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Age -Dependent Regulation of MUC1 and MUC4 Gene Expression and Histochemical Mucin Distribution in the Trachea of Broiler Chickens (Gallus gallus)

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Abstract Background: This study demonstrates that the trachea of broiler chickens undergoes significant age-dependent mucosal remodeling, characterized by differential expression of mucin genes and variations in mucin composition. Notably, MUC1 gene expression exhibited a dynamic pattern, with a significant upregulation observed at 21 days of age compared to 7 days, indicating that this stage represents a critical period for enhanced epithelial protection. This peak likely corresponds to the rapid growth phase in broilers, during which the respiratory system becomes more exposed to environmental stressors and pathogens, necessitating stronger mucosal defense mechanisms. In contrast, MUC4 expression remained stable across all examined age groups, suggesting a constitutive role in maintaining epithelial integrity rather than responding to developmental or environmental changes.

Histochemical findings further supported these molecular results, revealing a predominance of PAS-positive neutral mucins over acidic mucins in the tracheal epithelium. This suggests that neutral mucins play a more prominent role in the protective barrier function of the trachea during broiler development. The combined molecular and histochemical evidence highlights the coordinated regulation of mucosal defense components in the respiratory system.

Overall, these findings provide important baseline data for understanding the development of mucosal immunity in broiler chickens. They also offer valuable insights into how the respiratory system adapts to growth and environmental challenges, which could have implications for improving poultry health management and disease resistance strategies.

Keywords: Age-related changes; Broiler chicken; MUC1; MUC4; Respiratory mucosa; Trachea

Introduction The avian respiratory system is one of the most adaptable biological systems in the face of external challenges and is also one of the most exposed systems. In the case of the broiler chicken, the trachea is a primary airway and is the first epithelial structure that defends against inhaled pathogens, and dust, and against various environmental stressors present in the intensive production systems (Oladokun & Sharif, 2024). While mammals have similar systems, birds, in particular, have highly specialized respiratory systems and have developed specialized systems of mucosal defenses that keep the respiratory system functioning.

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Mucins are the vertebrate tracheal defense system that is built to protect against pathogens. The coating of mucins on the tracheal epithelium also serves to lubricate and signal the presence of the innate immune system. These are the very high molecular weight glycoproteins that shield both physically and biochemically against the adhesion of pathogens and make a system of clearance work (Mach, 2024). Many families of mucins exist, and among them the transmembrane glycoproteins, MUC1 and MUC4, have received considerable focus for their adhesive properties and for their immune modifying activities. In particular, MUC1, as a transmembrane signaling molecule, is involved in the control of inflammation. MUC4, on the other hand, is involved in cellular communication, and also in epithelial surface stability.

Prior studies have established that tracheal mucosa can be altered significantly due to the presence of infectious agents and inflammation.

Researchers have noted that the responses of tracheal epithelial cells have a range and are affected across multiple dimensions including age, immune system development, and the environment. Changes due to aging in inflammatory response pathways and epithelial cell bl barrier function has been noted in the tracheas of juvenile birds, indicating a positive correlation to the enhancement of the mucosal immune system (Zhong et al., 2024). In the same time, improvements in the culture models of tracheal explants and epithelial cells have strengthened the view that the defense mechanisms of epithelia change over time and are virtually in step with the developmental phase of the host (Heo et al., 2024; de Bruin et al., 2025).

Even with the advances, very little is known about the physiology, aging, and regulation of mucins in the trachea in the absence of infection. The majority of the studies focus on the disease model, either on the viral and bacterial infections (including AI and IBV) where the mucosal responses could be more pathological than developmental (Mahmoud et al., 2024). Therefore, no, or very little, baseline information is available on the regulation of the key mucins, MUC1 and MUC4 during the normal growth of broilers.

Additionally, there are now some indications of a relationship between mucin expression and the maturation of the respiratory microbiome and the epithelial barrier. The tracheal microbial composition across broiler genotypes and growth stages has been reported, and that changes in the composition are linked with alterations in mucosal immune responses, revealing the importance of host protective factors across ages (Poudel et al. 2025). Thus, for the understanding of respiratory health, disease tolerance, and the impact of the environmental or nutritional factors, the establishment of age-related mucin expression profiles will be highly relevant.

The current study focuses on MUC1 and MUC4 gene expression and related changes of mucin in the tracheal tissue of broiler chickens at the ages of 7, 21, and 45 days, providing the first molecular and histochemical insights on the maturation of the mucosal barrier in the broiler chicken trachea at different growth stages.

Materials and Methods

Ethical approval

The project was approved (4914 in 14/11/2024) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

Study Design and Experimental Groups

A study was performed experimentally to understand the changes in the myosin gene expression and the myosin distribution pattern in the tracheal tissue of broiler chickens (Gallus gallus). Thirty chickens were grouped according to their age; group 1 was made up of 7-day-old chickens, while group 2 comprised chickens aged 21 days, and group 3 consisted of chickens aged 45 days. Ten chickens formed one group, and all animals were clinically healthy

Sample Collection and Processing of Tracheal Tissues

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Broiler chickens were humanely euthanized in compliance with institutional ethical procedures. The trachea was removed aseptically postmortem. Each tracheal specimen was sectioned into two parts. One portion was snap-frozen in liquid nitrogen and stored at −20 °C for molecular studies. The other portion was embedded in 10% neutral buffered formalin for histochemical studies.

Extraction of Total RNA from Tracheal Tissue

Approximately 100 mg of tracheal tissue was used for extracting total RNA using a TRIzol® reagent (AccuZol™, Bioneer, South Korea) the same as in previous studies. To begin tissue homogenization, I used 750 µL of the TRIzol reagent and mixed it vigorously. I then added 200 µL of chloroform (Chem, Belgium), shook the sample for 15 seconds and then incubated the sample on ice for 5 minutes. I achieved phase separation through centrifugation (Eppendorf, Germany) at 12,000 rpm for 15 minutes at 4 °C.

The aqueous phase was transferred into a new tube, added 500 µL of isopropanol, then centrifuged the sample at 12,000 rpm for 10 minutes at 4 °C. To complete the process, the RNA pellet was washed once with 1 mL of 80% ethanol, air dried, and dissolved in 100 µL of New England Biolabs (UK) nuclease-free water. I then stored the extracted RNA samples at -20 °C until I was ready for further analysis.

NanoDrop (TM) spectrophotometer (Thermo Scientific, UK) was used to assess the concentration of the extracted RNA samples. I calculated the A260/A280 ratio from the absorbance which I measured at 260 nm and 280 nm to evaluate the RNA samples.

RNA samples were treated with the enzyme DNase I (Promega, USA) to remove genomic DNA contamination. Each reaction was prepared as follows: 10 µL RNA (100 ng/µL), 1 µL DNase I, 4 µL 10× reaction buffer, and 5 µL DEPC treated water (Bioneer, South Korea) to obtain a total volume of 20 µL. Incubate the samples at 37 °C for 30 min, then inactivate the enzyme at 65 °C for 10 min using the provided stop solution.

An M-MLV Reverse Transcriptase kit (Bioneer, South Korea) was used for the synthesis of complementary DNA (cDNA). RNA that had been treated with DNase was mixed with random hexamer primers and then denatured at 65 °C for 10 min and subsequently cooled on ice. Reverse transcription was done at 42 °C for 1 h in a thermal cycler (T100 Thermal Cycler, Bio-Rad, USA) and then the enzyme was inactivated at 95 °C for 5 min. The cDNA was synthesized and stored at 20 °C until needed.

Designing Primers and the Sequences

Using the Primer3Plus software and the gene bank sequences of NCBI GenBank, the gene-specific primers for the housekeeping gene (GAPDH), MUC1 and MUC4, were prepared and produced by Macrogen (South Korea). Below are the primers sequences and other details associated with the amplicons (Table 1).

Table 1. Primer sequences used for RT-qPCR analysis of mucin genes in broiler chicken trachea

Gene	Primer orientation	Primer sequence (5′–3′)	Amplicon size (bp)	GenBank accession number
MUC1	Forward	TCTGCTGCTCATCTCAAGTCC	143	XM_040680153.2
	Reverse	TTGCCCACAGTTGTCACAAG		
MUC4	Forward	ACCCAAAACAAACCGACTGG	118	XM_040678655.2
	Reverse	TCTACATCCCTGTTCCCATGG		
GAPDH	Forward	ATTCCTCCACCTTTGATGCG	103	NM_204305.2
	Reverse	ACAACACGGTTGCTGTATCC		

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Real-Time Quantitative PCR (RT-qPCR):

The GoTaq PCR Master Mix with SYBR Green Dye (Promega, USA) was used in this experiment as the qPCR mix and included 12.5 µL of master mix, 1.0 µL template cDNA (100ng), 1.0 µL of forward primer (10pM), 1.0 µL of reverse primer (10pM), and 9.5 µL of deionized water (New England Biolabs, UK). This reaction was vortexed on an Exispin vortex centrifuge (Bioneer) and transferred to a MiniOpticon Real-Time PCR System (BioRad, USA). The qPCR run's first step was an initial denaturation of 95°C for five minutes, followed by forty-five cycles of denaturing at 95°C for 20 seconds and annealing/extension at 60°C for 30 seconds. Fluorescence values were collected during the extension stage of the PCR run. The specificity of each amplification and thus the quality of the products was determined with a dissociation curve run after each amplification. Relative levels of expression for MUC1 and MUC4 genes are compared to the expression of the housekeeping gene, GAPDH, using relative quantification by the ΔCt method, where ratios are determined using the following equation:Gene expression = 2^(Ct GAPDH − Ct target gene)

. The efficiencies of each amplification product were determined with a calibration curve generated from a series of dilutions of the cDNA template, with efficiencies between 95% and 105% recorded for the various assays. Each sample and no template control were run in three technical replicates (triplicates) for each assay.

This research is done under committee for research ethics No. 1181 Date 7.3.2026

Results

Age-Related Variations in MUC1 Expression Within Tracheal Tissue

Quantitative RT-qPCR analysis of broiler chickens reveals the existence of an age-related variation in the expression of the MUC1 gene within the trachea. MUC1 expression in the G1 (7 days of age) chickens revealed expressions of lower MUC1 levels, suggesting that during this early post hatching period, the tracheal mucosa is likely not fully activated. MUC1 expression in G1 chickens increased in a statistically significant manner by G2 (21 days of age). MUC1 expression increased the most in G2 when compared to all other age groups. This reflects a solid active period in the epithelial maturation process, a sustained enhancement of the mucosal protection process, and rapid growth.

In G3 (45 days of age), the expression of the MUC1 gene diminished when compared to G2 and was comparable to the early age levels. Statistically significant differences existed between G1 and G2 and G2 and G3, whereas an absence of statistically significant differences existed between G1 and G3. The data indicates that the expression of the MUC1 gene in the trachea of the chickens during mid-growth stages is at a peak. Subsequently, expression of the MUC1 gene stabilizes as the chickens age (Fig. 1).

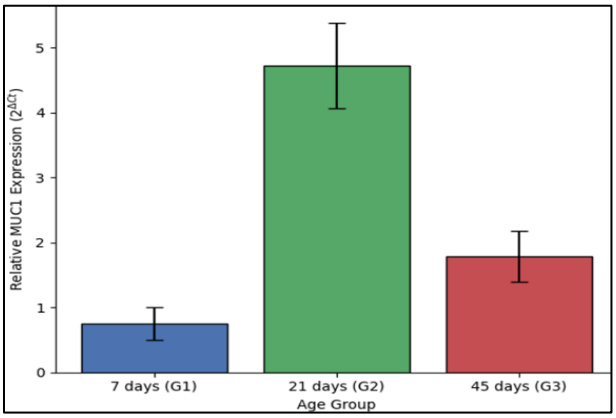

Fig. 1. Bar graph showing RT-qPCR analysis of the expression of the MUC1 gene in the tracheal tissue of broiler chickens (7, 21, and 45 days: G1, G2, and G3, respectively).

Age-Related Expression of MUC4 in Tracheal Tissues

Unlike MUC1, MUC4 retained a consistent expression pattern across all ages examined in this study. When MUC4 expression levels were

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compared among the three ages (7, 21, and 45 days), no significant differences were detected, indicating a profile of constitutive expression. The lack of significant changes in the levels of MUC4 expression suggest that this mucin may provide a consistent structural or supportive function in the tracheal epithelium, and does not take part in altered, age-associated immune responses.

There were, indeed, a few nominal differences in expression levels among the age groups, but the differences were all below the least significant difference threshold confirming that MUC4 does not undergo age-dependent transcriptional regulation in the trachea under normal physiological conditions (Fig. 2).

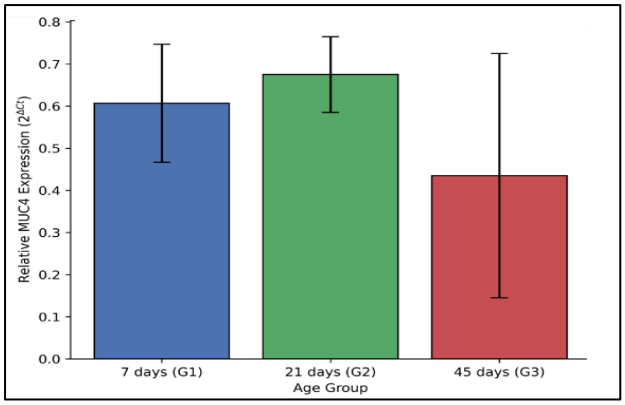

Fig. 2. Bar graph showing the expression of the MUC4 gene in the tracheal tissue of broiler chickens at the ages of 7, 21, and 45 days.

Comparative Analysis of the Expression of MUC1 and MUC4 in the Trachea

The direct comparison of MUC1 and MUC4 expression in the tracheal tissue reveals the predominance of MUC1, particularly during the mid-growth stages. At 7 days, expression of MUC1 was just slightly greater than that of MUC4 and the difference was not significant. However, at 21 days, the expression of MUC1 was much greater than that of MUC4 suggesting a progressive shift towards enhanced epithelial signaling and sensing. Interestingly, at 45 days, expression of MUC1 was lower than at 21 days, but was still greater than that of MUC4 (Fig. 3).

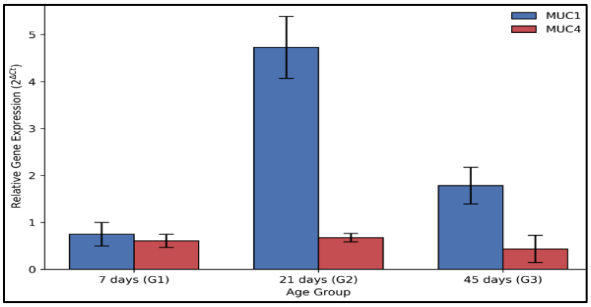

Fig. 3. Bar graph showing the expression of the MUC1 and MUC4 genes in the tracheal tissue of broiler chickens at multiple ages.

MUC1 and MUC4 qPCR Amplification Profiles in Tracheal Tissue

In the RT-qPCR analysis, the MUC1 and MUC4 targets were reproducibly and consistently amplified. MUC1 amplification curves had lower cycle threshold (Ct) values at 21 days than at 7 and 45 days, and this is evidenced transcriptional activity in the quantitative analysis. The amplification curves for MUC4 were closely clustered together in all age categories, reflecting the stable expression level of the gene. The consistent spacing among the amplification curves for each gene indicates that the RT-qPCR reactions had the same efficiency and great reliability (Fig. 4).

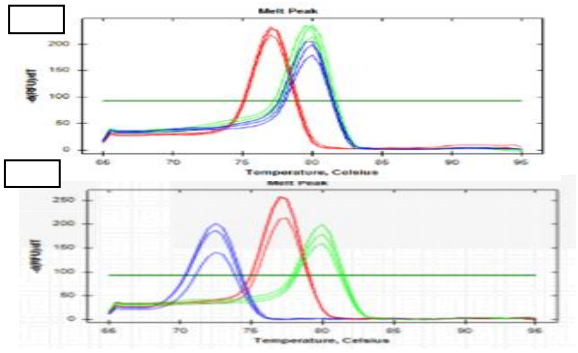

Fig. 4. RT-qPCR plots for amplification of MUC1 and MUC4 genes in tracheal tissues of broiler chickens at ages 7, 21, and 45 days.

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MUC1 and MUC4 qPCR Melting Curve Analysis in Tracheal Tissue

To confirm the specificity of RT-qPCR amplification, melting curve analysis was performed. The MUC1 and MUC4 melting profiles exhibited single, sharp, and well-defined peaks, suggesting that there were no amplification products that were not specific, or primer-dimers. The consistency across age groups for the melting temperature values lends further verification of the specificity and reproducibility of the assays (Fig٥).

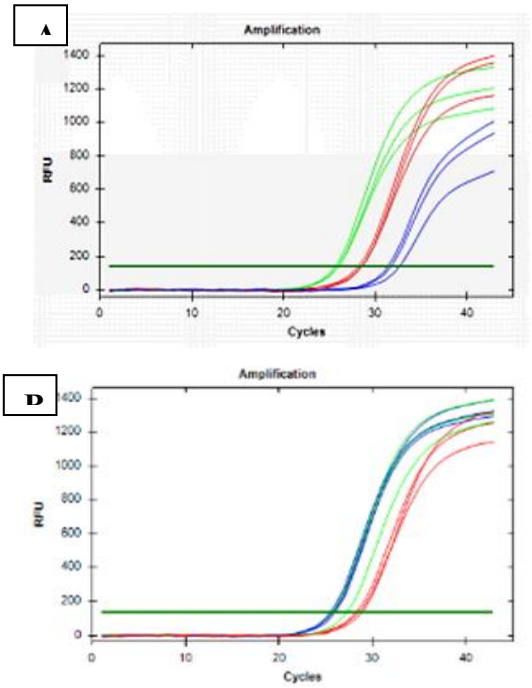

Fig 5 MUC1 and MUC4 gene melting curve data in tracheal tissue of broiler chickens at ages 7, 21, and 45 days.

Changes in Histology and Histochemistry in Mucosa of Trachea Across Age Groups

Tracheal tissues histology showed notable structural and histochemical changes with age in the mucosal layer of the broiler chickens. At seven days (G1), the main feature of the tracheal mucosa was a single layer of pseudostratified ciliated cells with a few goblet cells. The sub-epithelial surface and sub mucosal glands were immature barrier of the mucosa. At 21 days (G2), there was a notable maturation of the tracheal mucosa and epithelium. The epithelial layer was thicker. The goblet cells were more numerous and better organized. The cilia were more organized and the overall cellularity of the lamina propria of the mucosa was even greater, indicating that the functional activity of the mucosal barrier was even more developed. These changes structurally inform that there is an active multi-phase, longitudinal buttressing and fortifying of the mucosa in relation to the animal's age and environmental factors (Fig. 7).

By G3 at 45 days of age, the tracheal epithelium had stabilized its architecture. Though epithelial thickness and goblet cell density were greater than those at 7 days, they were comparable to or slightly less than those at 21 days. smooth and immature, reflecting an Transverse section of trachea with tracheal lumen (a), mucosa (b), submucosa (c), and adventitia (d). Lamina propria (e) under a thickened pseudostratified ciliated columnar epithelium (f) with more goblet cells (g). Defines perichondrium (i), hyaline cartilage (h), and chondrocytes in lacunae (k) indicating active maturation of tracheal structures. Panel C (45 days of age): Trachea transverse section showing the tracheal lumen (a), the mucosa (b), submucosa (c), and adventitia (d). A mature pseudostratified ciliated columnar epithelium (f) with a stabilized population of goblet cells (g) is supported by a lamina propria (e) that is well organized. Fully developed hyaline cartilage (h), perichondrium (i), and chondrocytes in lacunae (k) suggest the completion of tracheal maturation and structural stabilization. Stain: Hematoxylin and Eosin. Magnification:١٠٠and 400x add scale bars to the 400X

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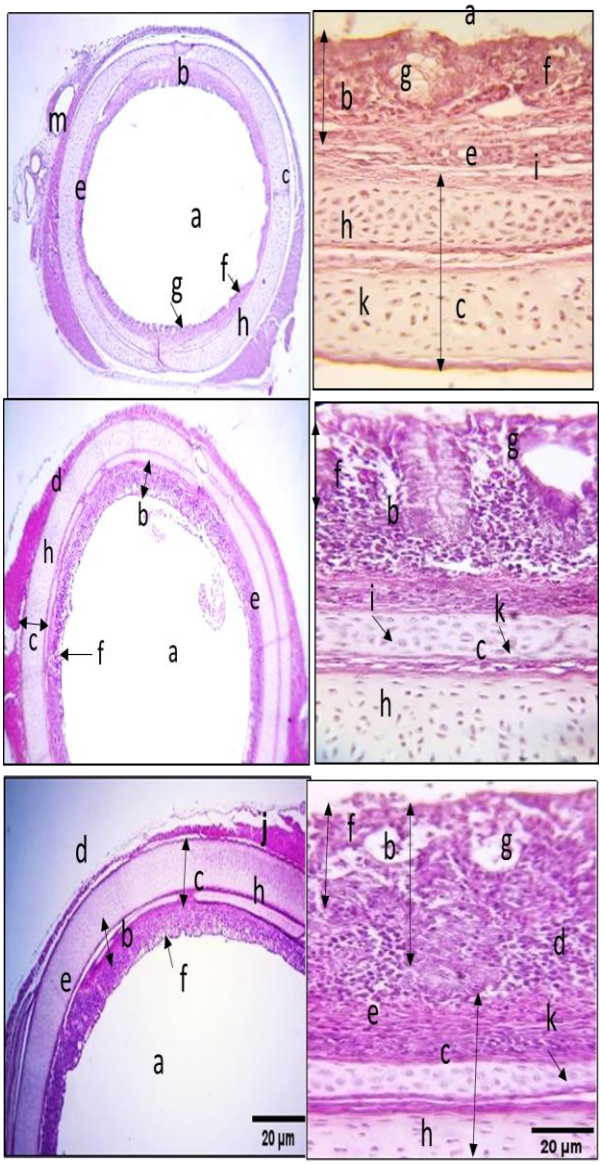

Fig. 7. Hematoxylin and eosin-stained transverse sections of trachea from broiler chickens at different ages. Panel A (7 days age). Transverse section of trachea with tracheal lumen (a), mucosa (b), submucosa (c), adventitia (d). Lamina propria (e) under a thin pseudostratified ciliated columnar epithelium (f) with sparse goblet cells (g). Immature hyaline cartilage (h) encased in perichondrium (i), with chondrocytes in lacunae (k), indicates early developmental stage of tracheal wall. Panel B (21 days age).

Distribution of Neutral Mucins in Tracheal Tissue (PAS Staining)

With each age group, there was a noted increase in the number of neutral mucins within the epithelium of the trachea, which was highlighted by staining for Periodic Acid-Schiff (PAS). In the 7-day group, PAS staining was limited and described the positive staining of a small number of epithelial goblet cells.

In the 21-day group, Goblet cells of the tracheal epithelium were PAS positive, and this positivity was greater than in the 7-day group. The number of PAS staining within a group increased and this correlated to the increased production and accumulation of neutral mucins of that group.
At day 45, PAS staining was still observed with a slight decrease in comparison with the 21-day group, however, PAS positivity was still much greater than the 7-day group, indicating the retention of the mucosal secretory capacity in the fully developed trachea (Fig. 8).

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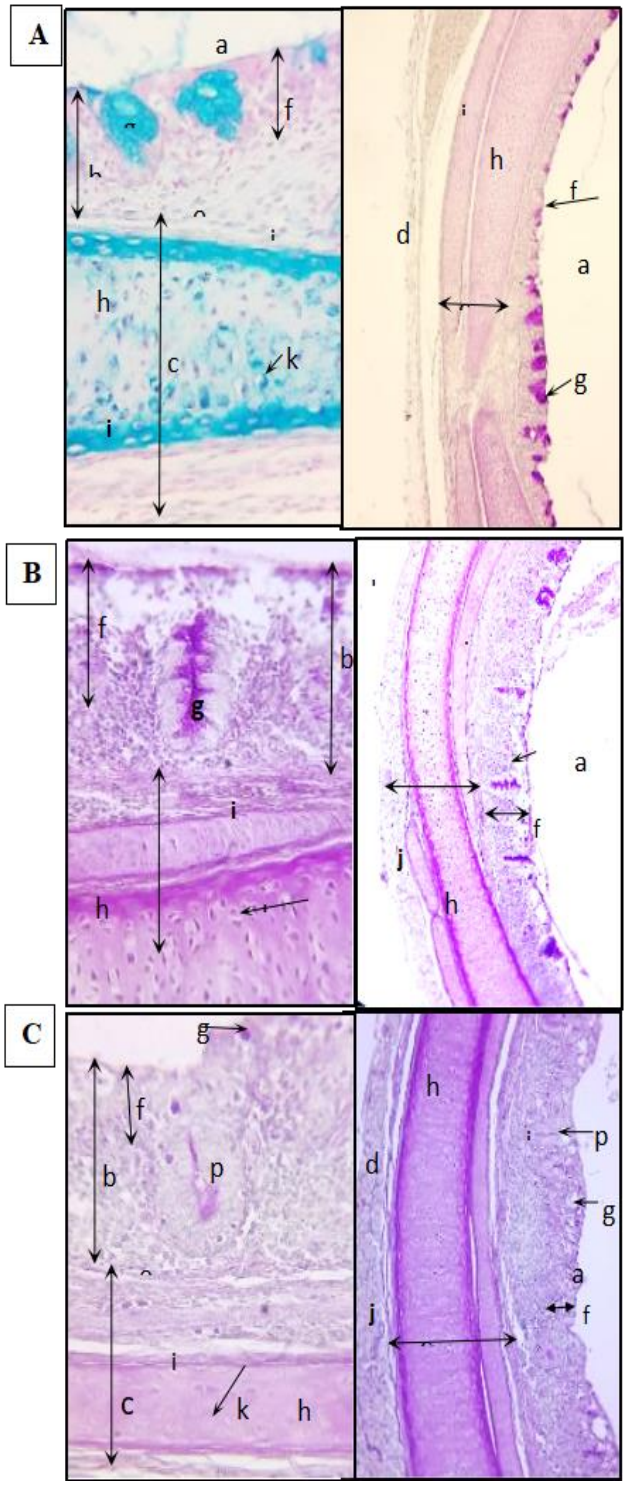

Fig. 8. Transverse histological sections of the trachea of broiler chickens at different ages (PAS stain). Panel A (7 days of age): Transverse section of the trachea. Tracheal lumen (a); mucosa (b); submucosa (c); adventitia (d). Lamina propria (e) supports a pseudostratified ciliated columnar epithelium (f) with few PAS-positive goblet cells (g). Hyaline cartilage (h) and perichondrium (i) with chondrocytes in lacunae (k) indicating the rudimentary structure of the immature trachea. Panel B (21 days of age): Transverse section of the trachea. Tracheal lumen (a); mucosa (b); submucosa (c); and adventitia (d). Lamina propria (e) supports a thickened pseudostratified ciliated columnar epithelium (f) with more PAS-positive goblet cells (g) than in 7d. Hyaline cartilage (h) and perichondrium (i) with chondrocytes within lacunae (k) demonstrating active maturation of the trachea as well as the thickening of the tracheal mucosal barrier. Panel C (days post hatch 45): Cross section of the trachea showing the tracheal lumen (a), mucosa (b), submucosa (c), and adventitia (d). A matured pseudostratified ciliated columnar epithelium (f) with a well distributed lamina propria (e) and preserved PAS positive goblet cells (g) was recorded. Full development of the hyaline cartilage (h), perichondrium (i) and chondrocytes in lacunae (k) confirm the structural stabilization of the tracheal wall. PAS stain; 100× and 400× magnification.

Presence of Acidic Mucins in Tracheal Tissue (Alcian Blue Staining)

Alcian Blue Staining confirmed the presence of acid mucins within the trachea epithelium at all ages examined, however, the presence of mucins was at a much lower level than the neutral mucins. Alcian Blue positivity at 7 days was minimal and confined to a few goblet cells.

At 21 days, an increase in Alcian Blue positive cells was observed, which may indicate an increase in acidic mucin production, and a more mature mucosa. Although the 21-day Alcian Blue staining was not as extensive as that of the PAS stain, it further endorsed the hypothesis that neutral mucins are in greater abundance in the trachea as compared to acid mucins (Fig. 9).

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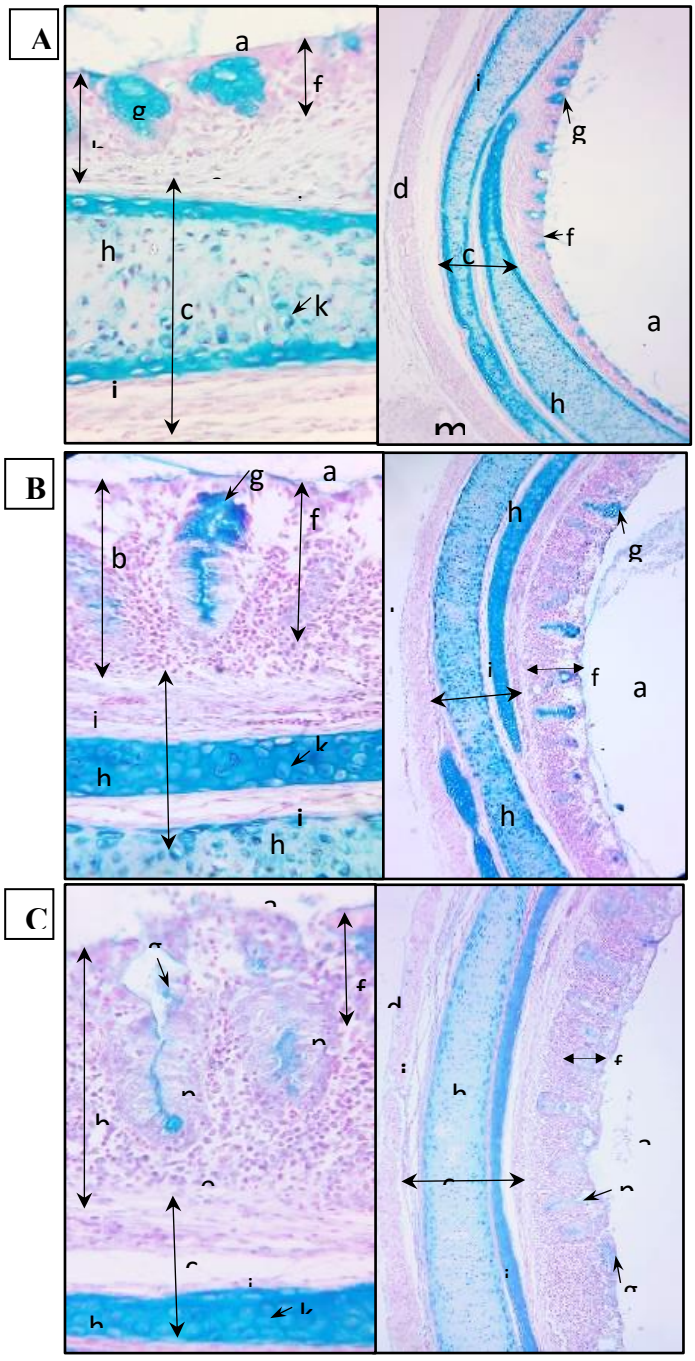

Fig. 9. Transverse histological sections of trachea of broiler chickens at various ages (Alcian blue stain). Panel A (Age 7 days): Transverse section of the trachea that illustrates tracheal lumen (a), mucosa (b), submucosa (c), and adventitia (d). Lamina propria (e) underlies a pseudostratified ciliated columnar epithelium (f) with Alcian blue positive goblet cells (g). Hyaline cartilage (h) with chondrocytes in lacunae (k) and perichondrium (i) are noted. Blood vessels (m) are located in the submucosal layer, which shows the early stages of vascular development in the tracheal wall. Panel B (Age 21 days): Transverse section of the trachea illustrating tracheal lumen (a), mucosa (b), submucosa (c), and adventitia (d). Lamina propria (e) underlies a pseudostratified ciliated columnar epithelium (f) which is considerably thickened with a greater number of Alcian blue positive goblet cells (g). Hyaline cartilage (h), perichondrium (i), and chondrocytes in lacunae (k) reflects maturation of trachea, both in mucosal and cartilaginous components. Panel C (Age 45 days): Transverse section of the trachea illustrating the tracheal lumen (a), mucosa (b), submucosa (c), and adventitia (d). A well-structured lamina propria (e) underlies a fully mature pseudostratified ciliated columnar epithelium (f) with Alcian blue positive goblet cells (g) that are fully preserved. Hyaline cartilage (h), perichondrium (i), and chondrocytes in lacunae (k) show fully developed tracheal components and structural stabilization of the tracheal wall. Alcian blue staining at 100× and 400× magnification.

Discussion

The predominant role of MUC1 in the present study coincides with the increasing recognition of membrane-associated mucins as not merely structural components, but as modulators of epithelial signaling and immune response. Immune and pathogen-associated stress in the epithelium has been shown to tightly regulate the expression of epithelial defense genes in the trachea transcriptomics during viral and bacterial infections (O'Dowd et al., 2024; Kamathewatta et al., 2024). MUC1 increased at 21 days of age showing normal development in healthy birds versus a disease-related change, and provides a measure to assess future disease changes. However since MUC4 remains constant throughout all ages MUC4 likely is mainly responsible for providing structure. These findings are consistent with earlier work demonstrating both MUC1 and MUC4 are present

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and can be measured in the respiratory tracts of other avian species such as Iraqi common quail (Al-Baghdadi and Al-Mamoori 2023). Therefore MUC1 and MUC4 likely serve conserved and distinct mucosal defense mechanisms and provide structural support in different species of poultry.

The histological data of the present study is corroborated by the molecular data in a similar manner. The increases in epithelial thickness, goblet cell hyperplasia, and mucin secretion-which is especially marked at 21 days, indicates synchronized epithelial differentiation and secretory cell maturation. These structural changes mirror Faldynova et al. (2024) reporting the rapid post-hatch development of the tracheal mucosa and its changes due to the microbes and environment. It has been documented that early exposure to the diverse gut microbiome significantly shapes the immune response and function of the respiratory epithelium, and the increased mucin production in this study may be a respiratory adaptive response to the colonization by microbes.

The findings from PAS and Alcian blue staining affirm that neutral mucins are predominant in the broiler trachea and suggest that tracheal lubrication and barrier function in broilers occurs primarily through neutral mucins under standard physiological conditions. Acidic mucins present in the broiler trachea appeared to function in a subordinate role. Considering the evidence that a variety of infectious agents can disrupt the mucosal barrier and change mucin composition (Wang et al., 2024; Wang et al., 2024), it is plausible that this balance may change. The unchanging profile of acidic mucins further validates the non-pathological state of the birds, establishing these findings as useful baseline reference data.

Some recent studies have highlighted the tracheal mucosa as a key location for vaccine-induced immunity for certain respiratory viruses, such as the infectious bronchitis virus, infectious laryngotracheitis virus, and avian influenza viruses (Cuadrado et al., 2024; Mumu et al., 2026; Tabynov et al., 2025). The tracheal mucosa is a key location for vaccine-induced immunity for certain respiratory viruses; recent studies have highlighted the importance of the tracheal mucosa as a key location for vaccine-induced immunity. Mucosal immunity involves the epithelial barrier, which can regulate antigen presentation and immune response signaling. Therefore, the increase in the expression of mucins and the maturation of the epithelium observed at 21 days may indicate a time period for optimal strategies for mucosal immunization, especially given that several studies support the premise that there are heightened immune responses as a result of the delivery of vaccines targeted to the trachea or mucosal sites (Tran et al., 2025).

The role of microbiota, mucins, and immune modulation interplay in the maintenance of the respiratory system has garnered the attention of several researchers. The modulation of epithelial signaling pathways and mucin dynamics has shown the influence of probiotics in restoring the function of mucosal barriers and reducing inflammation of the respiratory tract (Wang et al., 2024). Nutritional interventions of the type, including phytochemicals that improve the respiratory Th2 response, have also been shown to restore the epithelial barriers (Wang et al., 2024). The study provides an age-dependent mucin profile, which serves as an essential framework to interpret interventions of various types and to determine the best time for their use.

Conclusion

The researchers found that there is an increase in expression of the mucus protein MUC1 in the tracheal mucosa of broiler chickens with age, reaching maximum levels at approximately 21 days old. In contrast, MUC4 expression remained relatively constant in each case, indicating a consistent regulatory relationship between the two. In addition to these molecular and histological changes, there is evidence that the respiratory epithelium of broiler chickens undergoes important changes as they mature to respond to environmental and immunological challenges during the growth phase. Establishing these basic cellular and molecular representatives provides important information regarding the

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functional integrity of the respiratory system. In addition to providing more information about how to assess the risk of developing respiratory infections and the effectiveness of vaccination efforts, these findings will also assist in improving poultry production systems.

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