

Breathprints of the Barn: The Future of Livestock Research and Monitoring with Exhalomics

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Keywords

exhalomics, breath, noninvasive, health, physiology, microbial dynamics

Abstract

Livestock farming faces increasing demands for sustainability and improved animal welfare. Noninvasive approaches for monitoring animal health and physiology are of growing interest. Exhaled breath analysis, or exhalomics, has emerged as a promising tool for detecting volatile organic compounds and gases associated with metabolism, disease states, physiological processes, and microbiome in livestock. This review synthesizes current advancements in breath sampling and analytical technologies and evaluates applications in disease diagnostics, nutritional assessment, and physiological and microbial profiling across livestock species. Although progress is evident, key challenges remain, including sampling variability, incomplete metabolite annotation, and limited scalability for field use. Future efforts should prioritize standardizing protocols; expanding livestock-specific spectral libraries; and developing affordable, real-time sensors for on-farm deployment. Integrating exhalomics with multi-omics and artificial intelligence–driven analytics holds potential to enable earlier disease detection, improve production efficiency, and reduce environmental impacts, ultimately advancing precision livestock farming and animal welfare over the coming decade.

1. INTRODUCTION

The global livestock sector has undergone notable consolidation, with fewer but larger farms intensifying production to meet rising demand for animal-derived foods. This shift, driven by the pursuit of economic efficiency and higher yields, complicates the management of expansive operations, especially in tracking individual animal health, welfare, and productivity. At the same time, growing societal and regulatory emphasis on animal welfare is pressing producers to embrace innovative, welfare-focused strategies. Conventional invasive techniques for evaluating animal physiology and health, such as cannulation, blood sampling, and tissue biopsies, are proving less practical. Their invasiveness, labor demands, and unsuitability for ongoing monitoring in research and farm settings pose clear limitations (1–4). Precision livestock farming (PLF), harnessing data-driven tools for continuous oversight and management, provides a compelling solution to these issues. However, the implementation of effective PLF systems requires innovative, noninvasive tools capable of capturing accurate, real-time animal health and physiological data. Recently, exhaled breath analysis, or exhalomics, defined as the study of all-exhaled-volatiles, has emerged as a powerful noninvasive tool in research that analyzes volatile organic compounds (VOCs) and gases to gain insights into physiological and pathological processes, and it can be applied to both humans and animals. Still largely confined to experimental studies, exhalomics holds strong potential for integration into PLF systems, given its noninvasive nature, capacity for high-throughput analysis, and suitability for real-time monitoring (5–7).

Livestock exhale a complex blend of gases, including nitrogen (N_2), oxygen (O_2), hydrogen (H_2), carbon dioxide (CO_2), methane (CH_4), hydrogen sulfide (H_2S), and water vapor, mixed with a wide range of VOCs, as documented in early studies (8–11). These compounds, defined as organics with a vapor pressure above 0.1 Torr at 25°C at standard atmospheric pressure, originate from both internal physiological processes and external sources like diet, pollutants, and industrial emissions (12, 13). Internally produced VOCs act as potential biomarkers for physiological states and health conditions, whereas those from outside reflect influences such as diet or environmental stress. Ruminant livestock represent a unique example, as their exhaled breath carries VOCs from rumen fermentation (via eructation) alongside blood-borne compounds exchanged in the lungs. Analyzing these exhaled VOCs in ruminants can also provide valuable insights into microbial fermentation processes in the rumen, a crucial metabolic activity converting feed into essential nutrients and energy. For example, key VOCs such as short-chain fatty acids (SCFAs, e.g., acetic acid, propionic acid, and butyric acid) signal rumen fermentation patterns, digestive efficiency, and metabolic health in dairy cows (7, 14).

Early work tapped ruminal headspace gases like CH_4 and CO_2 as markers of fermentation and microbial activity in fistulated animals. However, technological limitations constrained detailed, real-time analysis, with most research reliant on gas chromatography–mass spectrometry (GC-MS), which lacked portability and practical application for continuous monitoring (15–19). Newer advances in analytical chemistry have widened exhalomics' reach beyond ruminants to key livestock species, such as swine, poultry, horses, goats, and sheep. Tools like high-resolution mass spectrometry (HR-MS), selected ion flow tube–mass spectrometry (SIFT-MS), proton transfer reaction–mass spectrometry (PTR-MS), and secondary electrospray ionization–mass spectrometry (SESI-MS) now offer precise, sensitive, and real-time detection of a diverse array of breath metabolites across multiple livestock species (20–25). These advanced analytical platforms enable comprehensive metabolic profiling, opening doors to health diagnostics, metabolic monitoring, stress evaluation, dietary analysis, and early detection of diseases across various livestock sectors.

This progress, paired with the rising needs for PLF and sustainable animal management, makes a thorough review timely. This review synthesizes the latest research and knowledge on exhalomics

in livestock, highlighting recent breakthroughs in breath sampling methods and analytical tools. It also examines breath as a diagnostic lens for health; probes links between breath markers, nutrient metabolism and physiological changes, and microbiome; and weighs prospects and hurdles for implementing exhalomics in both livestock research and production. The overarching goal of this review is to map out how exhalomics could elucidate animal physiology, enhance animal welfare, and foster sustainability across diverse livestock farming systems.

2. EXHALED BREATH SAMPLING AND ANALYTICAL PLATFORMS

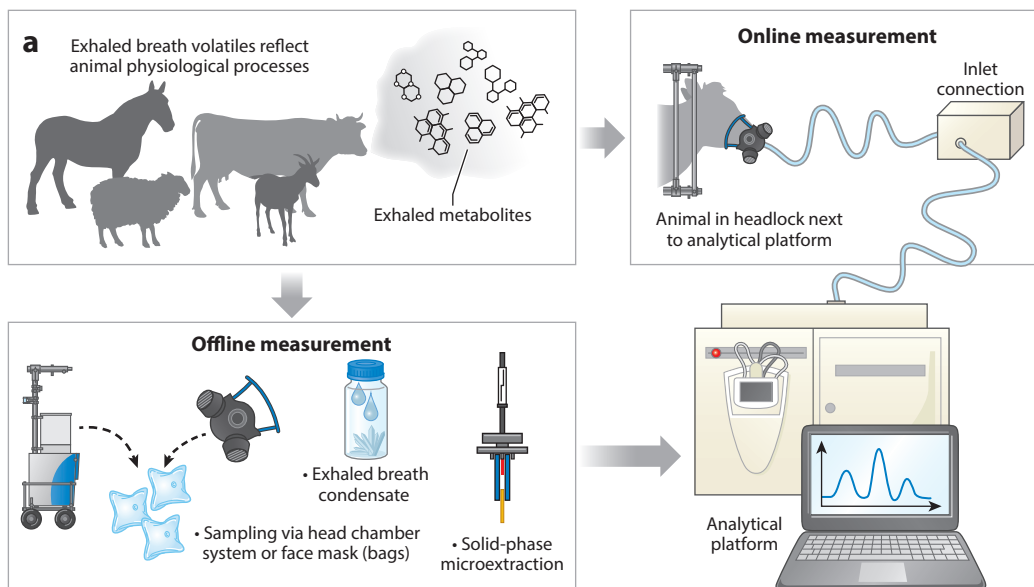
2.1. Exhaled Breath Sampling Methods

Breath sampling techniques fall into two main categories: online and offline, each with distinct strengths and constraints. Online sampling involves directing exhaled breath immediately into analytical instruments, enabling real-time measurements of VOCs; this method has been applied in livestock studies using systems like PTR-MS or SIFT-MS (26, 27). In contrast, offline sampling involves collecting exhaled breath samples in containers or onto sorbent materials [e.g., using canisters, bags, or thermal desorption (TD) tubes] for later analysis in the laboratory. A common offline method is the indirect trap approach, which, although used less frequently in animal studies, poses challenges such as the need for meticulous handling and the potential for degradation of VOCs between sampling and analysis.

Although real-time sampling holds clear potential, most livestock exhalomics studies lean on offline methods due to practical barriers—logistical challenges and the steep cost of advanced on-line equipment. Common devices for capturing exhaled breath of livestock animals include face masks (28, 29), head chambers (7, 14, 30), and nostril samplers (31) (**Figure 1a**). Additionally, offline approaches also include tube traps to temporarily store breath samples for subsequent analysis (32), albeit with minimal delay. These tools differ chiefly in how much ambient air mixes in, with head chambers diluting samples most due to air suction, blending exhaled and environmental gases. This dilution lowers VOC levels, limiting the detection capacity of analytical instruments like GC. Face masks and nostril sampling devices, by contrast, better manage air mixing, though their precision hinges on recognizing exhalation tidal events, as noted previously (33).

2.2. Analytical Platforms for Exhaled Breath Analysis

Once collected, exhaled breath samples require analysis to reveal the volatile metabolites tied to livestock's biochemical and physiological processes. Advances in chemical sensing and analytical technologies have broadened the scope and sharpened the accuracy of these analyses, deepening insights into metabolism, health, and productivity. Researchers employ a range of technologies as either standalone methods or combinations, each with specific strengths and trade-offs tailored to different research objectives (**Figure 1b**). These include MS-based approaches, ion mobility spectrometry, electronic nose, GC, and high-resolution laser-based spectroscopy (34–40). GC-MS, pioneered by Pauling et al. (41), is the most widely used technique for offline analysis due to its high sensitivity, specificity, and capacity for detailed chemical characterization of both VOCs and semi-VOCs (15–19). In GC-MS, compounds contained within a breath sample are separated in a capillary column using an inert carrier gas like helium. In this stage, compounds separate based on their interaction with the stationary phase and their volatility. These compounds then enter the mass spectrometer, where they are ionized, typically via electron ionization, resulting in fragmentation into distinct ion patterns. These fragments are sorted according to their mass-to-charge ratio (m/z) within a mass analyzer [such as a quadrupole, ion trap, or time of flight (TOF)], producing a unique mass spectrum for each compound. The resulting spectra are then compared to established libraries for compound identification and quantification.



b

	GC-MS	PTR-MS	SIFT-MS	SESI-MS	MI-MS
Commonly used analytical platforms for breath analysis					
Sampling mode	Offline	Online	Online	Online	Online
Ionization method	Electron ionization	Hydronium ion (H_3O^+)	H_3O^+ , O_2^- , NO^-	Electrospray ionization	Electron impact
Mass analyzer	Quadrupole, ion trap, TOF	Quadrupole, TOF	Quadrupole	Orbitrap, TOF	Quadrupole or TOF
Mass range	~10–600 m/z	~20–500 m/z	~10–300 m/z	50–500 m/z	10–200 m/z
Mass resolution	Unit resolution (quadrupole)	$\geq 5,000$ –60,000 (TOF)	Unit resolution	30,000–140,000 (Orbitrap)	Unit resolution (quadrupole)
Analysis type	Targeted and untargeted	Primarily untargeted	Primarily untargeted	Targeted and untargeted	Targeted and untargeted
Strengths	<ul style="list-style-type: none"> • High structural resolution • Detailed compound identification • Comprehensive chemical analysis 	<ul style="list-style-type: none"> • High sensitivity • Real-time monitoring • Low detection limits 	<ul style="list-style-type: none"> • Enhanced specificity • Selective ionization • Real-time sampling 	<ul style="list-style-type: none"> • High-resolution analysis • Real-time operation • Large molecular detection 	<ul style="list-style-type: none"> • Direct sample introduction • High sensitivity for certain VOCs • Reduced sample preparation
Challenges	<ul style="list-style-type: none"> • Requires offline preparation • Long analysis time • Labor-intensive sample processing 	<ul style="list-style-type: none"> • Lower structural resolution • Dependent on mass calibration • Requires soft ionization tuning 	<ul style="list-style-type: none"> • Lower structural resolution • Dependence on calibration • Potential interferences 	<ul style="list-style-type: none"> • Ionization optimization required • Humidity impact on efficiency • Complex sample handling 	<ul style="list-style-type: none"> • Limited molecular resolution • Dependency on membrane properties • Potential loss of volatile compounds

(Caption for Figure 1 appears on following page)

Figure 1 (Figure appears on preceding page)

Schematic representation of exhaled breath analysis in livestock using various MS platforms. (a) Exhaled breath from livestock contains VOCs that can be captured and analyzed either directly (online measurement) via breath masks or indirectly (offline measurement) using breath sampling devices, sorbent tubes, or gas sampling bags. (b) Captured breath samples are then analyzed using advanced MS platforms, including GC-MS, PTR-MS, SIFT-MS, SESI-MS, and MI-MS. The table summarizes key performance features of different analytical platforms, highlighting their ionization methods, mass analyzers, resolution, analysis type, strengths, and challenges. Abbreviations: GC, gas chromatography; MI, membrane introduction; MS, mass spectrometry; PTR, proton transfer reaction; SESI, secondary electrospray ionization; SIFT, selected ion flow tube; TOF, time of flight; VOCs, volatile organic compounds.

Sample collection for breath analysis is typically carried out using gas sampling bags, gas syringes, or specialized glass or stainless-steel canisters. Samples can then be introduced into the GC injection port either directly via a transfer line or after up-concentration on solid-phase microextraction (SPME) materials. Additionally, two-dimensional (GC×GC) combined with MS has been used to analyze both polar and nonpolar molecules in exhaled breath. TD units have also been integrated with GC-MS for breath analysis. In TD-GC-MS, breath samples are collected using a small, low-flow pump and absorbed onto sorbent-filled tubes (made of glass or stainless steel), which selectively trap VOCs based on their molecular properties and weights. Commercial and in-house spectral libraries can aid in interpreting volatile metabolomics data. Established databases, such as those from Wiley or the National Institute of Standards and Technology (NIST), contain mass spectral signatures for more than 600,000 chemical compounds, allowing for precise molecular identification. Additionally, custom-built libraries that integrate mass spectra of reference compounds significantly improve the accuracy of exhaled breath analysis in both research and clinical applications.

Beyond GC-MS, other advanced MS techniques have been used, such as PTR-MS, SIFT-MS, and membrane inlet-MS, as described earlier (36, 37, 42). These methods facilitate both online and offline measurements of VOCs in breath, skin emissions (volatilomics = study of endogenous VOCs emitted by a biological system), or headspace-based biological fluids, with applications ranging from biomedical research to forensic science and homeland security, as well as detection of doping in sports. PTR-MS uses hydronium ions (H_3O^+) to ionize VOCs and analyzes them using a TOF or quadrupole mass analyzer, offering high sensitivity, fast response time, and detection of low-concentration VOCs (down to parts per trillion), ideal for dynamic metabolic studies (43–47). Similarly, SIFT-MS employs a broader range of mass-selected reagent ions (H_3O^+ , O_2^+ , and NO^+) for ionization, providing enhanced specificity for different chemical classes (48–50). Both PTR-MS and SIFT-MS are soft ionization methods that preserve the molecular integrity of analytes and minimize fragmentation, enabling continuous exhaled breath sampling and capturing real-time metabolic changes during exercise, stress, sleep, or disease progression. However, their limitation lies in lower structural resolution compared to GC-MS and dependence on accurate mass calibration for compound identification. Alternatively, membrane inlet-MS using membrane-based pervaporation processes selectively absorbs VOCs for direct transfer into the mass spectrometer, proving beneficial for rapid, selective VOC screening in breath and other biological matrices (21, 42).

Recently, HR-MS techniques, including Orbitrap and TOF mass analyzers (22), have been combined with ambient ionization methods such as direct analysis in real time (51), atmospheric pressure chemical ionization (52), and SESI-MS (23–25) for exhaled breath or exhaled breath condensate (EBC) samples. These innovations have paved the way for real-time exhaled breath analysis, particularly in medical diagnostics. SESI operates by ionizing neutral VOCs from exhaled breath by introducing them into an electrospray plume, where they undergo ionization through charge transfer or protonation. When integrated with HR-MS platforms like Orbitrap and TOF analyzers, SESI-MS provides precise metabolite measurements with high mass accuracy

and resolution. It excels in detecting low-abundance compounds in complex mixtures, making it ideal for both targeted and untargeted metabolomics. Its real-time operation enables continuous monitoring of exhaled breath metabolites under dynamic conditions. In mouse and human research, longitudinal breath analysis with SESI-MS initially required high-frequency sampling within short intervals to mitigate measurement uncertainties (53, 54). Recently, these methodological constraints have been improved substantially by incorporating permeation oven-based or gas-mixing systems, which introduce precise, reproducible concentrations of gas standards into each breath sample or allow frequent instrument calibration by delivering defined gas standard mixes. Whereas Streckenbach et al. (54) focused on standard gases without isotope labeling, Wüthrich et al. (55) used isotope-labeled standards for improved accuracy in breath analysis. This innovation ensures accurate mass calibration, monitors ion suppression effects over time, and enhances data reliability, which is vital for robust longitudinal metabolic assessments. A key advantage of SESI-MS over PTR-MS and SIFT-MS is its ability to analyze larger molecules and low-volatility compounds, thereby broadening the range of detectable metabolites, though it requires optimizing ionization conditions and closely monitoring and regulating sample humidity, which can impact ionization efficiency.

Technologies such as HR-MS systems allow precise measurement of metabolites using targeted or untargeted approaches. Targeted metabolomics focuses on known metabolites for quantification, whereas untargeted or discovery-based metabolomics explores the molecular complexity, comparing the chemical and biological processes occurring in test versus control groups. Each analytical platform provides unique strengths and trade-offs depending on the research objectives and sample complexity. GC-MS enables comprehensive compound identification and structural assignment based on retention time and mass spectral data, but it requires offline sample collection, preparation, and long analysis time. PTR-MS and SIFT-MS excel in real-time quantitative analysis and dynamic monitoring, though lacking GC-MS's structural detail. SESI-MS, combined with HR-MS, bridges real-time analysis and high-resolution identification, but obtaining quantitative results is challenging, requiring precise tuning of ionization parameters and careful sample condition management. Platforms like PTR-MS and SIFT-MS are suited for large-scale population studies due to rapid throughput and high sensitivity, whereas GC-MS and SESI-MS are preferred for detailed biochemical pathway elucidation and clinical diagnostics. **Figure 1b** provides an overview of key performance features of these analytical platforms, including ionization methods, mass analyzers, resolution, analysis type, strengths, and challenges.

The adoption of exhaled breath sampling and analysis also faces several challenges. One major challenge with offline exhaled breath analysis is the variable degradation of different VOCs during storage over time. Immediate analysis of offline-collected samples may not always be possible, which creates additional variations and confounding factors when it comes to interpreting results. Recently, Fido et al. (56) conducted a study collecting breath samples in several types of sampling bags, including Tedlar, Kynar, Teflon, Nalophan, and EVOH (ethylene vinyl copolymer), to evaluate VOC integrity, particularly SCFAs. Their findings revealed significant SCFA losses, with recoveries dropping below 50% within 8 h. Ultraviolet exposure further altered VOC compositions, with all bags showing increased impurity levels, doubling after 48 h. According to Fido et al. (56), Teflon bags proved to be the most chemically stable, with low impurity levels, making them the best option for short-term storage (<8 h) of VOCs, though they are expensive and inflexible. In contrast, Tedlar, Nalophan, Kynar, and EVOH bags were unsuitable for short- or long-term storage due to VOC degradation. The work of Fido et al. (56) underscores the limitations of offline sampling and storage for VOC analysis, highlighting the need for direct, real-time monitoring to accurately capture the dynamic changes in exhaled metabolites in livestock.

2.3. Innovations in Sensor Technologies for Exhaled Breath Analysis

The evolution of sensor technologies for exhaled breath analysis has advanced significantly in recent decades, progressing from laboratory-based diagnostics to real-time, wearable, and noninvasive platforms (57). The use of exhaled breath in health assessment dates back more than 2,000 years to Hippocrates, who noted disease-related odors such as *fetor oris* and *fetor hepaticus* (58). A major scientific milestone occurred in 1857 when Wilhelm Petters first detected acetone in human breath, demonstrating the presence of measurable VOCs as indicators of metabolic state (58, 59). Building on these origins, exhalomics has transitioned from reliance on GC-MS, which remains limited by size, complexity, cost, and on-farm applicability, to the development of portable, affordable sensors suitable for practical livestock health monitoring. Among these, chemiresistive sensors, particularly those based on metal-oxide semiconductors and conductive polymers, have gained traction for their compact design, sensitivity, and potential for miniaturization (22). These devices transduce chemical interactions into electrical signals and, when arranged in arrays, can identify complex VOC profiles. Challenges such as humidity sensitivity, drift, and limited selectivity persist, but advances in materials engineering and sensor fusion techniques are addressing these limitations (60). In parallel, electronic noses, composed of sensor arrays designed to mimic olfactory systems, have been introduced to classify breath profiles based on VOC fingerprints rather than individual analytes (9, 61).

Efforts to identify disease-specific breath markers have also expanded. Ammonia, for example, has emerged as a key biomarker due to its link with renal and hepatic dysfunction, prompting the development of specialized polymer-based chemiresistors for its detection in humans (62). The COVID-19 pandemic catalyzed a leap in wearable diagnostics, leading to the integration of breath sensors into face masks. Designs incorporating porous membranes and SPME enabled passive, continuous sampling of VOCs during regular breathing (57), bridging the gap between laboratory-grade diagnostics and real-world usability. Furthermore, combining wearable sensors with artificial intelligence and Internet of Things technologies carries great potential for personalized, real-time interpretation of complex sensor outputs (57, 61). These integrated systems can provide robust application in livestock health monitoring, such as electrochemical breath sensor arrays to detect early signs of disease in dairy cows.

Sensors under development, for example using chemiresistive and flame-deposited nano-sensing, also show considerable promise and may in the future allow long-term, simple detection of microbial activity biomarkers in breath without the technical issues associated with MS (22, 63). These systems also have the huge advantage of low cost and size, allowing mass deployment under diverse conditions. Reaching this situation requires collaboration between biochemists understanding microbial metabolism, animal scientists with insight into the relevant animal conditions to monitor, and engineers/materials scientists developing appropriate sensor arrays. Overall, the trajectory of exhaled breath sensor development reflects a dynamic convergence of chemistry, engineering, and biosciences. From Hippocrates's clinical observations to modern artificial intelligence-driven wearables, breath sensing is poised to revolutionize personalized medicine and livestock health management.

3. BREATH FOR HEALTH ASSESSMENT

The analysis of exhaled breath for health assessment in livestock has progressed significantly over decades, evolving from a rudimentary technique for detecting VOCs toward a sophisticated, noninvasive tool for monitoring metabolic and physiological states. Advancements in analytical techniques have enabled the detection of a vast array of VOCs linked to specific physiological processes, expanding the potential of exhalomics for disease diagnosis and metabolic monitoring (53).

3.1. Early Developments in Metabolic Monitoring

The journey of breath analysis as a health assessment tool in livestock began with early studies targeting metabolic disorders, particularly in dairy cows. Dobbelaar et al. (64) conducted a pioneering study to assess the potential for detecting ketosis through breath analysis, focusing on the relationship between breath acetone levels and ketone body concentrations in blood and milk. Using four healthy lactating dairy cows, they induced ketosis by reducing feed intake and collected breath samples for analysis with GC-MS. Their findings revealed a positive correlation between elevated breath acetone levels and serum β -hydroxybutyric acid, as well as milk acetoacetate and acetone, suggesting that breath acetone could serve as a promising indicator for ketosis detection (64). This foundational work laid the groundwork for subsequent research. Elliott-Martin et al. (31) built on this by introducing a portable breath sampling device and exploring a gas sensor array for real-time analysis, demonstrating the feasibility of on-farm, noninvasive health monitoring in dairy cows. Their approach emphasized practicality and immediacy, addressing the need for accessible tools in farm settings. Mottram et al. (65) further refined these findings by focusing on induced hyperketonemia in dairy cows, showing that breath acetone levels increased more rapidly than changes in milk composition. This suggested that breath analysis could provide a noninvasive earlier marker for ketosis for timely intervention in lactating dairy cows.

3.2. Broadening Applications in Bovine Health

In the early 2000s, alongside the growing interest in exhalomics for metabolic disorders like ketosis, researchers began exploring its potential for monitoring a wide range of health conditions across livestock species (**Figure 2a**). Spinhirne et al. (8) took an important step toward identifying VOCs in the breath of healthy and sick steers as potential biomarkers for bovine respiratory disease (BRD). In their preliminary study, breath samples from three healthy and three sick cross-bred steers were collected using a face mask and analyzed with GC-MS from gas bags. They detected specific compounds, such as acetaldehyde and decanal, associated with sick steers, whereas heptane, octanal, and hexanoic acid were linked to healthy ones, highlighting the potential of VOC profiles to differentiate health states. Fend et al. (9) expanded this scope by assessing the feasibility of using an electronic nose to detect *Mycobacterium bovis* infection in cattle and badgers through serum VOC analysis. They collected serum samples from both experimentally and naturally infected animals, monitoring VOC release in headspace vials using an electronic nose. The resulting VOC patterns distinguished healthy from infected animals as early as three weeks post infection, demonstrating exhalomics' potential for early infectious disease detection. Burciaga-Robles et al. (10) introduced a new perspective by focusing on exhaled gases like nitrous oxide (N_2O) and carbon monoxide (CO) as biomarkers for BRD in 183 feedlot heifer calves. Using a custom bovine mask connected to a gas sampling line, they found that N_2O and CO ratios increased with BRD severity. However, these biomarkers alone were insufficient for predicting future BRD events, leading the researchers to recommend integrating breath markers with clinical assessments and blood inflammatory markers, such as haptoglobin, to enhance diagnostic accuracy and support earlier treatment decisions, ultimately reducing the economic impact of BRD.

3.3. Extending Exhalomics to Equine and Swine Health

As the scope of exhalomics research widened, its applications extended beyond bovines to equine and swine species, leveraging both exhaled breath and EBC. EBC is the liquid collected by cooling exhaled breath and is used to analyze nonvolatile components, such as pH or inflammatory markers (66, 67). Duz et al. (66) explored EBC as a method for monitoring lower airway inflammation (LAI) in horses, focusing on hydrogen peroxide (H_2O_2) concentrations and pH in EBC. Their

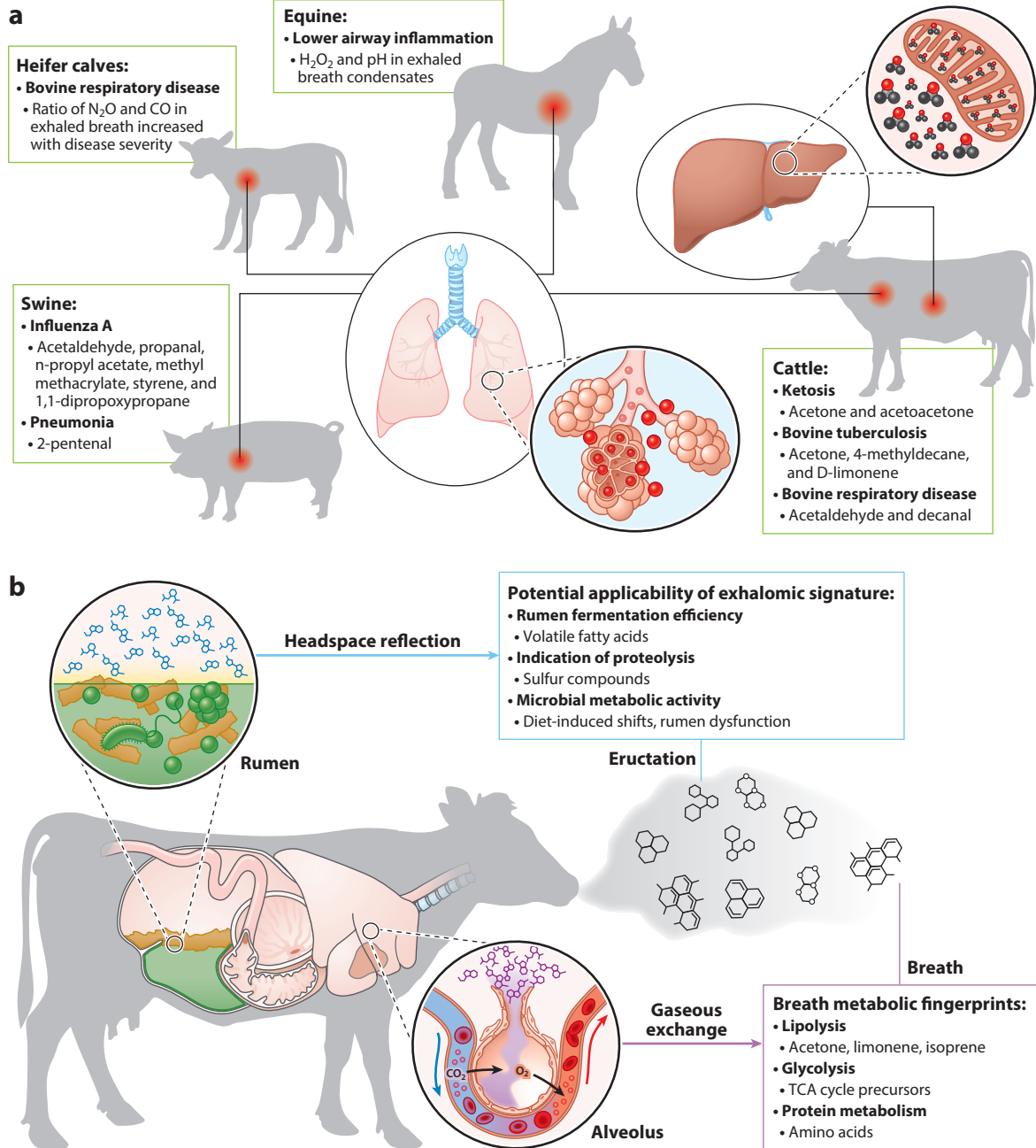


Figure 2

(a) The application of exhalomics for monitoring metabolic disorders and infectious diseases in livestock, highlighting various diseases across different species along with their key VOCs or disease-specific markers. (b) The role of exhalomics in studying nutritional physiology, protein or lipid metabolism, and rumen fermentation in livestock, using the cow as a model. Abbreviations: TCA, tricarboxylic acid; VOCs, volatile organic compounds.

study involved 15 healthy and 8 sick horses, with EBC collected over 10 min using an airtight face mask connected to a condensation chamber cooled to -80°C with an ethanol/liquid nitrogen slurry and nonbreathing valves to prevent contamination. Although LAI horses showed no significant difference in H_2O_2 levels, a trend toward lower pH was observed. The authors noted that high variability in H_2O_2 limited its reliability, but pH in EBC offered potential insights into airway health (66). Du Preez (67) advanced this work by analyzing EBC pH, H_2O_2 , and leukotriene B_4 (LTB_4) in 47 horses, comparing findings with clinical, endoscopic, and airway cytological results. They found higher EBC pH and H_2O_2 concentrations in LAI horses, positively associated with neutrophil percentages in bronchoalveolar lavage fluid, and LTB_4 linked to eosinophils, whereas pH was negatively associated with mast cell percentages. They concluded that EBC pH and H_2O_2 could serve as useful biomarkers for diagnosing and monitoring airway disease in horses, enhancing the understanding of respiratory health (67).

Expanding beyond cattle and horses, Traxler et al. (28) investigated VOCs in swine breath to monitor influenza A infection in 14 animals (3 controls, 11 infected) during a complete infection cycle under high safety conditions. The authors reported that breath VOC profiles revealed six compounds—acetaldehyde, propanal, n-propyl acetate, methyl methacrylate, styrene, and 1,1-dipropoxypropane—associated with disease progression, enabling differentiation between control and infected animals as early as day four post inoculation. More recently, Katsaros et al. (68) explored VOCs in exhaled breath to detect pneumonia in a porcine model using 60-kg adult pigs inoculated with methicillin-sensitive *Staphylococcus aureus* or a control solution via bronchoscopy. Breath samples, collected at multiple time points and analyzed with ultrahigh-performance liquid chromatography–MS, identified 2-pentenal as a key biomarker, increasing tenfold from baseline by day 9 post infection. By day 6, 2-pentenal levels distinguished infected from noninfected pigs with 88% sensitivity and 92% specificity, reaching 100% accuracy by day 9, suggesting its potential as a noninvasive biomarker for pneumonia detection in veterinary and human medicine.

3.4. Advancing Techniques and Addressing Challenges

Whereas earlier studies focused on identifying specific biomarkers, more recent work has emphasized refining techniques for broader, real-time applications. Ranade et al. (69) assessed oxidative stress biomarkers in EBC from 18 healthy Holstein–Friesian calves at birth and 6, 12, and 18 weeks, finding that H_2O_2 concentrations peaked at weaning. This indicated a progressive development of antioxidative defenses, marking H_2O_2 as a potential biomarker for respiratory oxidative stress during early development. Gierschner et al. (26) shifted their focus to real-time VOC profiling in dairy cows using an online PTR-TOF-MS system in a crowded farm setting, identifying significant VOC shifts linked to postpartum metabolic changes and paratuberculosis infections. This demonstrated the feasibility of on-the-spot health assessments in large groups. Moreover, Brebu et al. (70) aimed to identify VOC biomarkers for bovine tuberculosis (TB) in 18 TB-positive and 27 TB-negative Holstein cows, analyzing breath, feces, and skin samples. They reported 3 breath biomarkers (acetone, 4-methyldecane, D-limonene), alongside 9 fecal and 3 skin biomarkers, linked to TB-induced metabolic or infection changes, broadening the multi-sample approach to disease detection.

Despite significant progress, the precision and accuracy of breath analysis for diagnosing specific livestock illnesses remain underoptimized. Research has shown promising associations between VOC biomarkers and conditions like ketosis, BRD, TB, and pneumonia, but challenges in reliability and sensitivity persist. For example, Burciaga-Robles et al. (10) noted that N_2O and CO alone were insufficient for BRD prediction, requiring integration with other markers, whereas Duz et al. (66) highlighted H_2O_2 variability limiting its reliability for LAI monitoring. These inconsistencies stem from factors like biomarker variability, environmental influences,

sampling issues, and limitations in current analytical sensitivity, hindering widespread adoption in routine veterinary practice. To overcome these barriers, large-scale, data-driven studies are essential, ideally spanning field and veterinary hospital-based settings, to build comprehensive spectral libraries tailored to various livestock diseases. The integration of these libraries with advanced machine learning models holds transformative potential. By training algorithms on diverse data sets, researchers can improve the precision of disease detection, enabling real-time, noninvasive diagnostic tools capable of identifying diseases at their earliest stages. This approach could also address variability by identifying patterns across populations, enhancing sensitivity and specificity. The future of exhalomics in livestock health lies in optimizing these systems, moving beyond promising concepts to become indispensable tools for precision agriculture and animal care, ultimately improving welfare and productivity.

4. BREATH FOR NUTRITIONAL AND PHYSIOLOGY ASSESSMENT

Exhaled breath analysis offers a noninvasive approach for assessing metabolic fingerprints associated with drug metabolism, energy expenditure, and protein metabolism in livestock, offering insights into nutritional and physiological states (5–7, 40, 71). During respiration, volatile compounds are exchanged between capillary blood and the alveolar gas phase, reflecting blood concentrations and serving as direct indicators of metabolic changes across different phenotypes (71–73).

4.1. Early Foundations and Nutritional Insights

The concept of using exhaled gases to study metabolism dates back to Antoine Lavoisier, who in the eighteenth century demonstrated that respiration is a form of slow combustion by measuring O_2 consumption and CO_2 production in animals. This foundational work laid the groundwork for indirect calorimetry and the idea that gas exchange reflects metabolic activity (74). In livestock, the use of exhaled breath to assess nutritional status in livestock began with indirect calorimetry, measuring gases like CO_2 , O_2 , and CH_4 to understand energy requirements and redox status. Studies in horses, goats, sheep, and dairy cows established foundational correlations between these gases and nutritional needs (75–78). For example, Pagan & Hintz (75) used breath analysis to evaluate energy expenditure in horses, whereas Noziere et al. (76) assessed CH_4 emissions in ruminants as proxies for feed efficiency. In pigs, horses, and dairy cows, monitoring exhaled gases has also aided in evaluating energy redox status and performance, with studies like those of McKirnan et al. (79), Eaton et al. (80), Ohmura et al. (81), and Carroll et al. (82) linking CO_2 and O_2 dynamics to exercise and metabolic health. These early efforts, though limited by gas technology constraints, set the stage for more detailed exhaled breath profiling.

4.2. Ruminant-Specific Challenges and Innovations

In ruminant animals, phenotyping metabolism via breath analysis is complicated by ruminal eructation, which blends rumen-derived gases and VOCs with blood-borne exhaled metabolites (5, 83). Eructation is essential to relieve pressure from continuous microbial fermentation within the rumen, but it results in periodic mixing of ruminal headspace gases and compounds with alveolar air, complicating breath analysis and interpretation (84). Fischer et al. (85) observed a postprandial increase in hydrocarbons like hexane and pentane in goats' exhaled breath, suggesting origins from hepatic lipid peroxidation or intestinal bacterial metabolism, but could not distinguish these sources, highlighting the need for refined methods. Oertel et al. (5) addressed this by detecting up to a 36-fold increase in certain VOCs during eructation in cattle, developing the Burb Tracker algorithm to separate eructation from alveolar breathing cycles. However, this algorithm struggles

to detect changes in acetic and propionic acid, expected to rise during eructation, indicating limitations in capturing SCFA dynamics. More recently, Barrientos-Blanco et al. (30) advanced this further by establishing a head chamber–based sampling method using CH₄ as a marker to differentiate eructation from normal breathing. This approach allowed clear differentiation of eructation from normal alveolar breathing, revealing significantly higher concentrations of CH₄ and key SCFAs (acetate, propionate, and butyrate) during eructation, thereby minimizing rumen-derived interference and enhancing the accuracy of breath-based metabolic phenotyping.

4.3. Metabolic Pathways and Biomarkers

Exhaled breath reflects key metabolic pathways like the tricarboxylic acid (TCA) cycle, glycolysis, amino acid metabolism, and lipolysis, well-characterized in humans (72, 82, 86). In livestock species, similar profiles emerge, with energy metabolism studies in dairy cows identifying TCA cycle precursors and daily patterns (87). Metabolism of ketone bodies, particularly the production and exhalation of acetone as a key biomarker, has been profiled extensively in dairy cows, goats, and sheep, with early studies by Dobbelaar et al. (64), Elliott-Martin et al. (31), and Mottram et al. (65) linking acetone to ketosis and energy deficits. Eichinger et al. (88) recently identified gamma-hydroxybutyrate, 3-penten-2-one, and tetradecanal as biomarkers for fat and protein catabolism in dairy cows, expanding the metabolic scope. Exhaled H₂O₂ has also been used to assess oxidative stress in horses and sheep, reflecting energy redox status (66, 67). Cathcart et al. (89) integrated these findings in Thoroughbred racehorses, examining CO in exhaled breath and pH in EBC under field conditions. Analyzing 28 horses before and after high-intensity exercise in warm and cold seasons, they found CO decreased post exercise, especially in warmer conditions, whereas EBC pH increased with temperature, suggesting these as reliable indicators of physiological responses to exercise.

4.4. Rumen Fermentation, Dietary Impacts, and Exhaled Breath Profiling

The study of exhaled breath as a tool for assessing rumen fermentation originated with an interest in noninvasive monitoring of metabolic processes in ruminants. Early investigations focused on headspace gases like CH₄ and CO₂ in fistulated animals, using these as proxies for ruminal fermentation efficiency and microbial activity (90). Throughout the 1980s and 1990s, research centered on VOCs in ruminal headspace, though technological constraints limited detailed analysis (31, 64). Advances in GC and MS in the early 2000s enabled precise quantification of compounds like SCFAs, CH₄, H₂, and fermentation by-products. Dewhurst et al. (91) employed SIFT-MS to measure H₂S, methyl sulfide, and ammonia (NH₃) in the rumen headspace of fistulated Holstein cows, observing increased sulfur compounds pre-feeding and a post-feeding decrease, reflecting sulfur amino acid fermentation, whereas pH-dependent NH₃ levels indicated subacute ruminal acidosis (SARA). The SCFA profiles in rumen gas showed slight differences from those in rumen liquor, suggesting breath as a complementary measure. Following this, researchers began to explore the potential of using ruminal headspace gases to assess dietary and microbial shifts during rumen fermentation (92, 93). Specifically, Cai et al. (92) investigated rumen gas composition in three rumen-cannulated Angus steers via SPME and GC-MS-olfactometry. The authors identified 50 VOCs, including sulfides, SCFAs, ketones, and phenolic compounds, in rumen gas samples. The abundance of these VOCs and odors, particularly sulfides and SCFAs, was higher after feeding, indicating that rumen gases could be used to monitor digestion and fermentation patterns in cows. Dewhurst et al. (93) also identified interactions between rumen microbial processes (sulfur amino acid degradation) and plant defense mechanisms (cyanogenesis from white clover) in affecting rumen headspace gases. Fonseca et al. (94) proposed the use of rumen headspace gas

concentrations of H₂S as a better alternative to assess the key attribute of rumen protein degradation in feeds where high solubility negates the use of the traditional nylon bag *in sacco* technique.

The direct connection of a face mask with a PTR-TOF-MS in 2018 was a major innovation, enabling real-time exhaled breath analysis in ruminants (5). A more recent advancement by Islam et al. (7) set another milestone by using a head chamber system to monitor CH₄ during eructation in dairy cows. This method leveraged CH₄ as a marker to precisely identify eructation of cows for the collection of exhalome (total volatile metabolites contained in exhaled breath) samples, enabling exhalome characterization with HR-MS. They found that exhaled acetate, propionate, and butyrate peaked post feeding alongside CH₄, following a diurnal pattern. Islam et al. (14) validated exhalomics as a noninvasive method for assessing ruminal fermentation dynamics, showing strong correlations between exhaled and ruminal SCFA profiles. Eichinger et al. (88) demonstrated exhaled breath sensitivity to dietary-induced rumen metabolism changes, and Jorge-Smeding et al. (95) identified SARA biomarkers using SIFT-MS, reinforcing breath's potential for detecting dietary shifts.

Over three decades, studies have profiled more than 100 exhaled compounds in ruminants under various dietary and physiological states (5, 29, 31, 73, 85, 88, 96), as summarized in **Figure 2b**. These include hydrocarbons (e.g., isoprene, hexanoic acid), SCFAs (e.g., acetate, propionate, butyrate), ketones (e.g., acetone, 2-butanone), terpenes (e.g., limonene, p-cymene), and aldehydes (e.g., acetaldehyde, nonanal). Exhaled isoprene, acetone, and nonanal are recognized as biomarkers for metabolic and health assessments in humans (97). Isoprene, associated with lipolytic cholesterol metabolism, is modulated under increased physical activity (86, 98). Although isoprene has been studied extensively in humans, primarily as a biomarker of muscular lipolytic cholesterol and oxidative stress (86, 99), its metabolic origin and physiological role in livestock remain underexplored. Nonetheless, isoprene has been detected in the exhaled breath of dairy cows and goats, with concentrations reported to vary across lactation stages (73, 100). Nonanal, higher in early-life goats, correlates with elevated glucose and reduced protein levels (84). Limonene, a human biomarker for hepatic lipid clearance and liver impairment (101, 102), appears in exhaled breath of dairy cows with TB (70) and is excreted in ewe milk after inhalation (103), suggesting complex metabolic pathways.

5. BREATH ANALYSIS FOR UNDERSTANDING MICROBIAL DYNAMICS

Breath offers a significant advantage as a continuously produced biological matrix, enabling high temporal resolution to assess microbial dynamics. Under controlled laboratory conditions, bacterial metabolic processes can often be predictable, particularly during balanced growth (104). However, such conditions rarely occur naturally outside laboratory environments. Recent evidence demonstrates clear diurnal rhythmicity in gut microbial metabolism in laboratory mice, strongly associated with feeding cycles (105–107). Similarly, rumen microbial populations and metabolic activities fluctuate dynamically on both daily and intraday scales, making breath a potentially invaluable tool for real-time monitoring of these complex biological changes in ruminant animals.

5.1. Inferring Microbial Activity from the Volatilome

Technically, repeated sampling of breath or whole-animal volatilomes to infer dynamics requires additional considerations beyond single-time point measurements. To ensure that observed fluctuations represent genuine biological variations rather than analytical artifacts, internal standards, appropriate calibration, and careful mathematical treatment of data are indispensable. Moreover, the statistical analysis of diurnal breath-derived data poses considerable challenges

due to data volume and complexity and the inherent oscillatory nature of these signals. Such approaches have successfully tracked circadian fluctuations in whole-mouse volatilomes (unpublished observations).

Despite these advancements, directly linking the volatilome to microbiome dynamics remains challenging. Microbiomes are composed of numerous microbial taxa, many of which have not been studied beyond sequencing of their genomes. Additionally, microbiomes are distributed across diverse anatomical sites, which is expected to quantitatively and qualitatively alter their contribution to the whole-animal volatilome and/or the exhalome. To move beyond simplistic metabolic fingerprints linked to specific states of an animal, we need to develop a mechanistic understanding of host and microbial mechanisms responsible for metabolite concentrations observed. Gnotobiology offers a promising framework to achieve this, allowing comparisons of germ-free animals, with animals selectively colonized with individual microbiome-derived species or simplified defined microbial communities, and volatilomes obtained from growing microbes *in vitro*. For example, Lan et al. (107) recently demonstrated congruence between the volatilome of mice colonized by three specific bacterial species and the *in vitro* volatilome profiles of the dominant microbial species, suggesting potential for targeted exhalomics to elucidate metabolic activity of the dominant components of microbiomes. More recently, Hernandez-Leyva et al. (108) also discovered associations between gut bacterial metabolism and exhaled VOCs in healthy children, highlighting the potential of breath VOCs as diagnostic biomarkers for gut microbial dynamics. The identification of specific metabolites linked directly to particular microbial taxa or metabolic pathways should become possible with future mapping of the metabolic capacity of diverse microbial species.

5.2. Gas Sensor-Based Monitoring of Microbial Dynamics In Vivo

A much more established approach to linking microbial dynamics to breath metabolites is to use specific sensors for light gases. Metabolic cage systems for small rodents, in contrast to those for large ruminants, are identical to standard housing conditions, allowing animals to be housed for long periods of up to several weeks with continuous monitoring of light gas production and consumption. A recent study used this approach in mice to track the influence of feeding a microbiota-available carbohydrate, lactulose, on microbial fermentation. It clearly showed an increase in hydrogen emission over 3–6 h following feeding, corresponding to the kinetics of increased fermentation reactions in the mouse large intestine (109). The same approach has been used to examine differences in fermentation product production between mice with different gut microbiota compositions (105). Here, limitations include the availability of appropriate sensors and an understanding of the biodistribution of molecules of interest. H₂ sensing is useful because H₂ is an end-product of fermentation that is highly cell permeable, and therefore levels in ambient air quite reliably represent *in vivo* production/consumption. CH₄ can also be detected reliably with simple chemical sensors but is released largely via eructation and flatulence. Other compounds of interest, including NH₃, H₂S, and SCFAs, are more challenging to detect at relevant concentrations with high specificity and accuracy and will require further technological development.

5.3. Applications across Humans, Animals, and Livestock

Beyond laboratory animals, the analysis of exhaled breath VOCs is increasingly recognized as a valuable tool for noninvasive, real-time monitoring of host and microbial metabolism across various species (Figure 3). In humans, strong correlations between intestinal microbiota composition and volatile breath metabolites have been established, notably in gastrointestinal diseases

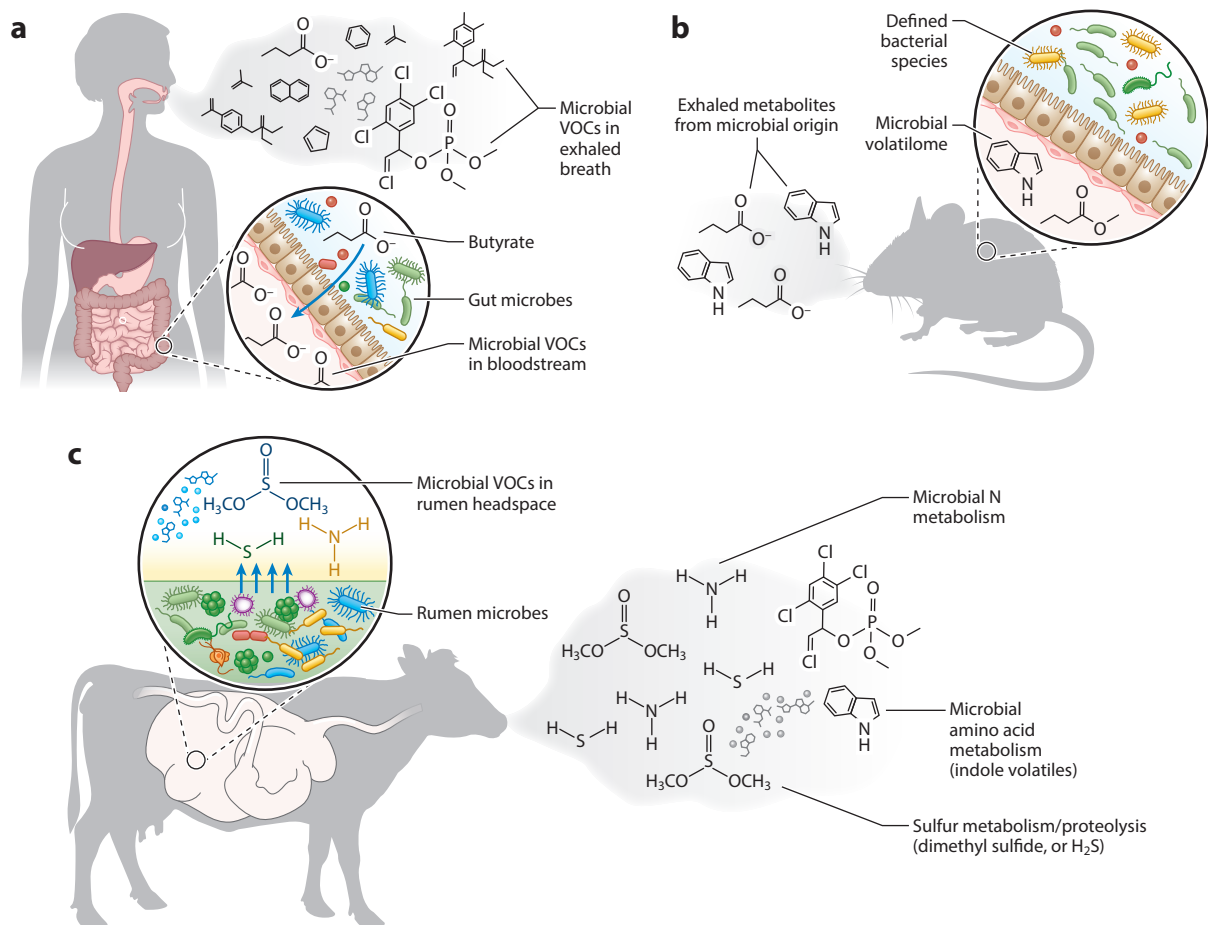


Figure 3

Schematic representation of microbial volatile organic compounds (VOCs) in host-microbiome interactions across species. (a) In humans, gut microbiota metabolize dietary components to produce short-chain fatty acids such as butyrate, along with a range of microbial VOCs. These compounds can enter the bloodstream and be transported to the lungs, where they are exhaled in breath and serve as potential biomarkers of gut health. (b) In laboratory animals, gnotobiotic mouse models with defined bacterial species are used to study the microbial volatilome. (c) In ruminants, complex microbial ecosystems in the rumen produce a rich array of VOCs during fermentation. These rumen-derived VOCs are released into the rumen headspace and potentially into exhaled breath, allowing study of rumen microbial activity and animal health status.

such as Crohn's disease, highlighting exhalomics' diagnostic potential (110). For instance, butyrate produced by gut microbiota during dietary fiber fermentation modulates immune and metabolic functions, with its production dependent on microbial composition and dietary intake, and is detectable in exhaled breath following systemic absorption (111). Additionally, gastrointestinal and hepatic diseases often involve metabolic shifts mediated by host-microbe interactions, yielding distinctive VOC patterns in exhaled breath. Such insights underscore the promise of integrating breath VOC analyses with microbiome profiling to facilitate biomarker discovery, inform dietary interventions, and advance noninvasive diagnostics.

A systematic review by Hintzen et al. (112) further emphasized the diagnostic value of breath VOC profiling in animal models of gastrointestinal disease, reporting consistently elevated VOCs

such as dimethyl sulfide, acetone, pentane, and putrescine as reliable biomarkers of microbial and host–microbial metabolic disturbances. Specifically, pentane, a lipid peroxidation and cytochrome P450 metabolic product, commonly increases in conditions like hepatic and intestinal ischemia (113, 114), whereas dimethyl sulfide, derived from microbial sulfur metabolism, is elevated during liver failure and dysbiosis (115). Furthermore, increased concentrations of microbial metabolites such as cadaverine, indole, and trimethylamine-*N*-oxide characterize sepsis and peritonitis, indicating microbial translocation and altered amino acid metabolism (116, 117).

Breath analysis directly captures these byproducts, offering real-time insights into microbial function. Methanogenic archaea convert H_2 and CO_2 to CH_4 , making eructed CH_4 a proxy for hydrogen metabolism (118). Field studies using the GreenFeed system and SESI-MS profiling show CH_4 correlates with methanogenic dynamics and SCFA profiles (14). Beyond CH_4 and SCFAs, rumen-derived VOCs include alcohols (e.g., ethanol, butanol from clostridial fermentation), esters, aldehydes, terpenes, phenolics, and sulfur compounds (e.g., H_2S , dimethyl sulfide), reflecting diverse microbial pathways (88, 119, 120). Short-chain alcohols (e.g., ethanol) are indicative of anaerobic microbial fermentation, whereas H_2S and dimethyl sulfide are associated with disruptions in sulfur metabolism or gut dysbiosis. Similarly, NH_3 , a byproduct of microbial nitrogen metabolism, exhibits elevated concentrations under high-protein diets, attributable to increased microbial deamination activity (121).

Despite technological advances, linking specific breath VOCs to rumen microbial taxa or functional pathways remains a significant challenge. High CH_4 emissions correlate with *Methanobrevibacter* abundance (118), but most VOC–taxa associations are unresolved due to the rumen’s microbial complexity and overlapping metabolic pathways. Data complexity from MS time series, combined with variable ionization and matrix effects, complicates interpretation (53). Sensor-based approaches are limited by the availability of high-specificity sensors for compounds like NH_3 and SCFAs (22). Integration of breath metabolomics with meta-genomics or meta-transcriptomics is a promising strategy to identify reliable marker compounds, enabling better monitoring of microbial function, fermentation efficiency, and diet–microbiome interactions in ruminant systems.

6. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Exhaled breath analysis, or exhalomics, is poised to become a transformative, noninvasive tool for monitoring livestock health, metabolism, and productivity, with particular relevance to PLF, as introduced earlier. Its demonstrated ability to detect biomarkers associated with rumen fermentation (e.g., SCFAs, sulfides), metabolic disorders (e.g., ketosis, SARA), and infectious diseases (e.g., BRD, TB, influenza A) and microbial dynamics (e.g., H_2 , CH_4) underscores its potential as a frontier technology. Yet, despite significant progress in sampling methods and analytical platforms, exhalomics remains largely confined to research settings. Transitioning it into practical, scalable applications (e.g., sensor arrays) for livestock management requires overcoming scientific, technical, and logistical barriers while seizing emerging opportunities.

A persistent challenge in exhalomics, as noted earlier, is the variability in breath sampling methodologies. Devices such as face masks, head chambers, and nostril samplers differ in design, sampling efficiency, and susceptibility to ambient air dilution, often yielding inconsistent or diluted VOC measurements. For instance, head chambers, although practical for ruminants, reduce VOC concentrations due to air suction (7), whereas face masks struggle to isolate pure exhaled breath from environmental contaminants (33). Standardizing collection protocols, potentially through automated tidal breath recognition or advanced sealing mechanisms, will be critical to ensure reproducible, high-quality data across farms and studies. Future research should prioritize developing rugged, livestock-specific sampling devices that minimize dilution and account

for species-specific behaviors (e.g., eructation in ruminants). Additionally, the instability of VOCs in offline sampling, as evidenced by Fido et al. (56) with SCFA losses in storage bags, highlights the need for real-time, on-site sampling solutions to preserve metabolite integrity.

The diversity and complexity of analytical platforms present another hurdle, as detailed previously. Technologies like GC-MS, PTR-MS, SIFT-MS, and SESI-MS offer high sensitivity and specificity, but their differing compound selectivity, ionization methods, and data outputs complicate cross-study comparisons and practical deployment. For example, GC-MS excels in structural detail but is impractical for real-time use, whereas SESI-MS enables dynamic monitoring yet struggles with quantification due to humidity effects. A major limitation across these platforms is the incomplete annotation of breath metabolites. Many m/z signals lack clear identities in existing spectral libraries (e.g., NIST, Wiley), hindering linkage to specific metabolic pathways or microbial taxa. Integrating untargeted metabolomics with condensed breath samples has improved annotation, but a significant proportion of VOCs remain uncharacterized compared to databases like the Human Metabolome Database or the Kyoto Encyclopedia of Genes and Genomes. Future efforts must focus on building livestock-specific spectral libraries, leveraging HR-MS and multi-omics approaches (e.g., metagenomics, transcriptomics). Artificial intelligence-driven tools, such as machine learning classifiers or network-based metabolite mapping, could accelerate identification by predicting molecular structures and inter-metabolite relationships from complex, nonlinear exhalomics data. Collaborative initiatives across research institutions to curate and share these libraries will be essential to standardize exhalomics outputs and deepen biological insights.

One of the most promising directions is the miniaturization and on-farm deployment of analytical devices. As suggested with sensor-based approaches, compact, portable systems, such as chip-based chemical sensors or miniaturized SESI-MS units, could bring high-resolution metabolic monitoring directly to livestock facilities. These technologies would enable continuous, real-time surveillance of health (e.g., early disease detection), nutritional status (e.g., rumen fermentation efficiency), and microbial dynamics (e.g., CH₄ production) without requiring off-site analysis or specialized operators. For instance, low-cost, nano-sensing arrays under development (22) could detect light gases like H₂ and CH₄, while future iterations might target SCFAs or ammonia with improved specificity. Achieving this vision demands interdisciplinary collaboration among analytical chemists, microelectronics experts, and agricultural engineers to design durable, user-friendly devices suited to farm conditions (e.g., dust, temperature fluctuations). Pilot studies testing these prototypes in diverse livestock settings, dairy barns, feedlots, or poultry houses will be crucial to validate their performance and refine their integration into PLF systems.

Another scientific frontier is establishing robust associations between exhaled breath and systemic physiological states, such as those reflected in blood or rumen fluid. Breath VOCs mirror metabolic pathways (e.g., TCA cycle, lipolysis), yet their precise relationship to blood-borne markers remains underexplored in livestock. Developing breath–blood correlation models could position exhalomics as a surrogate for invasive diagnostics, reducing animal stress and labor costs. This requires longitudinal studies with concurrent breath, blood, and rumen sampling across diverse herds, breeds, and environmental conditions to account for biological variability (e.g., lactation stage, diet). Integrating exhalomics with rumen microbiome profiling (e.g., microbe function) and plasma metabolomics could yield a systems-level understanding of host–microbe–diet interactions, identifying novel biomarkers for health and productivity. Artificial intelligence-based tools will play a pivotal role here, processing high-dimensional data sets to uncover predictive patterns, such as breath signatures for ketosis onset or feed efficiency, and enabling proactive management decisions.

Scalability and accessibility are critical for practical adoption. Current exhalomics technologies, although advanced, are often costly and complex, limiting their use to research rather than

routine farming. Developing cost-effective, farmer-friendly solutions, such as plug-and-play sensors with intuitive interfaces, will bridge this gap. Real-time alerts or predictive health scores derived from breath data, integrated into PLF dashboards, could empower farmers to implement early interventions (e.g., dietary adjustments, antibiotic reduction), enhancing welfare and sustainability. For example, a breath-based alert for SARA could prompt immediate feed reformulation, minimizing economic losses. Public–private partnerships could accelerate this transition, subsidizing technology development and training farmers in its use, particularly in resource-limited regions.

Looking ahead, exhalomics is set to redefine livestock management over the next decade. Advances in sensor miniaturization, metabolite annotation, and integrative data science will shift it from an experimental tool to a cornerstone of smart farming. Opportunities abound: Breath-based diagnostics could optimize antimicrobial use, aligning with global One Health goals; improve feed efficiency, reducing greenhouse gas emissions (e.g., CH₄); and enhance welfare by detecting stress or disease early. However, challenges remain, including validating biomarkers across species (e.g., swine, poultry), addressing environmental confounders (e.g., barn air quality), and ensuring equitable access to technology. By fostering interdisciplinary collaboration, spanning animal and veterinary science, analytical chemistry, engineering, and data analytics, and investing in large-scale field trials, exhalomics can move beyond promise to practice, delivering actionable insights that boost production efficiency, sustainability, and animal well-being on a global scale.

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