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The forecasting power of the mucin-microbiome axis in livestock respiratory diseases

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Abstract

Complex respiratory diseases are a significant challenge for the livestock industry worldwide. These diseases cause severe economic losses but also have a considerable impact on animal health and welfare. One of the first lines of pathogen defense combines the respiratory tract mucus, a highly viscous material primarily formed by mucin, and a thriving multi-kingdom microbial ecosystem, referred to herein as the mucin-microbiome axis. This axis can be considered a mighty two-edged sword, as its usual function is to protect from unwanted substances and organisms at one arm's length, while its dysfunction may be a clue for pathogen infection and respiratory disease onset. We further learned that the structure and function of this axis might be modulated by noncoding regulatory RNAs (e.g., microRNAs, long RNAs). This opinion paper unearths the current understanding of the triangular relationship of mucins, holobiont, and noncoding RNAs and the wide range of functions exhibited by the mucin-microbiome axis under respiratory infection settings. There is a need to look at these molecular underpinnings that dictate distinct health and disease outcomes to implement effective prevention, surveillance, and timely intervention strategies tailored to the different epidemiological contexts.

Keywords: livestock, mucin, microbiome, ncRNAs, respiratory diseases

Introduction

Complex respiratory diseases are a significant problem in livestock production, particularly in intensive systems. These diseases can cause significant economic losses due to reduced productivity, increased morbidity, premature mortality, treatment costs, and severe consequences for public health and the environment¹⁻⁵. In livestock physiopathology, there is a growing awareness that infectious agents frequently do not operate alone, and their virulence can be affected by multispecies synergic interactions^{1-4,6-8}. Consequently, a central finding of disease complexes involves interactions among holobionts (the host and the many other microorganisms living in or around it⁹) and multiple etiological agents⁵.

Undoubtedly, complex respiratory diseases entail multifactorial processes whose mechanisms are still not fully understood. New evidence has shown that the airway microbiota, defined as the complex community of microorganisms living in the respiratory tract, including bacteria, eukaryotes (especially yeast and protists), and archaea¹⁰, might act as a gatekeeper that provides resistance to infection on the mucosal surface^{11,12}. Under normal physiological conditions, the diverse community of commensal microorganisms maintains a mutualistic relationship with the host, acting as the prime educator and maintainer of the airway's innate and adaptive immune functions^{5,10}. Classifying healthy versus diseased individuals based on their airway microbiome has been done successfully for ruminants¹³⁻²², pigs²³⁻²⁶, horses²⁷, and chickens²⁸⁻³⁰. However, we can take one step further and broaden our view of the dynamics of respiratory diseases in livestock to include an additional aspect of the complex system: the respiratory mucus.

While mucus has historically been viewed as a simple physical barrier, recent work has suggested that mucins, the major gel-forming components of mucus, have many structural and functional roles in the respiratory system^{31–36}. Mucins are high-molecular-weight glycosylated proteins with hundreds of branching chain sugar (*O*-linked glycans), representing up to 80% of mucin weight. Through this rich biochemistry, *O*-linked glycans on mucins (collectively termed glycome) confer a wealth of physical and functional properties in the respiratory tract³⁷. First, the glycans on mucins provide a physical barrier that traps and clears inhaled particles and a chemical barrier that neutralizes toxins, allergens, pollutants, and pathogens^{32,33,38–41}. This limits the growth and colonization of pathogens and their adhesion and invasion of the respiratory epithelium. Thus, in addition to their role as a physical and chemical barrier, glycans on mucins have also been shown to have immune-modulating properties and may regulate inflammation³⁵. However, mucins' glycans are considered a double-edged sword, as they can aid invading pathogens to subvert host immune machinery. On top of these protective properties, mucin glycome affects the respiratory microbiota composition and functionality while serving as an environmental niche and a carbon and nitrogen source^{42–44}. Reciprocally, the respiratory tract microbiota's composition and activity can influence mucins' production and quality while stimulating the immune system and protecting against pathogen infection⁴⁵. Any disruption in the display or function of mucins and the glycosylation pattern of their glycans can lead to dysbiosis and respiratory diseases and potentially increase the risk of respiratory infections in livestock.

The next complexity is that mucin production, secretion, and glycosylation are highly regulated by noncoding ncRNAs (ncRNAs) at the genomic level. The glycans on mucins are products of multiple glycosyltransferases and glycosidases working in a coordinated manner to synthesize structures appended to proteins⁴⁶. Increasing evidence shows that ncRNAs are critical regulators of cellular and biological processes in living organisms, including mucin modifications^{46,47}. The research community has classified the ncRNAs based on their length: small ncRNAs (sncRNAs) and long ncRNAs (lncRNAs)⁴⁸. One type of ncRNA implicated in mucin glycome regulation is microRNAs (miRNAs)^{46,47,49}. miRNAs are small (~22 nucleotides) RNA molecules that bind to messenger RNAs (mRNAs) and regulate their translation and stability into proteins. Several miRNAs have been identified to fine-tune the glycan biosynthetic enzymes and regulate the expression of mucin genes in the respiratory tract of humans, including miR-34b/c⁵⁰, miR-146a⁵¹, miR-378⁵², and miR-141⁵³.

Determining the underlying causes of complex respiratory disease in farm animals is complicated. Species of veterinary interest are subjected to different host variables, environments, and pathogens, which could all play a role in disease, alone or in concert. From a One-Health-One Welfare perspective, this opinion paper aims to give insights into the potential avenues of complex respiratory disease, building on the surge of recent primary research to debate different aspects of the complex and intricate relationships between pathogens, the holobiont, mucins, and their genetic regulation. Underpinning these mechanisms will be crucial to determine how these can be harnessed to develop novel interventions to prevent disease infection and improve animal health and welfare.

Food-producing animal complexes: holobionts in a polymicrobial environment

Respiratory complex diseases constitute a significant cause of morbidity and mortality in livestock, in which prevention and prompt diagnosis, and targeted treatments are essential¹⁻⁵. For example, the bovine respiratory disease complex (BRDC) is a leading cause of morbidity and economic losses in wealthy countries, especially for newly feedlot calves, ranging from 30% in Belgium,⁵⁴ to 49% in Switzerland, and up to 90% in the U.S.A.⁵⁵. Similarly, sheep respiratory disease affects many animals⁵⁶, leading to significant indirect losses, such as carcass condemnations, treatments, and decreased production⁵⁷. Analogously, the prevalence of the porcine respiratory disease complex (PRDC) in finishing pigs continues to grow⁵⁸, with a morbidity rate ranging from 10% in Denmark⁵⁹ to 40% in the U.S.A.⁶⁰. As for common livestock animals, the respiratory disease complex in commercial birds remains widespread^{61,62}. It has become endemic in different countries, causing subclinical infections, mild respiratory symptoms, and high production losses in birds raised for meat or eggs⁶³⁻⁶⁶.

Complex respiratory diseases involve interactions among holobionts and multispecies synergistic etiological agents⁵. More than one pathogen-one disease paradigm is needed to explain complex respiratory disorders⁶⁷. Synergistic interactions between pathogens often occur through mechanisms such as chemical signaling influencing gene expression or metabolic exchange/complementarity to avoid competition for nutrients and improve the metabolic ability of microbial consortium (Mach and Clark 2017b; Mazel-Sanchez et al. 2019). A plethora of examples in ruminants and swine illustrates the framework for co-infection between pathogens (Gaudino et al. 2023), especially under intensive production, where animal density and stressing conditions are increased, and breeding programs are overly focused on enhancing traits related to production instead of robustness and disease resistance. In this context, multiple viral agents can contribute to the development of BRDC^{13,15,17,18,70}, including bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine herpes virus 1 (BHV-1), influenza D virus (IDV) (Oliva et al. 2019; Lion et al. 2021), bovine coronavirus (BCoV)⁷³ and parainfluenza three virus (PI3V)⁷⁴. In particular, IDV increases the susceptibility to the respiratory pathogen *Mycoplasma bovis* (Lion et al. 2021; Gaudino et al. 2023). *Mannheimia haemolytica* is the primary causative pathogen leading to lung damage in sheep⁷⁷. Yet, *Mycoplasma ovipneumoniae* and PI3V combined with adverse physical and physiological effects of stress predispose *Mannheimia haemolytica* infection^{78,79}. The swine influenza virus also compensates for the lack of suilysin (cytotoxic protein secreted by *Streptococcus suis*) in the adherence and invasion process of suilysin-negative *Streptococcus suis*⁸⁰. A synergism between nasal *Staphylococcus aureus* and pathobionts such as *Pasteurella multocida* and *Klebsiella* spp. has also been reported in pigs⁸¹. Co-occurrence between the porcine reproductive and respiratory syndrome virus (PRRSV), *Haemophilus parasuis*, and *Mycoplasma hyorhinis* in pig lungs is repeatedly observed⁸². The swine influenza virus enhances the morbidity of *Streptococcus suis* infection by decreasing mucociliary clearance, damaging epithelial cells, and facilitating its adherence, colonization, and invasion in the lungs⁸³. Another case in point is the low pathogenic avian influenza viruses (LPAIV) that, during outbreaks, are coupled with co-infections by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*, avian pathogenic *Escherichia coli* (APEC) and *Staphylococcus aureus*, increasing the mortality rate (Much et al. 2002; Umar et al. 2020; Filaire et al. 2022) and a marked reduction in egg production of laying hens (Umar et al. 2016). Frequently, these polymicrobial infections significantly hamper therapy, prognosis, and overall disease management. Under such circumstances, prevention is essential.

The untapped potential of airway mucins: the frontline defense of the respiratory tract

The respiratory tract is resistant to environmental injury, despite continuous exposure to pathogens, particles, and toxic chemicals in inhaled air⁸⁹. Such resistance primarily depends on a highly effective defense provided by mucus⁸⁹, a discontinuous, thin, and viscoelastic complex biological fluid that shields the lungs from environmental insults through a process known as mucociliary clearance⁹⁰.

The mucus is mostly water (usually > 97%), mucins, non-mucin proteins, ions, lipids, and immunological factors^{89,91,92}. The major macromolecular mucus components are the mucin *O*-linked glycoproteins⁹³. Mucins have a unique structure that distinguishes them from other proteins. First, they have a central protein core rich in Ser, Pro, or Thr- repetitive and non-repetitive sequences^{93,94}. Second, these repeated sequences allow for extensive post-translational modifications, including adding glycans and their glycosylation⁹⁵. The glycans attached to mucins are highly *O*-glycosylated⁹³. *O*-glycosylation is increasingly revealed as a sophisticated informational system that underlies essential biological functions at the cellular and organismal levels⁹⁶. Glycomics, one of the latest omics system science fields, evaluate the structures and function of glycoproteins in a biological system⁹⁷. The study of the glycome is metaphorically akin to forestry; each glycoprotein comprises glycans (leaves) conjugated to a protein (tree trunk)⁹⁸. These *O*-glycans are primarily built from five *O*-linked monosaccharide components: galactose, *N*-acetylglucosamine (GlcNAc), and *N*-acetylgalactosamine (GalNAc), fucose, and sialic acid^{93,94} attached to the protein backbone through an oxygen atom. The process of *O*-glycosylation leads to remarkable *O*-linked glycan heterogeneity and diversity, with over 200 distinct forms identified on mucins, representing a wealth of biochemical information in a minimum of space. The mucin glycome is analogous to the genome, transcriptome, and proteome but even more dynamic, and it has higher structural complexity that has yet to be fully defined. The human and mouse airway mucin glycome has started to be determined through different omic technologies^{37,99,100}. However, information needs to be created for livestock. Only two studies have described the *N*-glycan patterns in pig¹⁰¹ or chicken¹⁰² lungs, with no information about the *O*-glycome.

Broadly, there are two classes of mucins, those that remain tethered to cell membranes and those secreted, usually by the goblet cells. The cell-tethered mucins form the basis of a gel-like layer surrounding the cilia (periciliary layer) essential for regular ciliary action to move mucus out of the airways⁹³ and help control the mucus hydration¹⁰³. In contrast, the secreted mucins constitute the mobile mucus layer¹⁰³. Currently, 22 mucins (from MUC1 to MUC21) are identified in humans (denoted with capital letters), and 16 are found in the respiratory tract¹⁰⁴. The major mucins produced in the airways are the secreted polymeric mucins MUC5AC and MUC5B¹⁰⁵ and the cell-tethered mucins MUC1, MUC4, MUC16, and MUC20¹⁰⁶. MUC5AC and MUC5B are large (5–50 Mda) polymeric mucins and underpin the structure and organization of the airway's mucus gel⁹³. MUC5AC is mainly produced in epithelial surface goblet cells in the upper airways, whereas MUC5B is primarily secreted from mucous cells in submucosal glands¹⁰⁵. Similar conformation is observed in pigs^{107,108}.

Most of the physical properties and functions of MUC5B and MUC5AC are governed by the glycosylation patterns of their *O*-linked glycan structures⁹³. Indeed, mucin glycosylation

patterns are partially responsible for mucus's ability to set protective physical barriers against mechanical and chemical damage from the external environment (Goto et al. 2016) and harmful microorganisms¹⁰⁹. They also prevent opportunistic microbes' virulence^{110,111}, aggregation, and biofilm formation, interfering with pathogen adhesion and cell receptor binding¹¹¹. Glycans on mucins also possess antimicrobial properties and modulate and maintain immune homeostasis³⁸⁻⁴¹. Most of these actions in health are regulated by MUC5B, the major gel-forming mucin in the lung¹⁰⁵. Conversely, MUC5AC production is much lower in healthy airways but is upregulated, for example, in response to viral infections⁹³. It acts as a decoy for viral receptors and is essential for an allergic inflammatory challenge^{31,93,108}.

Despite mucins' various defensive and tolerative properties, some pathogens have developed strategies to exploit or manipulate mucins while enhancing their survival and evasion of the immune system. Certain viruses have evolved surfaces that do not adhere to mucins¹¹². Other pathogens can degrade the mucus layer and penetrate, release toxins that disrupt the epithelial barrier or modify mucus pH, influencing its viscosity. This is possible thanks to mucin-degrading proteases, chemotaxis, and flagella, which allow pathogens to move inside the mucus, adhere to and produce infection¹¹³. Once secreted mucus barrier is surpassed, pathogens recognize and target particular classes of cell receptors, such as sialylated glycans, glycosaminoglycans, and cell adhesion molecules, to mediate cellular attachment and entry^{96,114}. Several RNA and DNA viruses use sialylated glycans, e.g., sialic acids (Sia), to access the host cells as initial anchors¹¹⁵. In particular, influenza and coronaviruses, two of the most critical zoonotic threats, use sialic acids as cellular entry receptors¹¹⁶. More than 60 different Sias are known, which differ in sugar structural modifications¹¹⁷⁻¹¹⁹.

The presence or absence of an appropriate Sia receptor is a significant determinant of the host tropism of a pathogen. Indeed, studying the glycan structures in the chicken trachea and lung, Suzuki et al^{102,120} reported that IAVs bind preferentially to terminal Sia on glycans that possess Sia α 2-3Gal, with or without fucosylation and 6-sulfation but not to α 2,6-Sia. At the same time, horses' Sia α 2,3-Gal is predominantly expressed on the surface of ciliated epithelial cells of the nasal mucosa, trachea, and bronchus¹²⁰. In contrast, the trachea of cows is deficient in both Sia α 2,6-Gal and SA α 2,3-Gal receptors¹²¹. All influenza viruses tested in pigs interacted with one or more sialylated *N*-glycans but not *O*-glycans or glycolipid-derived glycans¹⁰¹.

In addition to serving as receptors for viral attachment, sialic acids can also play a role in viral evasion of the host immune system. Pathogens can mimic or mask their surface glycans to resemble those of host cells, making it difficult for the immune system to recognize and target them, otherwise called molecular mimicry. Other pathogens often carry glycan structures on their surface, e.g., sialic acid-specific glycans, to decorate their cell surfaces, aid in host cell attachment, and assist in evading host immunity^{40,117,122-125}. In summary, pathogens exploit their own and host mucin glycans to establish infection and survival.

Airway mucins: sweet and well-coated partners for microbiota

Interestingly, mucins also contain, feed, and dictate the airway microbiome ecosystem⁹⁶ while tolerating their enormous diversity^{42,126} and dictating their relationship (Varki 2007a). The respiratory tract microbiome is less diverse than the gut, with Bacteroidetes and Firmicutes as the dominant phyla in mammals⁵. High microbial biomass colonizes the mammal upper respiratory tract¹⁰. Conversely, the lower respiratory tract exhibits low biomass, with a significant role in lower airway mucosal immunology¹²⁷. The colonization of the airways occurs shortly after birth,

with the maturation of the microbiome occurring fast⁵. The airway microbiota composition is mediated mainly by microbial immigration, microbial elimination, and the proliferation rate of bacteria⁵. The airway microbiota has been shown to have essential roles in lung development¹²⁸ and maintaining homeostasis^{10,129}.

The significance of microbial research, especially when the world is trying to combat deadly infectious viral diseases and microbial resistance, has opened possibilities to explore diseased individuals based on their airway microbiome with much ease. Progress in this branch of science has helped elucidate the holobiont dynamics under respiratory infections in ruminants¹³⁻²², pigs²³⁻²⁶, horses²⁷, and chickens^{28,29,130}.

Being both an environmental niche and a food source¹³¹, respiratory mucins are essential drivers of microbiota composition, diversity, stability, and functionality, which can, in turn, influence microbial behavior and community structure under respiratory infection. Mucins create ecosystem heterogeneity by binding certain nutrients, leading to gradient formation and spatial niche partitioning. This is especially true for the airway microbiota, which primarily extracts nutrients from the respiratory mucins because nutrients are scarce¹³¹. Many *Bacteroides* spp and *Akkermansia muciniphila* encode an extensive repertoire of carbohydrate-active enzymes (CAZymes) that sequentially cooperate in metabolizing the host mucins^{132,133}. The first targets for CAZymes are the terminal residues on the *O*-glycans, such as sialic acid, fucose, and glycosulfate¹³⁴. The complete degradation of mucin polysaccharides can be done by combining enzymes that a diverse range of microbes can express. Insight into the enzymatic capacity of the microbiota is essential to predict how glycans on mucin landscapes contribute to the microbiota assembly and to host-microbiome symbiosis in eubiosis¹³⁵ and how we can use them to prevent complex diseases. Notably, the first insights into the respiratory mucin-microbiome axis in the livestock show how microbiota modifications (due to different levels of ammonia concentrations) in growing pigs impacted the thickness and viscosity of the mucus layer and increased the colonization of harmful bacteria¹³⁶. Enriching the upper respiratory tract with the probiotic *Bacillus amyloliquefaciens* in chicken increased the sIgA levels, the count of goblet cells, and the expression of the *MUC2* gene in the tracheal epithelium and the overall respiratory mucosal barrier function¹³⁷.

Yet, the relationship between the microbiome and the mucins in the respiratory tract is mutualistic and implies a two-way traffic. Thus, reciprocally, airway microbiotas have a pervasive impact on the composition and functionality of mucins. Microbiota is required to synthesize large gel-forming mucins completely, including encapsulation, glycosylation, changes in fucosylation and sialidation patterns, and thickness^{134,138,139}. Looking deeper at the sheer amount of data we have gathered from the mucin-microbiome axis in the gut, we know that microbiota metabolites regulate gut mucin synthesis at the transcriptional and epigenetic levels. Indeed, butyrate and propionate epigenetically regulate *MUC2* gene expression in the human goblet cell-like LS174T cells¹⁴⁰ and help maintain intestinal barrier function. In line with these findings, an elegant study by Bergstrom and Xia¹⁴¹ showed that the SCFAs resulting from *O*-glycan fermentation regulated intestinal mucin barrier function. Bioactive SCFA administration (primary butyrate) also promotes *MUC2* and *MUC5AC* gene expression and increases epithelial cell integrity after damage¹⁴². A study in piglets concluded that gastric infusions of SCFAs maintained intestinal barrier function by increasing the expression of intestinal tight junction proteins occludin and claudin-1 genes and decreasing the gene and protein abundances of IL-1 in

the colon, coupled with reduced intestinal epithelial cell apoptosis¹⁴³. Interestingly, co-culturing experiments of *A. muciniphila* with non-mucus-degrading butyrate-producing bacteria¹⁴⁴ indirectly stimulated intestinal butyrate levels near the intestinal epithelial cells with potential health benefits to the host. The mucin-microbiome axis study undoubtedly opens possibilities to explore *terra incognita* in health and disease contexts for animals of veterinary interest.

The mucin-microbiome axis in respiratory infections

The importance and problems mucus and mucins encounter are evident for the general public during upper respiratory tract colds⁹⁴. Persistent mucus accumulation and plugging of the airways pervasively impair microbial clearance, enhance the transition from the microbiome to the pathobiome^{31,89}, and inflammation^{43,145}. Thus, excessive mucin production can no longer attenuate microbial virulence and pacify opportunistic pathogens¹⁴⁶. The conversion from healthy to pathologic mucus occurs through multiple mechanisms⁸⁹, including abnormal secretion of salt and water, increased submucosal gland mucus secretion¹⁴⁷, mucus infiltration with inflammatory cells, and heightened broncho-vascular permeability with respiratory distress⁸⁹.

In line with this, a first study in pigs indicates that infections primarily drive changes in the quantity and physicochemical properties of airway mucins and may enhance pathogen biofilm formation and promote survival in nutrient-limited conditions, as reported for *Streptococcus mutans* in pigs¹⁴⁸. Similarly, the expression of MUC5AC has been induced in cells exposed to a wide variety of Gram-negative and Gram-positive bacteria, including *P. aeruginosa* and *Staphylococcus aureus*, evoking that mucin secretion is a host defense response to infection^{149,150}. Viruses can prominently stimulate MUC5AC expression *in vitro* or *in vivo* on airway epithelial cells³⁶.

Beyond mucin expression alterations, glycan glycosylation patterns have also been linked to respiratory physiopathology^{37,151}. For instance, mucin glycans exhibit reduced chain length, sulfation, fucosylation, and increased sialylation during inflammation¹⁴⁶. It is important to note that seemingly minor differences in glycan structures may result in significant pathophysiological outcomes. As an illustration, a shift in the nine-carbon backbone monosaccharides of the sialic acid might enhance the virus binding and infection of cells or can act as decoy receptors that bind virions and block viruses¹⁵² or bacteria, facilitating increased colonization and the development of lung disease^{153,154}. The specific roles of mucins and the glycome during infections can vary depending on the pathogen, tissue, and host factors involved¹⁵⁵. Yet, these early results give states good reason to continue investigating how respiratory pathogens shift host mucins and their associated glycome and the microbiome.

Given the intertwined relationship between pathogens, the airway microbiota, and mucins, and the considerable malleability of the microbiota relative to host genomes, the possibility of influencing respiratory tract health via microbiota manipulation seems possible¹⁵¹. Today, controlling pathogens' access to glycans on mucins via microbiome modifications has proven to be a promising method to prevent infection¹⁵¹. Elegantly, Pereira et al.¹⁵⁶ demonstrated that the administration of a synthetic bacterial consortium could decrease the availability of sialic acid from mucins by cross-feeding microbes and, thereby, protect against an infection caused by microbial pathogens that use these sugar groups as binding sites, e.g., *Clostridiodes difficile*¹⁵⁶

or viruses such as influenza virus, reovirus, adenovirus, and rotavirus¹¹⁴. Therefore, synthetic microbial communities expressing sialidase activity might improve mucosal health and prevent complex respiratory diseases¹⁵⁷. Complementary to this approach, nebulizing heparan sulfate-consuming commensal bacteria consistently contained SARS-CoV-2 attachment in higher-risk individuals¹⁵⁸. At the same time, nebulized fucose has been shown to reduce bacterial adhesion and improve lung function in animal models of respiratory infections¹⁵⁹. Determining the ideal microbial composition for optimal respiratory health and immune function in livestock is still a topic of ongoing research^{14,17,18,26,70,160–166}. The nature and the mechanisms behind respiratory microbiome and its interactions with the mucins and glycome profiles in livestock are not understood. Yet, it seems likely that there is a substantial amount of untapped potential concerning the airway microbiome-mucin axis in livestock and how external factors, such as the administration of nebulized synthetic bacterial consortium or the use of food containing synthetic and natural dietary glycans designed to target microbial activity at the mucosa. In the gut, such approaches have shed light on how alterations to the biochemistry of mucins and mucus impact their protective capacity and restore healthy mucosal function^{135,167,168}.

The non-coding RNAs: an engaged control of the mucin production and glycome patterns

Next to the use of microbial consortia or dietary glycans to control mucins glycome, understanding the genetic regulation of mucin production and glycosylation patterns offers a way to modulate mucin levels, affecting specific signaling pathways or transcription factors involved in mucin synthesis, glycosylation inhibitors, and glycosyltransferases, among others. However, transmembrane and secreted mucins have a complex glycosylated nature and extreme size¹⁶⁹. They are products of an orchestrated collection of enzymes working in a coordinated manner to synthesize structures appended to proteins and lipids^{95,170}.

As conserved regulatory agents, ncRNAs have started to gain attention as crucial regulators of mucin and glycome regulators at the transcriptional, post-transcriptional, and translational levels. The ncRNAs are RNA molecules that do not encode proteins but have regulatory or structural functions within cells¹⁷¹. ncRNAs are mainly divided into small ncRNAs and long non-coding RNAs. microRNAs, circular RNAs, and their precursor pri-miRNAs are sncRNAs ranging from ~22 bp in their mature form to ~70 bp in their premature stem-loop form. LncRNAs are noncoding RNAs transcribed by RNA polymerase II (RNA Pol II) and are longer than 200 nucleotides. Like miRNAs, lncRNAs have emerged as new regulators of gene expression and have become a focal point of biomedical and veterinary research¹⁷². MiRNAs regulate gene expression at the post-transcriptional level by binding (usually with imperfect complementarity) to the 3-UTR of a target mRNA, resulting in translation degradation or inhibition. A single miRNA can have hundreds of target genes, and multiple miRNAs can converge on a single mRNA. Unlike transcriptional regulators, the role of miRs is not to turn a gene on or off but instead to tune protein expression⁴⁶. The lncRNAs regulate the genomic output at many levels, from transcription to translation¹⁷³. The effect of lncRNAs is mainly achieved by interfering with the expression of downstream genes, supplementing or interfering with the mRNA splicing process, and regulating protein activity¹⁷⁴. Besides, a growing body of research has found that lncRNAs can regulate gene transcription through the function of competing endogenous RNAs (ceRNAs)¹⁷⁴. An individual transcriptome contains more lncRNAs than mRNA molecules¹⁷⁵. For livestock, the most significant number of identified lncRNA transcripts is available for pigs and cattle¹⁷⁵. Poultry is represented by less than half of the records. Genomic annotation of

lncRNAs showed that most are assigned to introns (pig, poultry) or intergenic (cattle)¹⁷⁶. The number of detected miRNAs in farm animal species is lower than lncRNAs, from 1064 in cattle to 267 in goats and 406 in pigs (miRbase release 22 (<http://www.mirbase.org/>)).

Given the emerging importance of ncRNAs in conditions and their potential to identify genes that underlie specific biological processes, it is clear that more attention should be paid to ncRNA and mucins interactions. To this point, data on the roles of ncRNAs in mucin regulation has been mainly generated by well-controlled murine models and humans. For instance, several miRNAs have been identified that regulate the expression of mucin genes in the respiratory tract of humans, namely miR-34b/c⁵⁰, miR-146a⁵¹, miR-378⁵², and miR-141⁵³. Additionally, modifications of miRNA expression can result in significant alterations of glycosyltransferases and glycome^{47,170}. Jame-Chenarboo et al. (2022) established that miRNAs are substantial regulators of cell glycosylation. Therefore, changes to mucin glycosylation patterns, such as increased sialylation, will likely alter mucin's binding properties with pathogens and their protective functions. miRNAs also play a crucial role in regulating mucin barrier functions, which has mainly been studied in the gut and the context of inflammatory bowel diseases¹⁷⁷⁻¹⁷⁹. Although these studies have elegantly displayed the impact of the regulatory ncRNAs on mucin structure and function, much work is needed to see whether these outcomes directly reflect livestock's complexity (genetic, environmental, comorbidities). The proposed mechanisms and results based on laboratory animals have yet to be validated in animals of veterinary interest due to the limitations regarding obtaining samples and the complexity of production systems, hosts, biotic and abiotic stressors, and infectious etiology. This complicates the application of human and laboratory animal-based findings to livestock. Therefore, the role of ncRNA on mucin glycome remains an open question in livestock. Coupled with this heterogeneity, the disease is known to change ncRNA behavior in livestock. For instance, PRRSV, one of the most important viral pathogens in the swine industry, affects host homeostasis through changes in miRNA expression^{180-182,183} (reviewed elsewhere¹⁸⁴). Profiles of lncRNA expression in disease were also affected by porcine circovirus-associated disease^{185,186}.

An extra difficulty lies in the fact that the presence and activity of microbiota might also regulate the host ncRNAs^{187,188}. Indeed, the microbiomes and microbial metabolites such as secondary bile acids and SCFAs have been shown to regulate the expression of miRNAs¹⁸⁹ and lncRNAs in the intestine epithelial cells¹⁹⁰, macrophages¹⁹¹, and other metabolic organs¹⁹⁰ in adult mice or *in vitro*¹⁹², evoking that the gut microbiome regulates the expression of both coding RNAs and ncRNAs regionally and systemically¹⁹³, especially during pathogen infection¹⁹⁴. For instance, a study investigating early rumen development in neonatal dairy calves revealed that nearly 46% of miRNAs expressed in the rumen are responsive to SCFA¹⁹⁵. Analogously, another study focused on rumen microbiota¹⁹⁶ suggested that the microbiota influences the host's miRNA expression pattern and that the host potentially helps shape the gut bacterial profile by producing specific miRNAs. Minimal knowledge exists about the ncRNA regulatory mechanisms behind mucin-microbiome interactions. Still, these emerging nuances illustrate that microbiome interacts with the host directly (direct modulation of transcriptome) and indirectly via the expression of ncRNA¹⁷³.

Concluding remarks

In livestock pathology, there is a growing awareness that infectious agents frequently do not operate alone, and their virulence can be likely affected by their interaction with the microbiome-mucin axis. Therefore, the microbiome-mucin axis is a significant player in the health of the respiratory tract. As such, they contain signals that can be used to forecast airway pathophysiology. There are fundamental gaps for species of veterinary interest, yet, these signals can be harnessed by combining microbial and glycomics omics, two fields that have tremendously developed in recent years. Based on the emerging evidence evaluated in this opinion paper, ncRNAs may play a vital role in the microbiota-mucin crosstalk via modifying the holobiont. The next phase in the livestock research area should focus on understanding whether particular microbiome-mucin glycome structures confer resistance and resilience to microbial infection and which are the critical factors controlling the mucin-microbiome axis, including the role of ncRNA. It is now time to harness the forecasting power of the microbiome-mucin axis.

Declaration of interest

The author declares no competing interests.

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Figures

Figure 1. The microbiome-mucin axis in the respiratory tract: mucin damage matters

Upper panel: The mutualistic relationship between the airway mucins and the microbiota and derived metabolites under eubiosis. On the one hand, the microbiota and their metabolism induce the synthesis of large gel-forming mucins, including encapsulation, glycosylation, changes in fucosylation and sialidation patterns, and thickness. On the other hand, the mucin layer serves as an environmental niche and a food source for the microbiota. The high diversity of gut mucins impacts the gut microbiota composition, diversity, and stability but also influences immune homeostasis.

Bottom panel: The mutualistic relationship between the mucin glycome and the microbiota under a disrupted airway ecosystem and environment. The deterioration of the respiratory mucosal barrier enables virus binding to the cells, and the translocation of bacteria and lipopolysaccharides (LPS) outside the respiratory tract, triggering immune and inflammatory responses, often resulting in increased permeability and, eventually, endotoxemia. Changes in

the respiratory barrier integrity involve changes in the abundance, expression, and glycosylation of mucins, and thus immune dysregulation, dysbiosis, and risk of disease onset. This figure has been created with BioRender.com.