Meat and Muscle Biology™

Meat Animal Biologics Discovery Opportunities from the Gut Microbiome: Application of Metabolomics‡

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‡The material presented in this review was part of a symposium entitled: Welcome to the Gut Microbiome: Discovery and Innovation," Concurrent Technical Session III – The Other 40% – Innovation in Biologics and Biomaterials. Reciprocal Meat Conference, St. Paul, MN, June 26th, 2023.

Abstract: Metabolomics is a high-throughput technology that is widely used across disciplines to identify and quantify metabolites in biological samples; however, its use has been limited in meat and animal science. The use of metabolomics, especially in these fields, is often curtailed by challenges with data processing and analysis. Improvements in data analysis platforms have broadened metabolomics applications and offer promise for determining metabolic pathways that directly influence animal health and livestock production. This review will present an overview of metabolomics concepts and current applications of metabolomics techniques in meat and animal science. Furthermore, we present evidence of the need to incorporate metabolomics in a systems biology context for the improvement of livestock production with an emphasis on animal health and production efficiency.

Key words: livestock metabolomics, meat metabolomics, microbial profiling
Meat and Muscle Biology 8(1): 18261, 1–11 (2024) **doi:10.22175/mmb.18261**
Submitted 11 July 2024 Accepted 26 September 2024

Introduction

Meat animal processing generates tissues and compounds that can be repurposed for other uses such as medical applications are given the term biologics. An often overlooked source of biologics is the meat animal digestive tract along with the resident beneficial microorganisms and their respective metabolic activities. Detection and retrieval of these potential biologics in the form of metabolite compounds from the gut ecosystem requires analytical methods such as metabolomics, an approach to study or capture all small molecules in a biological system. Metabolomics involves the separation and identification of metabolites from a particular sample such as gut contents. As omics techniques such as metabolomics become more widely available and utilized in animal and meat sciences, there will be an increased need for improved methods for interpreting the plethora of data, especially as it relates to host-microbe interactions that influence growth performance, meat quality, product yield, and the overall health of livestock. In this review, we will present an overview of metabolomics concepts and current applications of metabolomics techniques in meat and animal science (Figure 1A). In addition, we will provide evidence of the need to incorporate metabolomics in a systems biology context for the improvement of livestock and meat production. To summarize, we aim to impart knowledge on the importance of incorporating metabolomics for a comprehensive and accurate understanding of the metrics such as trait selection, feed intake, and growth rate have on the

livestock metabolome and subsequently livestock and meat production.

Metabolomics Concepts and Methods

The metabolome, or set of metabolites, represents all substrates, intermediates, and products of catabolic and anabolic metabolic pathways within a cell (Nielsen and Jewett, 2007; Tomita and Nishioka, 2005). Most of these are sugars, organic acids, lipids, and amino acids, which are produced during the metabolism of food, drugs, or chemicals (Tomita and Nishioka, 2005). Metabolites are measured spectroscopically with nuclear magnetic resonance (NMR), or mass spectrometry (MS) coupled with chromatographic separation (gas chromatography or liquid chromatography). Mass spectrometry is a technique used in chemistry since the early 1900s to measure the masses of individual ions and atoms (Dass, 2001). There are 3 main components of a mass spectrometer: the ionization source, the mass analyzer, and a detector (Dass, 2001; Siuzdak, 2006). The first step in MS is the ionization of the molecule. The ionization source allows for the molecule to be ionized and then fragmented into a gas-phase ion (Dass, 2001). Next, a mass analyzer separates each ion based on their mass ratios. Those various mass-to-charge (m/z) ratios are subsequently sent to the detector to be converted into a digital output of the relative abundance of each ion (Dass, 2001; Siuzdak, 2006).

Metabolomics can be used to characterize the profile of small molecules in a biological sample. It is often used to compare differences in metabolite composition to determine the effects of treatments or causes of abnormal biological phenotypes. The assumption is that the change or dysregulation of metabolites along samples is directly related to the biological activity underpinning the difference (Qiu et al., 2023). Overall, metabolomics has been used to elucidate unique biomarkers in various biological specimens (Lu et al., 2012; Metwaly et al., 2020; Mokkala et al., 2020), understand the function of metabolites (Schrimpe-Rutledge et al., 2016; Tomita and Nishioka, 2005), and identify metabolic pathways responsible for observed organismal phenotypes (Majumder et al., 2021).

When designing a metabolomics study, the first step is to determine if the purpose of the experiment is based on generating a hypothesis (untargeted metabolomics) or is hypothesis driven (targeted and untargeted metabolomics) (Schrimpe-Rutledge et al., 2016). Specifically, targeted metabolomics validates the presence and quantity of specific molecules of interest in a biological specimen whereas untargeted metabolomics is used for global detection of metabolites in a biological specimen (Tomita and Nishioka, 2005). Based on this determination, the type of mass analyzer and separation technique can be selected to best fit the experimental goals. Selection of the mass analyzer is a vital component of method development as the performance can vary. Among the common analyzers are quadrupole mass analyzers, time-of-flight mass analyzers, ion trap mass analyzers, and magnetic sector mass analyzers (Balogh, 2009; Dass, 2001; Siuzdak, 2006). Performance characteristics such as scan speed, mass range, resolution, and detection sensitivity (Dass, 2001; Siuzdak, 2006) are major factors in their selection for a metabolomics experiment (Siuzdak, 2006). Typically, targeted metabolomics studies are performed with single quadrupole or triple quadrupole mass analyzers. Untargeted metabolomics studies require greater resolution and are often performed with quadrupole time-of-flight, ion mobility, or orbitrap mass analyzers. Ultimately, selecting the correct mass analyzer will ensure good coverage for a metabolomics study.

Once techniques and mass analyzers are selected, metabolite extraction can be performed. Like all biological research studies, sufficient replicates and controls should be incorporated into the experimental design to allow proper analysis of analyte molecules. Typically, untargeted metabolomics studies compare 2 or more groups to allow for accurate analysis of metabolite dysregulation (Majumder et al., 2021). As previously mentioned, dysregulation refers to the change or altered quantity of a given metabolite. This change is then assumed to directly correspond to the biological activity related to differences among treatment groups. It is imperative that, for studies analyzing complex biological samples like animal gut microbiome samples, there are at least 10 biological replicates as well as a pooled quality control sample (QC). The QC sample is necessary to validate that there is no variance between samples; however, the only way to ensure good coverage of metabolites is to select solvents that extract polar and non-polar metabolites (Mushtaq et al., 2014). This can be accomplished by first conducting method development trials to assess extraction solvents and if better coverage is achieved in positive or negative mode. As an example, Figure 1B describes our fast and reproducible procedure for metabolite extraction from the ceca of broiler chickens.

Use of metabolomics is often limited by challenges with data processing and analysis. Improvements in data analysis platforms have broadened metabolomics use in diverse applications and offer promise for determining gastrointestinal tract (GIT) contributions to animal health and production assessment (Figure 1A). Three widely used platforms include XCMS, MetaboAnalyst, MetaboScape, and Compound Discoverer. All extract metabolite features from the raw MS spectra files and perform statistical analyses (Cerrato et al., 2020; Huan et al., 2017; Pang et al., 2022). After spectral processing, the next step is annotating features, which means assigning chemical names to the peaks in the spectra. This remains one of the most challenging aspects of metabolomics as many metabolites are not yet represented in reference databases, even with the largest databases now exceeding one million compounds (Cerrato et al., 2020; Huan et al., 2017; Pang et al., 2022). For annotating and identifying meat and livestock metabolites, in 2017 the Livestock Metabolome Database (LMDB) was developed. The LMDB provides additional access to nearly 1,200 metabolites specific to bovine, ovine, caprine, equine, and porcine (Goldansaz et al., 2017). In conjunction with other updated databases such as KEGG (Kanehisa and Goto, 2000), PubChem (Kim et al., 2023), and MetaCyc (Caspi et al., 2008), resources like LMDB are enabling metabolomics to become a robust tool for meat and

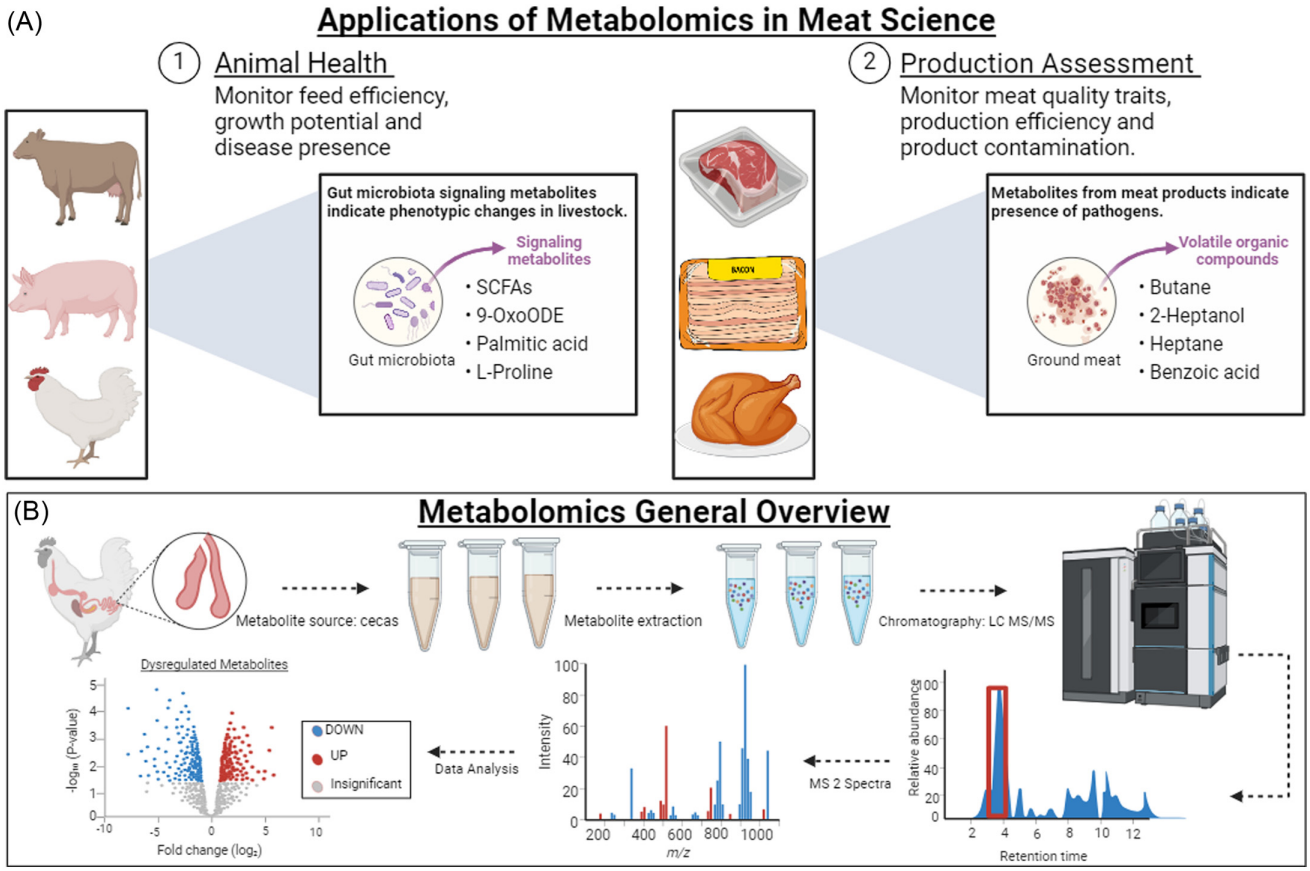

Figure 1. A) Livestock metabolomics can provide critical information on animal health and meat production. Specifically, GIT microbiome or meat samples can be utilized for identification and quantification of signaling molecules or volatile organic compounds associated with adverse health outcomes or pathogens. B) The metabolomics general overview is an example of our fast and reproducible protocol for cecal metabolome analyses using cecal digesta. First, cell extraction is performed on 0.5 mL of cecal digesta from each sample. At least 10 replicates per treatment is preferred to prevent or minimize the batch effect. Cell lysis is then performed using three freeze-thawing cycles at −80°C, which consist of thawing at room temperature for 10 min followed by a 30 s sonication on ice. Next, 1 mL of MS-grade acetonitrile:methanol:water (2:2:1 v/v) solvent mixture is added to cells extracts and sonicated for 30 s on ice and stored at -20°C from 1 h to overnight which allows cellular debris and protein to precipitate. Then samples are centrifuged for 15 min at 13,000 rpm at 4°C. The supernatant is then transferred to new Eppendorf tubes and dried for 4 h on a SpeedVac concentrator. Dried samples are reconstituted in MS-grade acetonitrile:water (1:1 v/v) solvent mixture based on the normalized protein concentration of all samples. Normalized volumes are determined using the Bradford method where the highest protein concentration is equal to 100 µL. Following this, samples are vortexed for 30 s, sonicated on ice for 10 min, and centrifuged for 15 min at 13,000 rpm at 4°C to remove any residual debris. Finally, the metabolite extracts are transferred into autosampler vials with inserts and stored in −80°C until analysis. Untargeted LC MS/MS is performed using an ultra-high performance liquid chromatography orbitrap. Data analysis and visualization is conducted on MetaboAnalyst. Created in BioRender. Chatman, C. (2024) https://BioRender.com/e51e259

animal science researchers. To maintain these robust metabolite search platforms, there is not only an increased need to utilize metabolomics for livestock production but also the development and application of innovative solutions for improving product yield, feed efficiency, growth performance, and meat quality.

Correctly, processing metabolomics data is essential for accurate interpretations. Following data processing, any of the previously described platforms can be used for comprehensive analysis of metabolomics data via statistical and functional analyses. Statistical analyses allow for the evaluation of significantly dysregulated metabolites using either univariate analysis (i.e., one-way analysis of variance, or volcano plots), multivariate analyses (i.e., principal component analysis or partial least-squares), or supervised classification (i.e., random forests) (Karaman, 2017; Pang et al., 2022). Performing these analyses allows users to identify metabolites and metabolic pathways correlated to a specific treatment group or disease state. When metabolomics data are used in a systems biology workflow users can holistically understand complex biological questions. Systems biology refers to the incorporation of various data sources to understand complex biological interactions broadly as opposed to individual genes or proteins (Huan et al., 2017). Regarding livestock production, metabolomics data can be used to identify metabolites associated with pathogens of concern, low residual feed intake (RFI), or breeding characteristics. This review will further highlight the current applications of metabolomics in meat and animal science while also emphasizing the value of incorporating metabolomics in a systems biology context within livestock production.

Metabolomics for Livestock Production

In the livestock industry, the use of metabolomics has not been widely adopted (Goldansaz et al., 2017), but it has been used recently for muscle and meat science (Artegoitia et al., 2022; Muroya et al., 2020; Wang et al., 2020). Current and future applications of metabolomics in animal and meat science are depicted in Figure 1, which can be categorized by either the assessment of livestock health or production efficiency. Current metabolomics methods for evaluating livestock health typically utilize the noninvasive collection of milk, serum, urine, or feces (Artegoitia et al., 2022; Imaz et al., 2022; Wang et al., 2020; Zhu et al., 2021), whereas metabolomics for production assessment is typically focused on determining nutritional content or carcass quality as seen in Table 1. Goldansaz et al. (2017) reported that the field of animal and meat science faces challenges with establishing noninvasive, fast, and effective analytical techniques and consistently designing studies with an appropriate sample size (Goldansaz et al., 2017). These challenges remain at the forefront of livestock metabolomics to date. In addition, the meat and animal science industry has seldom incorporated metabolomics into its production systems; however, the use of metabolomics in a systems biology or diagnostic workflow would help in the implementation of applications geared towards improving breeding, feed efficiency, product yield, and more.

Animal health

For assessment of livestock health, untargeted metabolomics of serum, fecal and urine samples provide a non-invasive method for assessing growth rate (Feng et al., 2021; Imaz et al., 2022), feed efficiency (Connolly et al., 2019; Su et al., 2022), pathogen detection (Zandkarimi et al., 2018; Zhu et al., 2021) and other health metrics of livestock as outlined in Table 1. For instance, a comparison of the rumen microbiome and serum metabolites revealed serum metabolite, pantothenate, and the gut bacteria, *Flavobacteria*, were notably higher in low RFI cattle (Clemmons et al., 2019). A separate study reported that distinct fecal metabolites expressed in broiler chickens with Tibial dyschondroplasia were correlated with differentially abundant fecal microbes (Huang et al., 2022). In these examples, metabolomics were coupled with 16S rRNA gene sequencing and provided an extensive evaluation of perturbations that corresponded to non-ideal phenotypes for livestock. This non-invasive workflow is cost effective and reproducible but more importantly does not require the slaughter of animals needed for breeding purposes.

Analyzing the gut metabolome via ruminal fluid or cecal digesta is an effective but invasive or sometimes terminal method for confirming metabolite dysregulation or health biomarkers. When comparing low-RFI cattle to high-RFI cattle, Liu et al. (2022) reported 11 significantly dysregulated rumen metabolites that are associated with linoleic acid metabolism, fatty acid degradation, and protein digestion and adsorption (Liu et al., 2022) (Table 1). Interestingly, Malheiros et al. (2021) explored the similarities and differences between ruminal fluid and feces of Nelore steers, which demonstrated that fumarate and nicotinate were among

Table 1. Synopsis of livestock metabolomics studies discussed in this review paper.

Study Objective	Species	Sample Type	Analytical Technique	Key Result	Reference
Growth Rate	Cattle	Blood	[1]H-NMR	Low growth period associated with increased relative abundance of acetate, choline, and lipids	Imaz et al., 2022
	Lambs	Blood	LC-MS/MS	Lipids associated with high average daily gain lambs	Feng et al., 2021
Milk Yield	Cattle	Rumen fluid	LC-MS	Low milk yield associated with increased dopamine levels	Amin et al., 2022
	Cattle	Serum	UHPLC-MS/MS	Milk protein biomarkers identified included hippuric acid, nicotinamide, and pelargonic acid	Wu et al., 2018
Feed Efficiency	Cattle	Blood	[1]H-NMR	3-hydroxybutyrate, propionate, acetate, creatine, histidine, valine, and isoleucine were associated with marbling	Connolly et al., 2019
	Chicken	Blood	UHPLC/MS	Potential biomarkers for high feed efficiency selection included 7-ketocholesterol, dimethyl sulfone, epsilon-(gamma-glutamyl)-lysine, gamma-glutamyltyrosine, 2-oxoadipic acid, L-homoarginine, testosterone, adenosine 5'-monophosphate, adrenic acid, and calcitriol	Su et al., 2022
	Cattle	Blood	LC-MS	Higher abundance of pantothenate was associated with the low RFI group	Clemmons et al., 2019
	Cattle	Rumen fluid	LC-MS	Low RFI cattle associated with L-proline, L-phenylalanine, L-isoleucine, peperidine, Gamma-linolenic acid, 9,10-DHOME, 9-OxoODE, S-Glutaryldihydrolipoamide, 3S)-3,6-Diaminohexanoate, glutaric acid and palmitic acid	Liu et al, 2022
Pathogen Detection	Cattle	Serum	UHPLC-MS	Positive clinical mastitis diagnosis associated with N-methylethanolamine phosphate, choline, phosphorylcholine, free carnitine, trimethyl lysine, tyrosine, and proline	Zandkarimi et al., 2018
	Cattle	Milk	[1]H-NMR	Dimethylamine, tyrosine, lactate, leucine, proline, valine, arginine, and isoleucine were significantly higher in cattle with clinical mastitis	Zhu et al., 2021
Meat or Carcass Quality	Cattle	Urine	UPLC-MS	Performance and carcass quality associated with bile acids and steroid metabolites	Artegoitia et al., 2022
	Chicken	Chicken breast	[1]H-NMR	Wooden breast samples associated with higher expression of lactate, glutamate, and 5'-IMP	Wang et al., 2020
	Cattle	Meat and fat	HPLC MS/MS	Cattle provided pre-natal protein-energy supplementation had significantly lower levels of phosphatidylcholines but higher levels of threonine and arginine.	Fernandes et al., 2024
	Sheep	Meat	UHPLC-QTOF-MS	Linoleic acid metabolism and biosynthesis of unsaturated fatty acids pathways were downregulated in metabolomes of stall-fed sheep	Zhang et al., 2022
	Cattle	Muscle	[1]H-NMR	Muscle from cattle in high growth and high precocity groups associated with muscle protein metabolism	Cônsolo et al., 2020
Common Diseases or Disorders	Chicken	Fecal	UPLC-MS/MS	Distinct fecal metabolites expressed in broiler chickens with Tibial dyschondroplasia	Huang et al., 2022
	Cattle	Serum	GC-MS	Cattle identified as "pre-retained placenta" had higher expression levels of 5 amino acids, oleic acid, phosphoric acid, myo0inositol, urea, and cholesterol	Zhang et al., 2021
Nutrient Content	Cattle and plants	Plant-based meat and beef patties	GC/EI-MS	22 metabolites differentially expressed in beef patties and 37 differentially expressed in plant-based patties	Vliet et al., 2021

[a]1H-NMR: Proton nuclear magnetic resonance

[b]LC-MS: Liquid chromatography–mass spectrometry

[c]GC-MS: Gas chromatography–mass spectrometry

[d]UHPLC: Ultra high-performance liquid chromatography–mass spectrometry

[e]QTOF-MS: Quadrupole Time-of-Flight

the metabolites detected in the ruminal fluid but not the feces (Malheiros et al., 2021); however, they emphasized that regardless of this difference the metabolites corresponded to the same metabolic pathways. Despite this finding, the difference between the ruminal and fecal metabolome provides valuable information on biochemical interactions occurring within the various GIT compartments since the fecal material likely

represents the lower gut of the ruminant animal. Therefore, the detection of unique metabolites of interest in the rumen compared to the fecal metabolome indicates that samples from specific regions of the GIT are helpful for initially determining the relevance and function of associated metabolic pathways and molecular mechanisms occurring within livestock's GIT. This initial assessment will allow for a systems-level evaluation of the GIT, which can aid in evaluating livestock health long-term.

In addition, metabolomics data from GIT samples can be coupled with GIT community sequencing to evaluate the relationship between pathogens and native microbial communities. A common practice for understanding this relationship is with *in vitro* models. For example, Olson et al. (2024) determined that *in vitro* poultry systems inoculated with *Campylobacter jejuni* did not alter the metabolome but systems inoculated with *Campylobacter jejuni* and protein did alter the metabolome (Olson et al., 2024). Similar systems can also be utilized for assessing feed additives or supplements. *In vitro* models such as this will aid in developing targeted strategies for pathogen intervention strategies or improving dietary supplements.

Production assessment

There are few studies that have evaluated production efficiency via metabolomics. When metabolomics is utilized, meat quality (Artegoitia et al., 2022; Wang et al., 2020) and milk yield (Amin et al., 2022) are common metrics evaluated. Meat quality evaluation has historically focused on meat color and water-holding capacity whereas carcass quality traits focus on moisture content and crude protein (Muroya et al., 2020). In recent years, metabolomics has been incorporated to detect metabolites associated with meat and carcass quality traits. When metabolomics is incorporated, the finished products are homogenized and metabolites are extracted for the analysis. An example is the study by Kodani et al. (2017), which elucidated the influence of postmortem aging, crude protein, and fatty acid composition on beef tenderness, flavor, and juiciness using NMR-based metabolomics. In this study, it was determined that lactic acid, which was the most abundant compound detected in sirloin extracts that underwent a 2-week aging process, corresponds to the sour taste of beef (Kodani et al., 2017). Zuo et al. (2022) also explored postmortem aging of beef but focused on its impact on water-holding capacity. Examples of the most enriched pathways that could impact water-holding capacity included fructose and mannose metabolism, purine metabolism, and the pentose phosphate pathway (Zuo et al., 2022). In both studies, metabolomics methods were coupled with traditional techniques to monitor pH, drip loss, and/or energy metabolism and allowed for a comprehensive evaluation of changes to the water-holding capacity, tenderness, and color of beef.

Finished products have also been used for elucidating the effects myopathies have on meat quality. For example, Wang et al. (2020) performed NMR analysis on homogenized pectoralis major breast samples from broiler chickens with and without wooden breast myodegeneration to understand the influence this disease has on the meat metabolome (Wang et al., 2020). Wooden breast myodegeneration is a disease that results in hardening of the pectoralis major muscle (Sihvo et al., 2017) and greatly impacts broiler processing and meat quality. Wang et al. (2020) determined that wooden breast samples were associated with higher expression of lactate, glutamate, and inosine 5'-monophosphate compared to the healthy broiler chicken breast samples. Alternatively, Artegoitia et al. (2022) evaluated the production efficiency and beef carcass quality of low average daily gain and high average daily gain steers using urine samples (Artegoitia et al., 2022). This noninvasive method allowed them to identify bile acid and steroid metabolites associated with carcass quality and performance. Both studies effectively utilized metabolomics for meat quality assessments. Table 1 highlights other studies that have evaluated either meat and carcass quality or product yield with invasive or noninvasive metabolomics techniques.

Metabolomics can also be used to evaluate breed selection and its influence on meat quality and production efficiency (Goldansaz et al., 2017). For poultry, breed selection has resulted in broilers that grow at faster rates and have inadvertently led to increased incidences of wooden breast myodegeneration, spaghetti meat, and white stripping (Kuttappan et al., 2016). By using metabolomics, metabolites associated with genetic parameters that result in these myopathies can be easily identified. Therefore, resulting in improved animal welfare, improved meat quality, and reduced product loss and economic burden. In cattle, Cônsolo et al., (2020) demonstrated that meat samples from high-precocity and high-growth groups contained a higher quantity of metabolites associated with protein metabolism (Cônsolo et al., 2020), thus explaining why cattle within these groups had higher muscle development (Cônsolo et al., 2020). This demonstrates that breeding traits have a direct impact on the meat

metabolome. Ultimately, the inclusion of metabolomics analysis provides a robust and streamlined process for improving production parameters.

Emerging Applications of Metabolomics

Evaluation of stress-induced changes to GIT metabolism

Aside from typical metrics such as product yield or growth rate, metabolomics can be useful for understanding the effects stress has on the gut metabolome. Stress has been documented to alter the gut microbiome (Kurchaba et al., 2020; Pearson-Leary et al., 2020), which would greatly impact livestock health and consequently production efficiency. Specifically, heat stress has been documented to result in increased reactive oxygen species (Chauhan et al., 2014; Kikusato and Toyomizu, 2013) and heat shock proteins (Gabler and Pearce, 2015). It has also been reported that in growing pigs, the GIT is among the first organs affected (Gabler and Pearce, 2015). Therefore, it is reasonable to assume that metabolic activity in the GIT metabolome is altered as well. To give an example, Jiang et al. (2020) reported that stress due to weaning piglets resulted in distinct changes within the colon microbiome and metabolome (Jiang et al., 2020). Livestock research has explored the effects of temperature stress-induced health effects (Gabler and Pearce, 2015; Guinn et al., 2019; Pardo et al., 2021; Shakeri et al., 2018; Toghiani et al., 2020; S. Wang et al., 2023); however, few have incorporated metabolomics to evaluate the effects temperature stress has on the GIT and/or finished products (Li et al., 2022; H. Liu et al., 2022).

An important consideration for future studies evaluating the effects of the rearing process on livestock health is the effect on the gut–brain axis via microbial functional analysis with metabolomics. The gut–brain axis is the connection that allows the nervous system to communicate and relate to GIT functions. For example, in germ-free mice the gut microbiota influenced stress behavior and memory dysfunction (Carabotti et al., 2015). Incorporating analyses that can evaluate effects on the gut-brain axis in livestock metabolomics studies could greatly improve current efforts to meet production demands while ensuring animal health and welfare are still a priority.

Evaluating the GIT microbiome's function as opposed to only the taxonomic composition is vital for understanding gut health. Focusing on microbial activity within the GIT will enhance our understanding of the mechanisms that allow microbes to react to endogenous and exogenous factors. In human populations, changes to the gut microbiome have been linked to positive and negative health effects. As a result, there has been an increased emphasis on finding techniques that can aid in understanding the impact microbial activity has on overall gut health (Guinane and Cotter, 2013). Although many animal science studies have deciphered which microorganisms are present within the GIT of livestock, few have used metabolomics to elucidate changes to microbe function or activity (Goldansaz et al., 2017). A limiting factor in evaluating livestock health via GIT biomarkers is that an all-inclusive evaluation requires euthanasia of the animal (Pietro et al., 2019).

To alleviate this issue, we propose incorporating metabolomics into animal science studies and livestock production that can allow for long-term assessment of animal health and welfare if using noninvasive methods. An example is the use of metabolomics to evaluate how diet or dietary supplementation can alter the GIT metabolome of livestock and subsequently meat quality. For example, Chen et al. (2020) detected alterations in the cecal metabolome of broilers fed plant essential oils (Chen et al., 2020). Other studies have explored the beneficial effects of probiotic and postbiotic supplementation on the GIT metabolome of livestock (Mo et al., 2022; Wang et al., 2023). These approaches allow for thorough and all-encompassing evaluation of the microbial activity as related to dietary supplementation.

Metabolomics for assessing animal health and product quality

Emerging applications in the meat industry would ideally combine noninvasive metabolomics analyses followed by an invasive methodology for an overall evaluation of changes to animal health and product contamination as seen in Figure 1A. In this proposed workflow, urine or serum metabolites would be monitored during the animal's lifespan. For instance, for poultry production, serum can be collected at designated intervals to monitor feed efficiency. In this scenario, key biomarkers may include 7-ketocholesterol, dimethyl sulfone, or epsilon-(gamma-glutamyl)-lysine as documented by Su et al. (2022). Table 1 highlights several other animal studies that provide potential biomarkers for assessing livestock health and production using non-invasive methods.

Following processing, metabolomics can then be used to assess product quality. The use of

metabolomics in meat science has increased in recent years due to its ability to provide greater insight into the biochemical reactions that influence flavor, tenderness, spoilage, and marbling (Muroya et al., 2020; Ramanathan et al., 2023). Metabolomics studies using raw meat products have identified byproducts produced by pathogenic bacteria (Carraturo et al., 2020; Cevallos-Cevallos et al., 2011; Jadhav et al., 2018). Specifically, Carraturo et al. (2020) detected several volatile organic compounds including butane and 2-heptanol in raw beef contaminated with *Salmonella enterica* Typhimurium, while heptane and benzoic acid were detected in raw chicken contaminated with the same pathogen (Carraturo et al., 2020). Such thorough approaches are then capable of detecting metabolites associated with known pathogens more rapidly.

Metabolomics has been utilized to monitor active changes in metabolites in meat during storage and processing. This allows for a better understanding of the mechanisms that contribute to changes in meat quality and helps improve meat storage technology (Castro-Puyana et al., 2017; Lana et al., 2015; Wen et al., 2020) assessed the effect of metabolomic changes on beef stored at 1°C for up to 44 d, focusing on meat quality. They indicated that serine, arginine, and glutamic acid could serve as indicators of meat flavor. In another study, Subbaraj et al. (2016) employed hydrophilic interaction liquid chromatography–mass spectrometry to compare polar metabolites associated with meat color under different storage conditions, meat aging (length of postmortem aging days), and display time (Subbaraj et al., 2016). They found that compounds related to myoglobin chemistry and antioxidant properties were more abundant in color-stable meats. Wen et al. (2020) used Liquid chromatography–mass spectrometry (LC-MS) to identify metabolites associated with the quality of chicken meat stored at 4°C for up to 10 d (Wen et al., 2020). Their findings highlighted the role of carbohydrates, nucleotides, amines, amino acids, and other compounds in the quality changes of chicken meat. Linking the identified metabolites to core microbial populations can help determine the origin of these metabolites, aiding in improvement strategies and quality control. For instance, spoilage microorganisms such as *Proteus*, *Lactobacillus*, and *Pseudomonas* have strong decarboxylation abilities, converting amino acids into bioamines. These processes generate ketones, sulfur compounds, alcohols, and aldehydes associated with microbially mediated proteolysis in chilled meat (Santos, 1996; Wen et al., 2020). Ultimately, integrating metabolomic analysis with microbial profiling offers an exhaustive approach to understanding and enhancing meat quality during storage and processing. This combined methodology not only identifies key metabolites linked to flavor and color but also elucidates the microbial contributions to spoilage, providing valuable insights for developing improved meat storage technologies and quality control measures.

In addition, metabolomics has been used to evaluate finished meat and meat-alternative products for their respective nutritional content. In recent years, demand for plant-based meat alternatives has increased due in part to consumers preferring more sustainable and lower sodium diets (De Marchi et al., 2021; Vliet et al., 2021). In a study comparing the metabolome profiles of a popular meat-alternative brand and grass-feed beef, Vliet et al. (2021) noted 171 out of 190 metabolites differed between these products, but total protein and several key vitamins and minerals were similar when comparing the products (Vliet et al., 2021). Notably, creatinine, arachidonic acid, and pentadecanoic acid were among the metabolites detected solely or in greater abundance in the grass-fed beef samples (Vliet et al., 2021). On the other hand, plant-based products had a higher amount of several metabolites including lauric acid, spermidine, vitamin C, and sorbic acid (Vliet et al., 2021). Likewise, De Marchi et al. (2021) detected higher levels of lauric acid in plant-based meats, which was suggested to be derived from coconut oil (De Marchi et al., 2021). Despite similar nutrition labels for meat products and meat alternatives, metabolomics was capable of elucidating molecular-level differences among the products. Ultimately, plant-based products are highly processed and may not offer the same nutritional value to consumers (Vliet et al., 2021). Therefore, future incorporation of metabolomics can provide the necessary information to allow producers to better adapt meat alternatives for consumers' needs and allow consumers to make more informed decisions regarding consuming meat alternatives.

Conclusions

This review paper highlights the importance of incorporating metabolomics in a systems biology or diagnostic context, as host interactions play a significant role in changes to the livestock metabolome which may greatly influence meat quality, growth performance, feed efficiency, and other important metrics. The studies highlighted also demonstrate how metabolomics is a high-throughput technology that can be

easily incorporated into a production assessment workflow especially if using non-invasive methods. In conclusion, increased utilization of metabolomics in meat and animal science can improve livestock health and production efficiency by directly contributing to biomarker discovery.

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