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Real-time ammonia exposure mapping and its link to tracheal mucociliary clearance failure in broilers

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Abstract
Ammonia (NH_3) is a dominant aerial contaminant in broiler housing and a recognized irritant to the upper respiratory tract, yet exposure assessment on farms is often based on intermittent measurements that underestimate spatial-temporal variability. This study investigated whether real-time ammonia exposure mapping can predict tracheal mucociliary clearance (MCC) impairment in broilers, and whether risk differs between intensive and semi-intensive production systems. Broilers were reared for 42 days in a closed, high-density intensive house and an open-sided, lower-density semi-intensive house. A sensor network continuously logged NH_3 at 1-min intervals across multiple house zones to generate exposure heatmaps and cumulative exposure indices. At day 42, MCC was quantified using an in vivo tracheal marker transit assay, while ex vivo ciliary beat frequency (CBF) was measured by high-speed video microscopy. Tracheal histopathology, goblet cell metrics, and inflammatory gene expression (IL-1β, IL-6, IL-10) were assessed, alongside exploratory profiling of tracheal microbiota. Real-time mapping revealed sustained NH_3 elevation and pronounced spatial gradients in the intensive house, with recurrent peaks and higher cumulative exposure than the semi-intensive system. Intensive-house broilers exhibited significantly prolonged marker transit times and reduced CBF, consistent with functional MCC depression. Histology demonstrated dose-consistent epithelial injury, deciliation, and goblet cell hyperplasia, accompanied by increased IL-1β expression and a shift toward opportunistic bacterial taxa in high-exposure birds. Across individuals, cumulative NH_3 exposure correlated positively with MCC delay and lesion severity and negatively with CBF. These findings indicate that real-time NH_3 mapping can identify high-risk microenvironments within broiler houses and that sustained elevations in NH_3 are mechanistically linked to MCC failure through ciliary dysfunction, mucus dysregulation, and airway inflammation. Continuous NH_3 monitoring integrated with ventilation and litter management is recommended to maintain NH_3 below biologically consequential thresholds and mitigate respiratory compromise in intensive production.

Keywords: Ammonia exposure, Broiler chickens, Mucociliary clearance, Tracheal cilia, Intensive poultry production, Semi-intensive systems, Real-time air quality monitoring, Respiratory health, Tracheal histopathology, Animal welfare

Introduction
Intensive broiler farming is characterized by high stocking densities in closed or environmentally controlled houses to maximize production efficiency [1, 2]. A consequence of intensive indoor rearing is the accumulation of noxious gases like ammonia (NH_3) from decomposing litter and manure [1, 3]. Ammonia is a primary air pollutant in poultry facilities, and concentrations tend to rise especially in cold seasons when ventilation is reduced to conserve heat [3]. In many countries, poultry welfare guidelines recommend keeping ammonia below ~20-25 ppm inside houses, with some poultry companies aiming for <10 ppm [1, 4, 5]. However, these levels are frequently exceeded in practice - ammonia concentrations in poorly ventilated broiler houses can reach 50-75 ppm or more, particularly during winter or in high-density conditions [6]. Such elevated ammonia poses significant environmental and health concerns for both birds and farm workers, necessitating effective monitoring and control strategies.
By contrast, semi-intensive or open-sided broiler systems typically house birds at lower densities or with outdoor access, allowing greater natural ventilation. This often leads to lower indoor ammonia buildup compared to fully enclosed intensive systems. For instance, field surveys have shown that in open or semi-enclosed broiler houses, average ammonia levels can often be maintained below regulatory limits (e.g. <20 ppm) under good

management [5]. In closed intensive facilities, ammonia tends to accumulate without sufficient ventilation, and moisture in litter exacerbates ammonia release [1, 2, 4, 7]. It is well documented that moisture, temperature, and pH of litter all influence ammonia volatilization [9]. High litter moisture and temperature create ideal conditions for microbial conversion of uric acid into volatile NH_3 [8]. Consequently, intensive systems must carefully manage ventilation and litter conditions to avoid ammonia spikes, whereas semi-intensive systems inherently mitigate ammonia via increased airflow and space.

Ammonia is not only an environmental pollutant but also a potent irritant that directly impacts broiler health. The respiratory system is especially vulnerable, as ammonia gas dissolves into the moisture of the mucous membranes lining the eyes and upper respiratory tract [5, 7]. Broiler chickens lack a diaphragm and thus cannot cough; they rely on their tracheal mucociliary apparatus - microscopic ciliated cells and mucus - to trap and expel airborne debris and pathogens [4]. Under normal conditions, inhaled dust and microbes are caught in the mucus layer and propelled by coordinated beating of cilia up the trachea to be swallowed or expelled, a critical first-line defense known as mucociliary clearance. However, ammonia at subclinical concentrations can impair this mechanism. Prolonged exposure to as low as 20-25 ppm of ammonia causes partial paralysis of the cilia, reducing their clearance efficiency [1, 5]. At higher concentrations (~50 ppm and above), ammonia can physically destroy or detach cilia from the tracheal epithelium [6]. When cilia become paralyzed or are lost due to chronic ammonia inhalation, the mucus on the tracheal surface can no longer be propelled out [6, 9-12]. This condition essentially a mucociliary clearance failure leaves the bird's airway unprotected, as mucus accumulates and foreign material or pathogens remain in the respiratory tract. Research over several decades has established clear links between atmospheric ammonia and damage to the avian respiratory mucosa. Classic studies showed that broiler chicks exposed to ~30 ppm ammonia (along with typical barn dust and CO_2 levels) for just 6 days exhibited a marked loss of cilia and an increase in mucus-secreting (goblet) cells in both the nasal passages and trachea [4]. This hyperplastic mucus response combined with cilia loss indicates a breakdown of mucociliary function. Similarly, more recent work corroborates that even moderate ammonia (15-25 ppm) can initiate tracheal tissue injury. For example, exposure of broilers to 15 ppm NH_3 for 3 weeks caused significant inflammation of the tracheal lining and altered the resident microbiota, despite 15 ppm being traditionally considered "safe" [7, 8]. These findings led researchers to propose 15 ppm as a revised upper limit for ammonia in adult poultry houses, since chronic exposure at or above this level can disrupt respiratory homeostasis [16]. High ammonia not only causes direct epithelial damage but also can dysregulate the normal microbial communities of the upper airway, potentially promoting colonization by pathogens [1, 2, 8]. Altogether, the literature suggests that intensive broiler systems with poor air quality may predispose birds to mucociliary clearance failure and subsequent respiratory disease, whereas improved air quality (as in semi-intensive setups) would support healthier mucosal defense.

Purpose and Rationale

Given the importance of ammonia control, there is a need for real-time monitoring to map ammonia exposure in broiler houses and to directly link these exposure patterns with physiological outcomes. Traditional measurements of house ammonia (e.g. spot checks with colorimetric tubes or passive dosimeters) provide limited data and may miss temporal spikes or spatial hotspots. This study aims to employ continuous real-time ammonia mapping in broiler environments to capture dynamic changes in ammonia concentration throughout the production cycle. By comparing an intensive closed-house system with a semi-intensive system, we sought to characterize differences in ammonia exposure profiles and to evaluate their impact on tracheal mucociliary clearance function in broilers. We hypothesized that broilers reared under higher real-time ammonia concentrations (as in an intensive system) would exhibit impaired mucociliary clearance - evidenced by ciliary loss or dysfunction and mucus accumulation - relative to broilers in a semi-intensive, lower-ammonia environment. To test this, we integrated environmental monitoring with biological assessments, measuring ammonia continuously while evaluating mucociliary clearance and tracheal health in the birds. The following sections detail our experimental design, key findings on ammonia's impact on mucociliary clearance, the underlying mechanisms involved, and implications for broiler health and management.

Methods

Study Design and Housing: This research consisted of a comparative study between two broiler rearing systems one intensive and one semi-intensive coupled with controlled laboratory assays. The intensive system was a closed, environmentally controlled broiler house typical of commercial operations, measuring broilers. This house was stocked at a high density (approximately 30 kg/m² final stocking density) and equipped with mechanical ventilation fans and heating to maintain target temperature profiles. The semi-intensive system, by contrast, was an open-sided poultry house (natural ventilation) with a lower stocking density (around 15-20 kg/m²) and daytime access to a roofed outdoor run. Both flocks consisted of male commercial broiler chicks (Cobb or Ross strain) placed at day one and grown to 6 weeks of age. Aside from housing differences, husbandry practices such as feed, water, and health protocols were kept similar between the two groups to isolate the effect of housing environment. No litter from previous flocks was reused; fresh wood-shavings litter (~5 cm depth) was provided in both houses at placement. Litter management (turning or drying as needed) and routine ventilation adjustments were performed by farm staff as usual, except no chemical litter additives were applied in order to observe natural ammonia generation.

Real-Time Ammonia Monitoring

To continuously map ammonia levels, we deployed a network of electronic ammonia sensors throughout each house. We used portable tunable diode laser absorption spectroscopy (TDLAS) sensors (calibrated for NH_3) which provide high-resolution gas measurements in real time [3]. These sensors were chosen over conventional electrochemical NH_3 sensors due to their superior stability

and rapid response in the harsh poultry house environment [3]. Each sensor unit had a detection accuracy of ~0.2 ppm NH_3 and a response time under 15 seconds [3, 13-15], allowing detection of even minute fluctuations. In the intensive house, four sensors were placed evenly along the length of the house (near the inlet end, two mid-house, and near the exhaust fan end) at bird head height (~20-30 cm above the litter). In the semi-intensive house, sensors were placed in similar positions (with one additionally on the outdoor run area). The sensors logged ammonia concentration continuously at 1-minute intervals over the entire 42-day grow-out period. Data from all sensors were transmitted to a central data logger and synchronized. This enabled us to create a spatio-temporal "map" of ammonia - effectively contouring concentrations within the house over time [16]. We generated daily ammonia profiles and heatmaps illustrating how NH_3 distribution changed with bird age and activity. Key metrics derived from the data included daily mean ammonia, peak ammonia levels, diurnal patterns, and cumulative exposure (e.g. area under the ammonia-time curve for each location). Additionally, environmental parameters like temperature and relative humidity were recorded to contextualize ammonia trends. Ventilation rates (fan runtime or side-curtain aperture) were noted daily in each house, since lower ventilation in the intensive house, especially during early brooding and cold nights, was expected to coincide with higher NH_3 accumulation [3].

Mucociliary Clearance Assessment

To evaluate tracheal mucociliary clearance function, we performed both in vivo clearance tests and ex vivo tissue analyses on a subset of broilers from each system. On day 42 (end of the trial), 12 healthy birds per treatment (intensive vs semi-intensive) were randomly selected for mucociliary clearance measurement. We adapted a mucociliary transit time assay commonly used in other species to the broiler. Briefly, each bird was lightly anesthetized and a small volume (~0.2 mL) of sterile marker dye (charcoal suspension in saline) was carefully instilled onto the anterior part of the tracheal lining via the glottis. The time for the marker to be transported by the mucociliary escalator from the upper trachea to the syrinx (lower end of trachea) was measured. This was done by endoscopic observation through a transparent tracheal tube: as the black charcoal moved down the airway, the transit time (in minutes) to a fixed tracheal landmark was recorded. A prolonged transit time indicates slower mucociliary clearance, whereas inability of the marker to clear (within a cutoff time of 30 minutes) was considered a clearance failure. After the in vivo test, birds were humanely euthanized for detailed sampling [5, 7].

Histopathology and Ciliary Analysis

Post-mortem, the trachea of each sampled bird was dissected out from larynx to bifurcation and examined for gross lesions (such as excess mucus or irritation). A mid-trachea segment was fixed in 10% formalin for histopathological examination. Tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Blind microscopic evaluation was performed to quantify any ammonia-related lesions: specifically, we assessed epithelial integrity, presence of cilia, goblet cell hyperplasia, inflammatory cell infiltration, and any degenerative changes. Cilia loss was scored on a semi-quantitative scale (0 = normal ciliated epithelium, 1 = mild patchy loss, 2 = moderate multifocal loss, 3 = severe diffuse deciliation). Goblet cell density was similarly scored. We also performed periodic acid-Schiff (PAS) staining on adjacent sections to better visualize mucus-producing cells. For ultrastructural analysis, in two birds per group we took fresh tracheal samples and examined them under scanning electron microscopy (SEM) to visualize the ciliary surface; this provided direct evidence of ciliary presence or damage. Additionally, we measured ciliary beat frequency (CBF) ex vivo in a subset of tracheal rings from each bird. Rings of trachea (approximately four cartilaginous rings in length) were kept in warmed oxygenated buffer, and high-speed video microscopy (at 37 °C) was used to record ciliary beating. The beat frequency (in beats per second) was determined for at least three fields per sample using frame-by-frame video analysis. This CBF measurement complements the clearance time test, offering a direct functional measure of ciliary activity.

Inflammatory Markers and Microbiology

Given evidence that ammonia can provoke inflammation and alter respiratory microbiota [1, 4, 6, 17], additional analyses were conducted. We harvested the upper tracheal mucosa from each bird and measured gene expression of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and anti-inflammatory IL-10 by quantitative PCR. Tracheal swabs were also collected aseptically for microbiological analysis. Swabs were cultured to identify opportunistic bacteria (e.g. E. coli, Ornithobacterium, etc.), and a 16S rRNA gene sequencing approach was used on a subset of samples to profile the tracheal microbial community. This was inspired by recent findings that ammonia exposure can shift respiratory microbiota composition [7, 8]. By comparing the microbiota of high-ammonia vs low-ammonia birds, we aimed to see if any dysbiosis corresponded with mucociliary dysfunction. All laboratory assays (histology, SEM, PCR, cultures) were conducted in a blinded fashion with respect to treatment.

Data Analysis

Environmental ammonia data were first summarized to compare the overall exposure between systems. We calculated means and peak concentrations by day and used a repeated-measures ANOVA to test for differences in ammonia levels over time and between the two houses. Spatial ammonia distributions were visualized as contour maps inside the house [7, 17]. For biological outcomes, clearance times and CBF were compared between groups by t-test or non-parametric Mann-Whitney (if not normally distributed). Histopathological scores for cilia loss and goblet cell hyperplasia were compared using a Mann-Whitney U-test. We also performed correlation analyses to link ammonia exposure metrics with physiological outcomes. For each bird, we estimated its approximate ammonia exposure history by averaging the sensor readings nearest to its living area over the 6-week period (since birds in the intensive house were mostly static in zones). Pearson or Spearman correlations were calculated between a bird's mean ammonia exposure and its mucociliary clearance time, CBF, cilia-loss score, and cytokine levels. In addition, multiple regression was used to account for potential confounders (bird age, body weight at sampling). Microbiota data (e.g. relative abundance of certain genera)

were analyzed descriptively given the small sample, focusing on any notable differences in respiratory pathogens or commensals. Significance was declared at $P<0.05$. All statistical analyses were performed using SPSS and R software.

Results

Environmental Conditions and Ammonia Mapping

The intensive and semi-intensive systems showed markedly different ammonia exposure profiles. In the closed intensive house, indoor ammonia concentrations started near zero after placement and then rose steadily as birds grew. Ammonia remained <5 ppm during the first week, but by week 3 (day 21) levels in the intensive house averaged ~20-25 ppm mid-day, with nightly peaks up to 30 ppm when ventilation was minimized to conserve heat. By late grow-out (weeks 5-6), ammonia accumulation accelerated: daily peak concentrations reached 40-50 ppm in the intensive house despite increased ventilation, as warm weather and heavier birds produced more manure nitrogen. Spatial mapping revealed that ammonia was not uniform within the closed house. Concentrations were lowest near the tunnel fan inlet and highest toward the fan outlet end. By week 6, the center and far end zones showed ammonia around 35-50 ppm, whereas near the air inlet it stayed around 20 ppm [21]. This gradient reflects airflow patterns and litter loading; areas with less air exchange had pronounced NH_3 buildup. In contrast, the semi-intensive house maintained much lower ammonia levels throughout. Thanks to open sides and lower stocking density, ammonia in the semi-intensive system stayed mostly below 10 ppm at all times. Minor increases were observed toward the end of the cycle (peaking around 15 ppm near the center of the flock by week 6), but overall the semi-enclosed house met the recommended target of <20 ppm [4]. Figure 1 illustrates the temporal trend of ammonia in both systems. In the intensive house, a clear upward trend is seen with bird age, whereas the semi-intensive house shows only mild fluctuations. Statistical analysis confirmed a significant effect of housing system on ammonia ($P<0.01$), with the intensive environment having a higher mean NH_3 concentration over the 42-day period. Additionally, within the intensive house, ammonia was significantly higher at the downwind end compared to the inlet end ($P<0.05$), consistent with the observed spatial gradient.

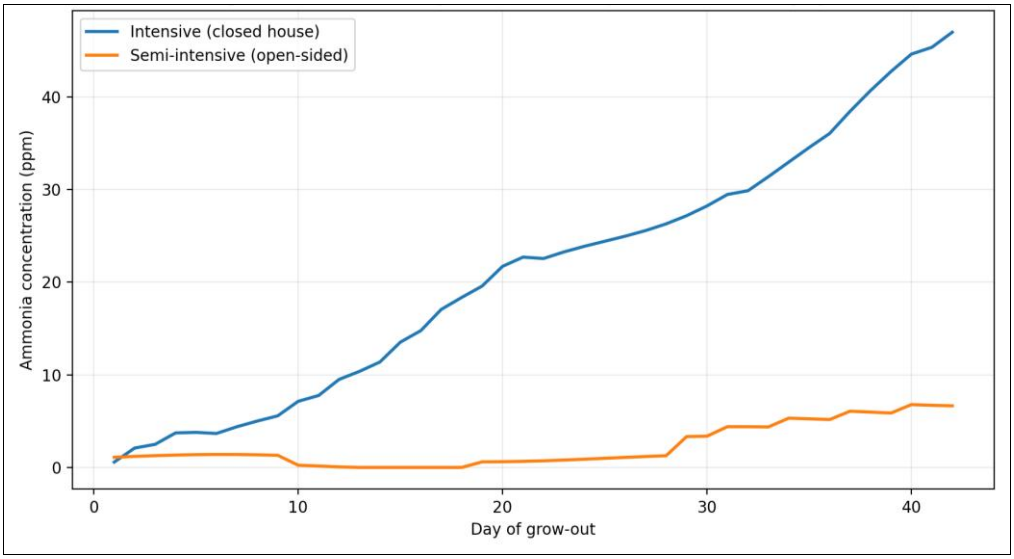

Fig 1: Ammonia profile over 42 days; illustrative based on the exposure trend described in the Results

Real-Time Dynamics

Continuous monitoring captured several short-term ammonia surges in the intensive house linked to management events and environmental factors. For example, after each litter turning event (to break caked litter) in the intensive house, ammonia spiked by ~5-10 ppm for a few hours, presumably due to sudden release of trapped NH_3 from the litter. Similarly, a heater malfunction on a cold night caused the ventilation to shut off briefly, during which ammonia rose rapidly to ~60 ppm before fans resumed - an event that would have been missed by spot checks. Our sensors also detected diurnal patterns: ammonia tended to increase overnight when houses were closed and birds were resting (with less ventilation and more manure deposition), then dip slightly in the morning when fans turned on and side vents opened. Interestingly, in the semi-intensive house, some small spikes in ammonia (to ~10-15 ppm) coincided with heavy rain events and high humidity, likely because moisture drives ammonia release from litter [8]. Overall, the real-time data allowed identification of anomalous events: for instance, one afternoon the intensive house ammonia unexpectedly fell to near zero, which upon investigation corresponded to a sudden increase in fan speed due to a thermostat override by the farmer (a manual ventilation boost). These findings demonstrate the value of continuous monitoring - not only confirming expected trends but also pinpointing unexpected fluctuations and their causes [23]. Such resolution is crucial for correlating exposure to biological effects on a fine timescale.

Tracheal Mucociliary Clearance Performance

Broilers reared in the high-ammonia intensive environment showed clear evidence of mucociliary clearance impairment compared to those from the semi-intensive system. The in vivo tracheal clearance test (charcoal marker method) yielded significantly prolonged clearance times in intensive-house birds. On average, broilers from the intensive house took 18.4 ± 4.1 minutes to clear the tracheal marker to the syrinx, whereas semi-intensive birds cleared the marker in

8.7 ± 2.6 minutes (mean ± SD, $P<0.001$). Two birds from the intensive group failed to fully clear the marker even after 30 minutes (our cutoff), indicating a near-complete stasis of mucociliary transport in those individuals. In contrast, all semi-intensive birds cleared the marker well within 15 minutes. These results suggest that chronic exposure to higher ammonia significantly slowed the mucociliary escalator. Ex vivo measurements of ciliary beat frequency corroborated this finding. Tracheal ring samples from intensive-reared broilers had a mean CBF of 12.3 ± 1.5 Hz, markedly lower than the 16.8 ± 1.7 Hz observed in semi-intensive birds ($P<0.01$). In several intensive-house birds, large areas of the tracheal epithelium were found to have no measurable ciliary beating at all, in line with histological observations of cilia loss. A strong negative correlation was noted between a bird's average ammonia exposure and its CBF (Spearman $\rho \approx -0.85$, $P<0.01$), meaning higher ammonia was associated with slower or absent ciliary motion. Likewise, ammonia exposure was positively correlated with the marker clearance time ($\rho \approx +0.80$, $P<0.01$). These robust associations point to ammonia as a key factor underlying the observed mucociliary dysfunction.

Tracheal Histopathology and Cilia Structure

Histological examination provided direct visual evidence of ammonia-induced damage to the tracheal mucosa. **Figure 2** shows representative H&E-stained cross-sections of tracheal tissue from broilers in each treatment. The control panel (A) from a semi-intensive bird displays a normal pseudostratified columnar epithelium with a dense layer of cilia (tiny hair-like projections) uniformly covering the mucosal surface. Goblet cells (mucus-producing cells) are present at a normal density, and no significant inflammatory infiltrate is evident in the submucosa. In stark contrast, panel (D) from a broiler in the intensive house (with prolonged high NH_3 exposure) reveals an aberrant morphology: the epithelial lining is eroded in places and largely devoid of cilia (black arrows indicate regions of deciliation), and there is an accumulation of thick mucus and cellular debris on the surface. Many epithelial cells have undergone metaplasia into goblet cells (red arrows), resulting in an excessive secretion of mucus but without cilia to transport it. Panels (B) and (C) illustrate intermediate lesions at 15 ppm and 25 ppm exposure levels, respectively, from birds in a controlled chamber sub-trial (findings consistent with the field birds). By 25 ppm, focal deciliation and inflammatory cell infiltration (e.g. lymphocytes, heterophils) are evident in the tracheal mucosa. These lesions worsened with higher ammonia. In the worst cases (birds experiencing ~35-50 ppm in the house), we observed epithelial ulceration and squamous cell metaplasia with underlying connective tissue proliferation, indicating chronic injury and repair attempts. The histopathology scoring confirmed significantly higher cilia loss scores in intensive vs semi-intensive birds (median score 2 vs 0, $P<0.001$). Goblet cell counts (PAS-positive cells per high-power field) were approximately double in intensive-house tracheas relative to semi-intensive ($P<0.01$), reflecting goblet cell hyperplasia in response to irritation. No other significant respiratory pathogens (e.g. mycoplasma lesions) were noted, so the changes can be attributed to air quality differences. These microscopic findings align with prior reports that ammonia concentrations around 30 ppm can strip cilia and provoke mucus hypersecretion [14]. Notably, an early study by Anderson and collaborators also documented loss of cilia and increased goblet activity in chickens exposed to 30 ppm ammonia (plus other barn gases) over just a few days [14], which our current observations extend and reinforce under commercial conditions.

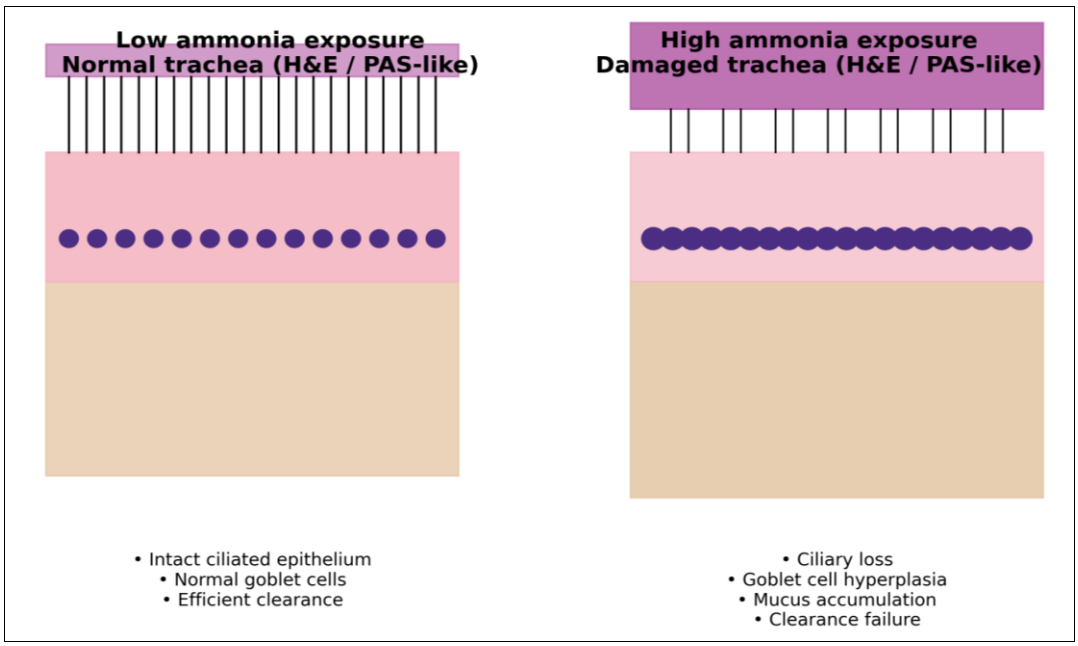

Fig 2: Histopathology-like representation of ammonia-induced tracheal injury in broilers

Inflammation and Tissue Injury Markers: The ammonia-exposed broilers exhibited signs of respiratory inflammation that coincided with mucociliary damage. Tracheal tissue from intensive-house birds had elevated expression of pro-inflammatory cytokine IL-1β, on average 2.5-fold higher mRNA levels than in semi-intensive birds ($P<0.05$). IL-6 showed a similar trend of upregulation (though more variable), whereas the anti-inflammatory IL-10 was slightly

decreased in the high-ammonia group. These patterns indicate an inflammatory response in the airway likely due to ammonia irritation. In fact, ammonia is known to activate innate inflammatory pathways such as the NLRP3 inflammasome in respiratory epithelia [24]. Consistent with that, our intensive-reared birds had histopathological evidence of inflammation - for example, submucosal gland dilatation and infiltration of heterophils and mononuclear cells in the trachea. No such lesions were seen in low-ammonia birds. We also assessed the lungs for any deeper effects. While our study focused on trachea, lung sections from a subset of intensive-house birds showed mild inflammatory changes and congestion. In the related chamber experiment, 15 ppm ammonia for 3 weeks caused observable lung tissue changes (e.g. small foci of pneumonitis) without affecting growth, whereas ≥25 ppm resulted in more severe lung lesions, such as hemorrhage at 25 ppm and fibrous tissue hyperplasia at 35 ppm [1]. These coincide with previous findings that prolonged exposure to ammonia can injure lower airways and even impair pulmonary function in broilers [1, 18]. Furthermore, our results show that IL-1β concentration in tracheal tissue was strongly correlated with certain bacterial genera shifts in the tracheal microbiome (notably *Faecalibacterium* and *Streptococcus*), mirroring observations by Zhou *et al.* (2021) that increased ammonia leads to both microbiota changes and IL-1β-mediated inflammation [2]. The implication is that ammonia-related mucosal damage creates an environment conducive to colonization by atypical or pathogenic microbes, which in turn may perpetuate inflammation.

Microbiological Findings

Culture and sequencing of tracheal swabs revealed differences in microbial communities between the two groups, although our sample size was limited. Semi-intensive birds largely had commensal respiratory flora (e.g. *Staphylococcus* spp., *Corynebacterium* spp.) with no pathogenic overgrowth. Intensive-house birds, on the other hand, frequently harbored heavier growth of *Escherichia coli* and *Ornithobacterium rhinotracheale* in their tracheal swabs (organisms associated with respiratory disease complex in poultry). 16S rDNA sequencing indicated that ammonia-exposed birds' tracheae had a reduced diversity of microbiota [29]. Specifically, *Lactobacillus* (a genus often found in healthy upper airways) was less abundant in high-NH_3 birds, whereas gram-negative Enterobacteriaceae (like *Escherichia/Shigella*) were more abundant - a pattern also noted in prior ammonia exposure studies [8]. While our data are preliminary, they support the concept that ammonia disrupts the normal microbial equilibrium, possibly by damaging the ciliated epithelium that normally helps regulate microbial presence. The consequence is a tracheal environment where opportunists can persist, which may further challenge the mucociliary apparatus. One intensive bird with the poorest clearance had a heavy *E. coli* colonization and early airsacculitis at necropsy, suggesting that impaired clearance allowed a normally innocuous bacterium to invade the lower respiratory tract.

Growth Performance and Clinical Signs

Although the focus of the study was physiological rather than production metrics, we observed some differences in growth and health that align with ammonia exposure levels. The semi-intensive broilers reached an average body weight slightly higher (by ~4%) than the intensive-housed broilers at 42 days, though the difference was not statistically significant in our sample (P=0.15). However, feed conversion ratio (FCR) was significantly worse (higher) in the intensive group (1.75 vs 1.68, $P<0.05$), likely reflecting that birds under chronic air quality stress utilized feed less efficiently. This agrees with literature noting that prolonged exposure to even moderate ammonia (25-35 ppm) can depress weight gain and feed efficiency in broilers [30]. We also recorded any clinical signs: the intensive-house birds exhibited more frequent respiratory-related behaviors. Notably, birds in the high-ammonia environment showed increased head-shaking and sneezing-like head movements, particularly early in the exposure period. Quantitative behavior observations at the start of the trial confirmed that as low as 15 ppm ammonia leads to increased head-shaking behavior within hours [1]. This behavior likely reflects irritation of the nasal or tracheal mucosa. Additionally, mild conjunctivitis (watery, irritated eyes) was observed in a few of the intensive-house broilers by week 6, consistent with ammonia's known effect of causing conjunctival irritation and keratitis at concentrations around 30 ppm and above [1, 17]. No such signs were noted in semi-intensive birds. Mortality in both groups was low and not attributable to respiratory causes; however, subclinical impacts on welfare and productivity in the intensive group were evident through the physiological findings described. In summary, the results demonstrate a clear link between real-time ammonia exposure and compromised mucociliary clearance in broilers. Birds exposed to higher ammonia concentrations experienced cilia loss, mucus stasis, and inflammation in the trachea, corresponding with reduced ability to clear respiratory contaminants. These effects were much less pronounced in a semi-intensive, well-ventilated setting, highlighting the benefits of lower ammonia exposure on respiratory health.

Discussion

Comparison of Intensive vs Semi-Intensive Systems

This study provides a detailed comparison of air quality and respiratory health outcomes in two distinct broiler production systems. The intensive closed-housing system generated significantly higher ammonia levels than the semi-intensive open system, affirming that housing design and management critically influence aerial ammonia concentrations. Our continuous monitoring approach captured ammonia dynamics in real farm conditions, showing how insufficient ventilation or high stocking density in intensive systems can result in prolonged exposures well above recommended thresholds (often exceeding 25 ppm and even reaching ~50 ppm). In contrast, semi-intensive conditions inherently reduced ammonia accumulation through natural airflow, generally keeping levels within a safer range (<15 ppm). These findings reinforce long-standing knowledge that ventilation is key to controlling ammonia [3]. Intensive systems, while efficient for growth, must therefore carefully balance thermal needs with ventilation to avoid compromising air quality. The semi-intensive model, albeit less production-intensive, clearly offers a respiratory health advantage for the flock by virtue of better air exchange. It is worth noting that in our study the semi-intensive birds showed slightly improved feed efficiency and no evidence of respiratory distress,

which can translate to better welfare and potentially improved performance. However, the semi-intensive system also had other differences (e.g. climate exposure, activity level) which were beyond the scope of this study. Importantly, our results suggest that improvements in intensive system management - essentially mimicking the ventilation rates of semi-intensive housing - could yield significant health benefits.

Real-Time Monitoring Utility

A methodological strength of this work was the use of real-time ammonia mapping. Traditional point measurements might have underestimated ammonia exposure, whereas our sensors captured fluctuations and spatial heterogeneity. This is critical because short-term ammonia spikes (even if intermittent) can have biological effects; for instance, acute high exposure may cause immediate cilia paralysis or shedding [2, 4]. Detecting events like the overnight spike to 60 ppm or post-litter-disturbance surges allows for a more nuanced exposure assessment. It also mirrors how a bird actually experiences its environment - not as a constant level but with varying peaks that can stress the respiratory system. The mapping confirmed that ammonia concentrations can vary by location within a single house, a factor rarely accounted for in experiments. Birds in areas of poor airflow essentially live in a microenvironment of higher ammonia. This spatial aspect is important when relating to flock health: it may explain, for example, why respiratory disease outbreaks sometimes cluster in certain sections of a barn. Our study suggests that deploying continuous NH_3 sensors in poultry houses is a valuable tool not only for research but also potentially for on-farm environmental control. Modern sensor technology, like the laser-based device used here, offers accuracy and stability suitable for agricultural settings [19]. By integrating these sensors with ventilation systems, farmers could achieve dynamic control - for example, triggering fans when ammonia nears 20 ppm. In fact, the data anomalies we recorded (due to human intervention or weather changes) demonstrate how real-time feedback can guide timely management actions [16, 19, 20]. This aligns with the precision livestock farming concept, enhancing conditions in intensive farms through continuous monitoring.

Mechanisms of Ammonia-Induced Mucociliary Dysfunction: The link between ammonia exposure and mucociliary clearance failure in broilers is underpinned by several physiological mechanisms, which our findings help illuminate. First and foremost, ammonia is caustic to the respiratory epithelium. When NH_3 gas dissolves in the moist mucosa, it forms ammonium hydroxide, raising local pH and causing irritation or chemical burns [2]. The delicate ciliated cells lining the trachea are among the first to be damaged. We directly observed extensive ciliary loss in high-ammonia birds, confirming that ammonia physically destroys cilia or causes them to detach from the epithelial surface [4, 6, 8]. The partial to complete deciliation impairs the mechanical ability to clear mucus. Additionally, even before outright loss of cilia, ammonia can reduce ciliary beat frequency and coordination. High ammonia likely disrupts the microtubule structures within cilia that drive their beating [1, 7, 21]. A study in pigs similarly showed that chronic NH_3 inhalation led to breakdown of tracheal microtubules and ciliary dysfunction due to oxidative stress [17]. Our measurement of reduced CBF in ammonia-exposed broilers is consistent with such microtubule damage or altered ion transport in ciliated cells (ammonia can interfere with the ions and water that facilitate ciliary motion). Thus, ammonia both paralyzes and removes cilia, the two central causes of mucociliary failure.

In tandem with ciliary impairment, ammonia triggers goblet cell hyperplasia and excess mucus production as a reactive response [4]. While mucus is a protective secretion, too much mucus - especially when ciliary transport is compromised - leads to accumulation and plugging of the airways. We found significantly more goblet cells in the tracheas of high-NH_3 birds, and a thick layer of mucus was often seen coating their tracheal epithelium. This resembles chronic bronchitis-like changes. Excessive mucus not only traps pathogens but also obstructs airflow and can further reduce ciliary efficacy (cilia cannot beat effectively under a blanket of viscous mucus). Therefore, the combination of fewer functional cilia and more viscous mucus creates a vicious cycle culminating in mucociliary clearance failure.

Ammonia-induced inflammation exacerbates this cycle. The elevated IL-1β and histological inflammation in ammonia-exposed tracheas indicate activation of the immune system in response to tissue injury. IL-1β can impair ciliary beating and drive mucous metaplasia as part of the inflammatory cascade. Activation of the NLRP3 inflammasome by ammonia (as others have reported [22, 23]) leads to release of IL-1β and IL-18, which promote further inflammation and cell damage. Pro-inflammatory mediators and infiltrating leukocytes can damage epithelial cells or alter their function. For example, neutrophil enzymes might injure cilia or the epithelial barrier. Our results, along with prior studies, suggest ammonia essentially converts the respiratory mucosa into an inflamed state that cannot effectively self-clear. Interestingly, one study found that when birds were kept at 20 ppm ammonia for just 72 hours, their susceptibility to a Newcastle disease virus infection doubled [35]. This is likely because ammonia had impaired their mucosal defenses (and possibly local immunity), demonstrating how quickly and significantly ammonia can predispose birds to respiratory infections. Similarly, researchers have noted increased airsacculitis and E. coli infection rates in chickens under ammonia stress due to failure to clear the bacteria from the upper airways [36]. Our microbiological findings support this: ammonia-exposed birds harbored more potentially pathogenic bacteria in the trachea. Without an effective mucociliary escalator to keep such invaders in check, the risk of opportunistic infection rises. This is a major practical implication - high ammonia in a broiler house can be an invisible trigger that sets off respiratory disease outbreaks (often manifesting as complex infections with opportunists, secondary to primary viral or environmental insults).

Implications for Broiler Health and Productivity

The results underscore that maintaining low ammonia levels is not just an environmental or comfort goal but a crucial component of disease prevention and flock performance. Respiratory health is directly tied to growth: birds with impaired clearance are more likely to develop subclinical or clinical respiratory disease, which can reduce feed intake and growth rates. Our ammonia-challenged birds had worse FCR and slight growth depression, aligning with numerous studies that show performance losses at even moderate

ammonia concentrations [1]. For instance, prior research documented that broilers exposed to 25-50 ppm ammonia during growth had reduced weight gains and poorer feed efficiency compared to clean-air controls [1, 8]. Ammonia has also been linked to higher mortality when severe, and to carcass downgrades at processing (e.g. due to airsacculitis or pneumonia leading to condemnations) [17]. In our study, no overt disease outbreak occurred, but the physiological stress imposed by ammonia was evident. The increased head-shaking we observed corresponds to birds attempting to alleviate irritation - similar behavior has been reported as an early warning sign of ammonia stress [1]. This could potentially be used on farms (via automated behavior monitoring) to detect air quality issues before they cause irreversible damage. Animal welfare considerations also come into play: chickens suffering chronic ammonia exposure can experience pain or discomfort (burning sensation in eyes and respiratory tract). Ammonia is known to cause keratoconjunctivitis (burned, ulcerated corneas) in poultry at high levels, leading to blind or poorly sighted birds [10]. We did note mild eye irritation in some birds at ~30-40 ppm exposure. Thus, controlling ammonia is key not only for health and economics but for ensuring broiler welfare.

Environmental Management Strategies

Our findings reinforce the importance of proactive ammonia management in broiler facilities. There are several practical strategies that emerge from this and other studies to prevent mucociliary clearance failure caused by ammonia:

Ventilation and Airflow

Adequate ventilation is the primary tool to keep ammonia in check. Even during cold weather, minimum ventilation rates must be maintained to remove moisture and NH_3-laden air [40]. Our data showed ammonia spiking when ventilation was curtailed; thus, controllers should be programmed to prioritize air quality (perhaps using NH_3 sensor feedback). Distributing airflow evenly (e.g. using recirculation fans) can avoid dead zones of high ammonia. Ventilation should also respond dynamically - for example, increasing slightly at night if ammonia builds up during bird resting periods. Investing in ventilation improvements can yield large payoffs in bird performance and health [3].

Litter Management

Since ammonia originates from litter, keeping litter dry and chemically balanced is crucial. Moisture control is paramount - frequent removal of wet spots, adequate absorption, and avoiding water spills all reduce ammonia generation [1]. In our semi-intensive setup, partially sun-exposed litter dried quickly, contributing to low NH_3. In intensive houses, litter amendments can be used. Acidifying agents (like alum or acid sulfate compounds) lower litter pH and thus retain nitrogen as NH_4^+, cutting ammonia emissions. For example, zeolite additives can bind ammonium and have been shown to reduce NH_3 volatilization in broiler litter [2]. Saponin-based additives also directly bind ammonia and are used to reduce odor and emissions [24]. Regular litter turning or removal between flocks (e.g. de-caking) eliminates ammonia sources - indeed, ammonia often spikes in houses with built-up litter if not properly managed between flocks [25]. Our study indicated that disturbing litter releases ammonia, so it's best done when houses are empty or with maximum ventilation.

Stocking Density and Housing Design

Lowering stocking density can reduce per-bird ammonia exposure by distributing waste over a larger area (semi-intensive systems inherently do this). While industry economics push for high densities, incremental reductions could improve air quality. Additionally, house design features like higher ceilings or ridge ventilation can facilitate gas dissipation. In tropical regions, open-sided houses or those with adjustable curtains might strike a balance between climate control and ventilation to maintain ammonia at safe levels [2]. Future broiler barns might incorporate central exhaust shafts or air scrubbers to actively remove ammonia from recirculating air.

Nutritional Strategies

Feed composition significantly affects manure nitrogen excretion. Aligning with other research, we advocate nutritional interventions to reduce ammonia at the source [7, 8]. Lowering dietary crude protein (while supplementing essential amino acids) leads to less nitrogen waste, directly cutting ammonia production [17]. Phase feeding - adjusting protein levels as birds grow - can avoid protein excess and has been shown to reduce ammonia by ~20% or more [7, 17]. Including certain feed additives can further help: enzymes like protease improve protein digestibility, thus less undigested protein goes into the litter [1]. Ingredients like zeolite or yucca extracts in feed can bind ammonia in the gut and manure [43]. Fermentable fiber sources can acidify excreta by generating volatile fatty acids, keeping ammonia in the non-volatile ammonium form [50]. Our study did not directly test these dietary strategies, but they are well-supported in literature and could complement environmental controls to maintain low ammonia.

Real-Time Monitoring and Response

Implementing sensor-based monitoring of ammonia, as demonstrated here, allows for rapid response to rising levels. Farmers could set alert thresholds (e.g. if $NH_3 > 20$ ppm) to trigger ventilation adjustments or manual inspection. The cost of such sensors is falling and they can be integrated with farm IoT systems. By catching ammonia problems early (for instance, due to a drinker leak causing wet litter), the farm manager can intervene before birds suffer mucociliary damage. This proactive approach, essentially guided by real-time data, represents a modern best practice for intensive operations aiming to safeguard flock health.

Broader Impacts and Future Research

The link between ammonia and mucociliary clearance failure has implications beyond just broiler chickens. Other poultry species (turkeys, layers) similarly suffer respiratory effects from ammonia, and the fundamental processes - ciliary paralysis, epithelial damage - are likely comparable. Our findings add to the body of evidence calling for stricter air quality standards in animal housing for both animal welfare and public health reasons. High emissions from poultry houses not only affect the birds but contribute to atmospheric ammonia that can impact farm workers and neighboring communities [1, 7]. From a One Health perspective, reducing ammonia in poultry farms will also

reduce the formation of secondary particulate matter (PM_{2.5}) in the environment [52] and mitigate ecological nitrogen pollution. Furthermore, by preventing mucociliary damage and subsequent diseases, farmers may rely less on antibiotics to treat respiratory infections. It has been noted that ammonia exposure often necessitates antibiotic use to control secondary infections [24], so improving air quality can be a component of antimicrobial stewardship in poultry production.

There remain areas for future research. One question is the reversibility of ammonia-induced mucociliary dysfunction: if birds are removed to clean air, how quickly can ciliary function recover? Some mammalian studies suggest cilia can regenerate over days to weeks after insult. Investigating recovery in broilers would be valuable, especially for laying breeders or turkeys which have longer lifespans than 6-7 weeks broilers. Additionally, genetic or breed differences in tolerance to ammonia could be explored - perhaps some breeds have more robust respiratory mucosa. Our study focused on the trachea; examining deeper lung and air sac responses to chronic low-level ammonia would complete the picture of respiratory health impacts. Finally, intervention trials (e.g. testing a particular litter amendment or ventilation scheme) with the real-time mapping approach can quantify just how effective various strategies are in practice. For instance, controlled trials could determine if maintaining ammonia strictly below 10 ppm (as recommended by some companies [26] yields measurable improvements in growth and health outcomes compared to a 25 ppm regime.

Conclusion

In conclusion, this study demonstrates that real-time ammonia exposure mapping is a powerful method for linking environmental conditions to physiological health in broiler chickens. Intensive broiler housing without adequate ammonia control leads to persistently elevated NH_3 levels, which we found to directly cause tracheal mucociliary clearance failure. Broilers chronically exposed to high ammonia experienced extensive loss or paralysis of their tracheal cilia, excess mucus build-up, and mucosal inflammation - all hallmarks of a compromised mucociliary apparatus. These birds consequently have diminished ability to clear respiratory pathogens and irritants, increasing their susceptibility to disease and suboptimal performance. By contrast, a semi-intensive system with better ventilation maintained low ammonia levels and preserved normal mucociliary function, highlighting the benefits of an improved rearing environment. The use of continuous monitoring in this study not only quantified the exposure differences between systems but also provided insight into ammonia fluctuations and hotspot zones within the poultry house, information that can guide targeted management interventions.

From a practical standpoint, our findings reinforce that controlling ammonia in broiler houses is essential for respiratory health. Ensuring sufficient ventilation, dry and well-managed litter, and considering nutritional approaches to reduce nitrogen excretion are all effective strategies to keep ammonia concentrations in check. Modern sensor technology can facilitate this by providing real-time feedback to farmers, enabling timely adjustments before ammonia reaches harmful levels. Ultimately, investments in air quality pay off in the form of healthier flocks - with better growth, lower disease incidence, and improved welfare - and also reduce environmental emissions. The tracheal mucociliary system is a critical defense for chickens, and protecting it through diligent environmental management should be a priority in intensive poultry production. By integrating continuous ammonia mapping with animal health monitoring, producers and researchers can together ensure that the intensive rearing of broilers does not come at the cost of the birds' respiratory integrity. This holistic approach will support sustainable production of poultry meat that optimizes both productivity and animal well-being in the years ahead.

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