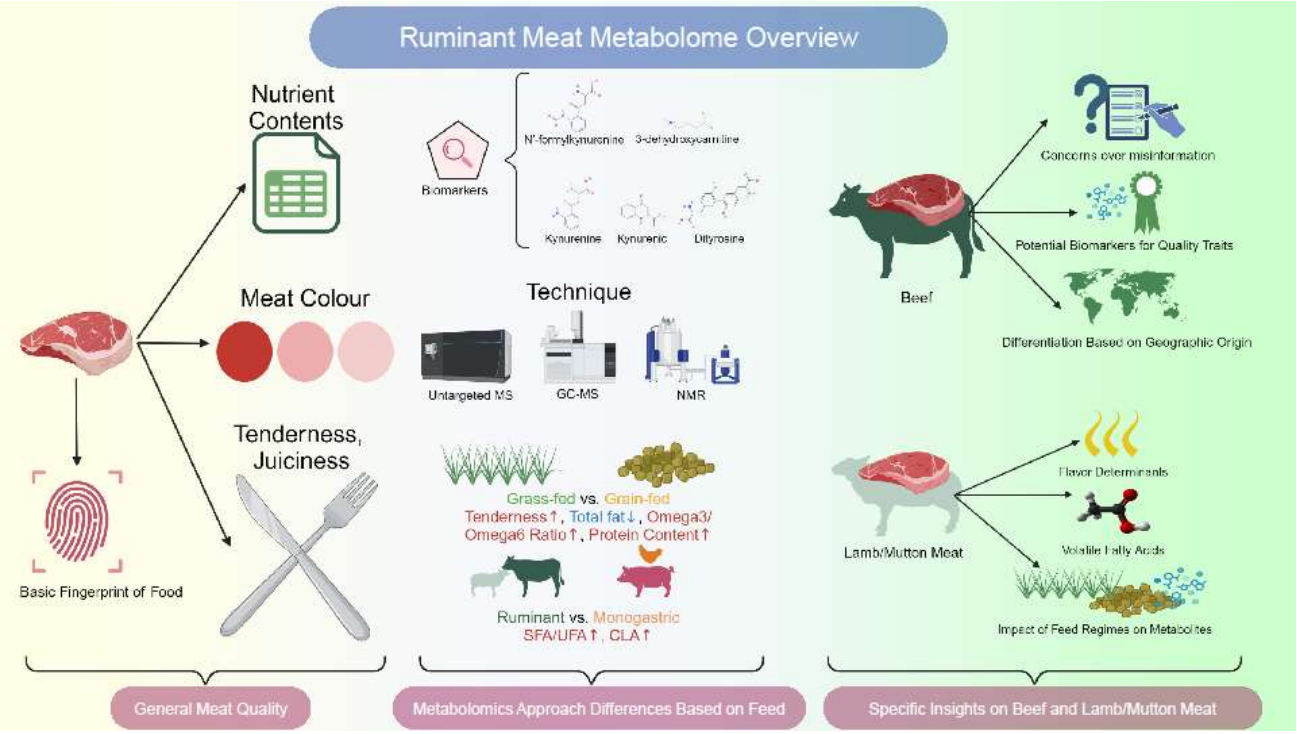

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Fig. 3. Metabolomic strategies for the development and quality control of ruminant food alternatives. (Created in BioRender. Zhang, B. (2024) BioRender.com/l16r397)

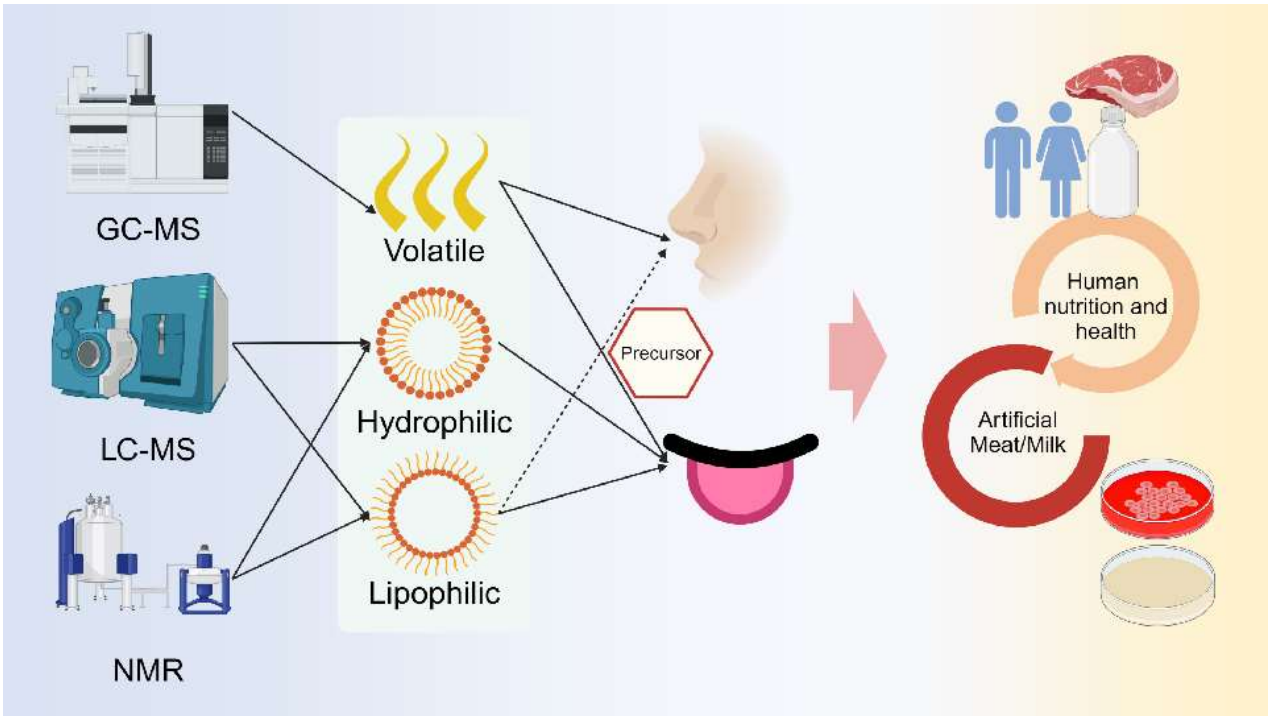

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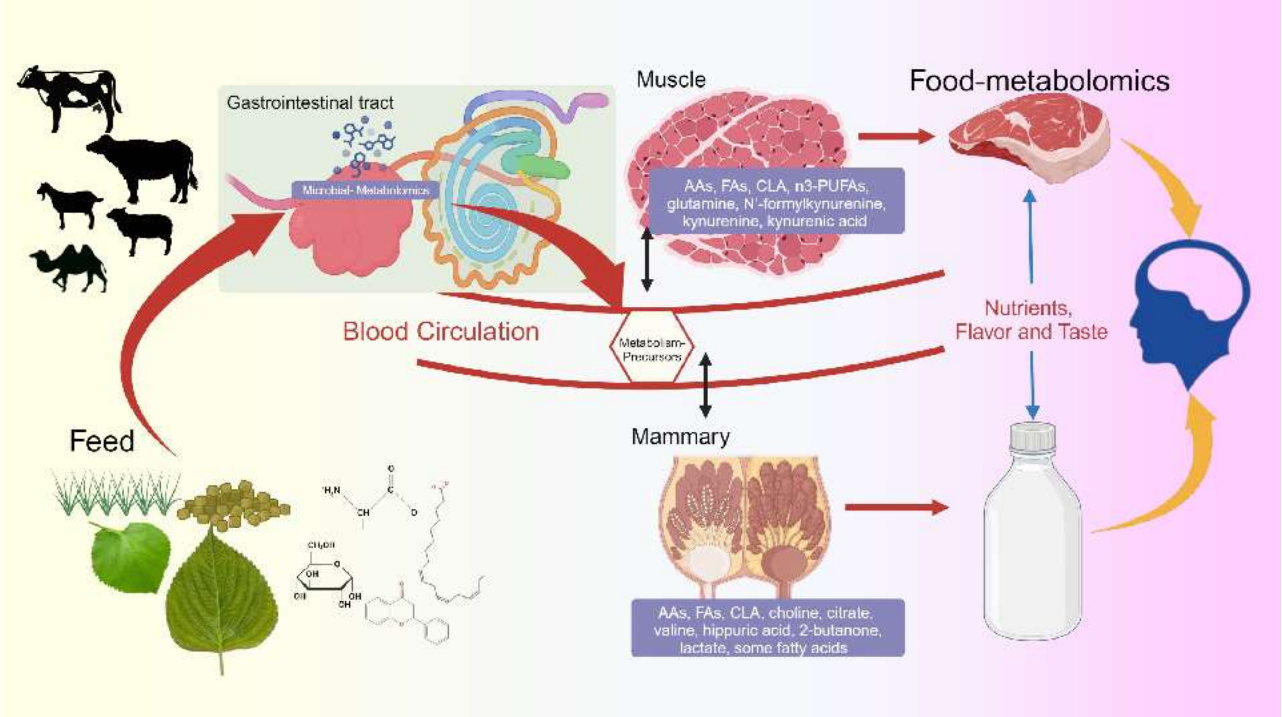
Fig. 4. The whole workflow and key points in determining ruminant meat and milk production. (Created in BioRender. Zhang, B. (2024) BioRender.com/k75h157)



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