AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 4, pp. 782-794
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Intensive animal farming

UDC 636.52/.58.033:611.21/.23:57.04:697.92

doi: 10.15389/agrobiology.2021.4.782eng
doi: 10.15389/agrobiology.2021.4.782rus

HISTOSTRUCTURE OF THE TRACHEAL WALL OF BROILER CHICKENS DEPENDING ON AIR CIRCULATION CONDITIONS IN CLOSED POULTRY HOUSES

V.I. FISININ[1], I.P. SALEEVA[1], A.K. OSMANYAN[2], V.P. PANOV[2], V.V. MALORODOV[2] ✉, N.G. CHEREPANOVA[2], V.Z. HAMITOVA[3]

[1]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail fisinin@land.ru, saleeva@vnitip.ru;
[2]*Department of Zootechny and Biology, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy,* 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail ptitsa@rgau-msha.ru, panovvp@rgau-msha.ru, malorodov56@gmail.com (✉ corresponding author), ncherepanova@rambler.ru;
[3]*Ltd «Chelny-Broiler»,* 8, ul. Stroybaza, Naberezhnye Chelny, Republic of Tatarstan, 423800 Russia, e-mail hamitova-valeriy@mail.ru
ORCID:
Fisinin V.I. orcid.org/0000-0003-0081-6336
Saleeva I.P. orcid.org/0000-0002-7446-1593
Osmanyan A.K. orcid.org/0000-0002-0677-4264
Panov V.P. orcid.org/0000-0002-7075-580X
Malorodov V.V. orcid.org/0000-0001-9033-7552
Cherepanova N.G. orcid.org/0000-0001-7484-9021
Hamitova V.Z. orcid.org/0000-0001-7834-7328

The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian State Agrarian University — Timiryazev Moscow Agricultural Academy (project No. 1.2.10)
Received May 25, 2021

Abstract

Currently, optimization of indoor microclimatic conditions in poultry houses is attracting considerable interest due to the intensification of broiler meat production. However, given the increase in flock sizes of broilers, little research has focused on the effect of microclimate parameters in poultry houses on the bird's respiratory system. Insufficient air exchange in the premises can cause functional respiratory disorders in broiler chickens. This paper is the first to report that air circulation in closed poultry houses contributes to maintaining productivity and improves the histostructure and histochemical properties of the tracheal wall in broiler chicks (*Gallus gallus domesticus*). Our work aimed to study the influence of different air circulation regimes in closed poultry houses on histostructure and histochemical characteristic of the trachea in Ross 308 broiler chicks and their productive performance. The study was conducted in 2020-2021 at the LLC Chelny-Broiler poultry farm (Republic of Tatarstan). Ross 308 cross broiler chicks were raised until 39 days of age in five closed premises under different airflow distribution and air circulation (five groups of 35 birds each). For morphometry, 525 preparations of 175 trachea specimens from of all broilers (2500 g bodyweight) were measured. Trachea sections were stained by hematoxylin and eosin procedure. For histochemical studies of acidic and neutral mucins, sections were stained by a combined method for detecting polysaccharides using the Schiff-iodic acid (PAS-reaction) and alcian blue according to the manufacturer's recommended (LLC Labico, Russia). In the control groups 2, 3, and 4, there was no air circulation; in the experimental groups I and V, circulation was provided by forced ventilation, capacity of 8.5 thousand m^3/h (SF-550-02, AgroKurs, Russia). Ventilation was run at the 10 day-age of the broiler chicks. Insufficient air circulation in the poultry rearing rooms caused destructive changes in the tracheal mucous membrane, i.e., its own lamina proliferation, edema, a decrease in the height of the epithelium, and destruction of cilia. This led to metaplasia of the epithelium and disruption of mucociliary transport. The thickness of the mucous membrane and its own lamina was minimum in the experimental group 1 (147.2±3.3 μm and 129.1±3.1 μm, respectively) and maximum in the control group 3 (404.7±9.4 μm and 395.7±9.4 μm) ($p \leq 0.01$). The thickness of the tracheal epithelial layer significantly increased in the experimental groups 1 and 5 (by 14 % on average) compared to the control groups 1, 2, and 3 ($p \leq 0.01$). The lack of indoor air circulation led to a significant decrease in the height of cilia in the control groups 2, 3, and 4 (by 39.5, 58.1, and 67.5 %, respectively) as compared to the experimental groups 1 and 5. The increase in birds' bodyweight at 5 weeks of age in the experimental group 1 increased compared to the control groups 2, 3, and 4 by 6.5, 3.2, and 7.1 %, respectively ($p \leq 0.05$). The histochemical characteristics suggests the presence of simple multicellular endoepithelial glands in the

tracheal epithelium layer of birds. Thus, with the provision of proper air circulation in an enclosed space, the thickness of the mucous membrane and its own lamina decreases, and the thickness of the epithelial layer and the height of the tracheal cilia increases. These characteristics are indicative of proper airexchange in the poultry houses.

Keywords: *Gallus gallus*, trachea, histostructure, tracheal mucosa, ciliated epithelium, microclimate, air circulation, ventilation system, respiratory tract, histochemistry, PAS-reaction, alcian blue

The morphofunctional structure of mammalian respiratory systems has been studied well, in particular the morphogenesis of ciliary and ciliated epithelium [1-3], the mechanisms of the mucociliary apparatus [4, 5], whereas the respiratory anatomy of birds remains relatively understudied [6, 7]. Earlier research has covered the histological structure of respiratory tracts in various avian species [8, 9], including tracheal and laryngeal morphology of quails [10], broilers [11], turkeys [12], and guinea fowls [13, 14], as well as the lungs and air sacs of geese, turkeys, chickens, and ducks [15].

Avian respiratory systems maintain gas exchange and temperature homeostasis in the body. The trachea carries air from the larynx to the lungs, moistens and warms up such air while also removing mechanical particles, bacteria, and viruses from it. Like mammals, birds' trachea consists of a mucous membrane, submucosa, fibrocartilage, and adventitia [9, 16]. The mucous membrane is lined with multirow ciliated epithelium that consists of several cell types. Those are mainly tall ciliated cells that reach the edge of the layer and bear cilia on the apical pole. Epithelial cells also include goblet cells, which are simple unicellular glands that produce a mucous secretion [1].

The ratio of cell types depends on the species, age, health status, and environment. Matveev et al. [11] studied the trachea in Ross 308 broiler chicks and found the goblet cells to be in a 1:10 ratio to the rest of the epithelial cells. Lamina propria, made of loose connective tissue, underlies the epithelium. It contains collagen, elastic, and reticular fibers with the amorphous ground substance in-between [12]. Then goes the submucosa, also of loose connective tissue. Some authors refer to lamina propria and the submucosa as dense connective tissue [9]. Then follows fibrocartilage consisting of hyaline rings with dense connective tissue in-between. Birds have closed overlapping tracheal cartilages that can ossify in some species [12, 16]. The mucus secreted by goblet cells and tracheal glands is viscous and contains PAS-positive glycoproteins and glycosaminoglycans that are stained with Alcian blue [1]. Glycosaminoglycans are highly sulfated polysaccharides that form proteoglycans with protein molecules [17]. This mucus is on top of the cilia and catches mechanical particles and bacteria carried by the inhaled air. The ciliated apparatus, goblet cells, and tracheal glands operate in sync and make the mucociliary transport system in the trachea and in the bronchi, thus completing the defense system. Abnormalities in this system cause inflammation [1].

As a defensive mechanism, the trachea is the first to suffer the effects of environmentally induced changes in, or damage to, the histological structure. Thus, the ciliated epithelium has been found to be able to convert into typical multilayered epithelium when exposed to aggressive external effects (toxic vapors, mechanical or thermal effects) [1]. There are reports on the suppressive effects of radiation on all tracheal wall elements [7], as well as on the negative carbon dioxide effects that cause mucosal edema [2]. Some papers note the effects of low temperatures on the anatomy and histology of the trachea [4, 18, 19].

In most cases, failure to comply with the physiological standards of poultry farming contributes to non-infectious respiratory diseases in broilers [20]. Respiratory allergies in birds might be caused not only by pathogenic microflora in the air-dust bioaerosol [21, 22] but also by insufficient air circulation in enclosed

facilities [23, 24].

As of today, the histological structure of the respiratory system in poultry has been studied well in the context of resistance to respiratory diseases [25], infectious bronchitis in chickens [26, 27], and laryngotracheitis virus [28, 29], air contamination [20], microclimate uniformity [30, 31], harmful gas concentrations [32, 33]; however, the histological and histochemical structure of tracheal walls in birds grown in enclosed facilities with different air circulation rates has not been studied yet.

This paper is the first to report that air circulation in enclosed poultry houses contributes to maintaining productivity and improves the histological structure and histochemical properties of the tracheal wall in broiler chicks.

The objective hereof was to determine the histological and histochemical structure of the tracheal wall, as well as the dynamics of live weight in Ross 308 broiler chicks (*Gallus gallus domesticus*) kept in ventilated vs. non-ventilated facilities.

Materials and methods. The research was carried out in 2020-2021 at Chelny-Broiler LLC (Republic of Tatarstan), which has a good epizootic status. Ross 308 cross broiler chicks were raised until 39 days of age in five enclosed facilities under different airflow distribution and air circulation conditions (five groups of 35 birds each, sampled by the analog-pair method). The poultry was kept on a deep litter. It was fed in seven phases with an all-in-one formula. Live weight was recorded specimen-by-specimen during Weeks 3, 4, and 5.

Tracheae were sampled from all 35 broilers (2500 g live weight on average) in all of the five groups, a total of 175 samples. The samples were fixed in 10% formalin, then washed and poured into paraffin. Five-micrometer-thick tissue sections (three for each sample, a total of 525 preparations) were made per the standard guidelines [17], stained with hematoxylin and eosin to make review preparations. For histochemical studies of acidic and neutral mucins, sections were stained by a combined method for detecting polysaccharides using the Schiff-iodic acid (PAS-reaction) and Alcian blue staining (acidic glycosaminoglycans are stained blue whereas PAS-positive glycoproteins are stained purple) [17]. For more accurate differentiation of glycoproteins and acidic glycosaminoglycan-containing proteoglycans, sections taken from the same samples were stained with the PAS reaction only. The researchers used reagent kits from Labico LLC and followed the manufacturer's manuals.

The preparations were then inspected with a Biolam M-3 optical microscope (LOMO JSC, Russia) using different magnifications (15×8, 15×20, 15×40), photographed, and described. Epithelial thickness and tracheal cilia were counted using an ocular micrometer; relative values were then converted into absolute values using an object micrometer.

In all facilities where poultry was kept, air exchange was provided by a supply-and-exhaust ventilation system using negative pressure. SF-550-02 circulation axial fans (AgroKurs, Russia), each rated at 8.5 thousand m^3/h (total capacity of 42.5 thousand m^3/h) in Chambers 1 (Group 1) and 5 (Group 5) were installed at the same height as gas generators and placed 10.8 m away from the gas generator outlets, sloped at 5° down towards the poultry. Circulation fans were powered together with the gas generators so that air heating and circulation would start simultaneously. In Chamber 1, air flow from the fans went towards the exhaust vents whereas in Chamber 5, it went from the exhaust vents. Circulation fans were started once the broilers reached 10 days of age. Facilities with the control groups (2, 3, and 4) had no air circulation. Control groups differed in the age of parent flocks that the broilers were brooded by: 28 weeks in Group 2, 47 weeks in

Group 3, and 38 weeks in Group 4.

The experiments were in line with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd edition (Federation of Animal Science Societies, 2010). Everything was done to minimize the birds' suffering and the number of euthanized specimens.

Data were processed by variational statistics using Student's *t*-test in Microsoft Excel 2010. For processing, the researchers calculated the means (*M*) and standard errors of the mean (±SEM). Significance thresholds were $p \leq 0.01$ for biological values, $p \leq 0.05$ for zootechnical values.

Results. In Ross 308 chicks, tracheal morphogenesis ends on Day 35 of postnatal ontogenesis [11]; thus, none of the histological structure changes observed were age-related. Tissue samples taken from Groups 1 and 5 differed from those of Groups 2, 3, and 4: they had thinner mucous membranes and laminae propriae.

1. Thickness of tracheal mucosa layers (μm) in Ross 308 broilers (*Gallus gallus domesticus*) as a function of air circulation in the facility (*M*±SEM, poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021)

Tracheal wall	Group (n = 105)				
	1	2	3	4	5
Mucosa	147.2±3.3	267.5±4.1*	404.7±9.4*	298.1±10.5*	161.2±2.9*
Lamina propria	129.1±3.1	253.9±4.2*	395.7±9.4*	285.3±10.4*	144.8±2.9*
Epithelial layer	16.1±0.4	16.2±0.3	13.2±0.3*	15.1±0.3*	18.4±0.4*
Cilia	4.3±0.1	2.6±0.1*	1.8±0.1*	1.4±0.1*	4.3±0.1*

N o t e. See the description of groups in the "Materials and methods" section.
* Differs statistically significantly from Group I at $p \leq 0.01$.

Group 1 birds had minimum mucous membrane and lamina propria thicknesses (147.2±3.3 and 129.1±3.1 μm, respectively), whilst Group 3 had the maximum values (404.7±9.4 μm and 395.7±9.4 μm, respectively) ($p \leq 0.01$). The epithelial layer was significantly thicker in Groups 1 and 5 by an average of 14% than in Groups 2, 3, and 4 ($p \leq 0.01$). Lack of air circulation in the facilities resulted in a statistically significant reduction in cilia height in Groups 2, 3, and 4 by 39.5%, 58.1%, and 67.5%, respectively, against Groups 1 and 5 ($p \leq 0.01$), see Table 1.

The thickness of the epithelium and height of the cilia correlate positively with the ability of the mucosa to retain particles of inhaled air. As organelles of movement, cilia have an important role to play in protecting the respiratory tract from exogenous particles; they are also involved in nonspecific immune responses [1]. A statistically significant increase in cilia size in the air-circulated groups was a sign of better growth conditions. The cilia size did not correlate with the thickness of the epithelium.

Micrographs of the tracheal wall histostructure are shown for Groups 1, 5, and 3 based on the minimum and maximum values.

Histological testing confirms the existing data on the tracheal wall structure (mucous membrane, submucosa, fibrocartilage, and adventitia [9, 16, 34]. The mucosa, in turn, consisted of multilayer ciliated epithelium and lamina propria of loose connective tissue. Beside the fibrous component and the amorphous ground substance, it also contained cellular forms characteristic of this tissue type, as well as leukocytes. The submucosa consisted of loose connective tissue, the fibrous component being predominant, which is also in line with earlier reports [9, 11-13]. Fibrocartilage consisted of closed hyaline cartilage rings and dense connective tissue in-between. Due to the anatomical overlapping of tracheal rings, sections (cuts) of two adjacent rings were visible in the preparation. Adventitia consisted of loose connective tissue, see Fig. 1.

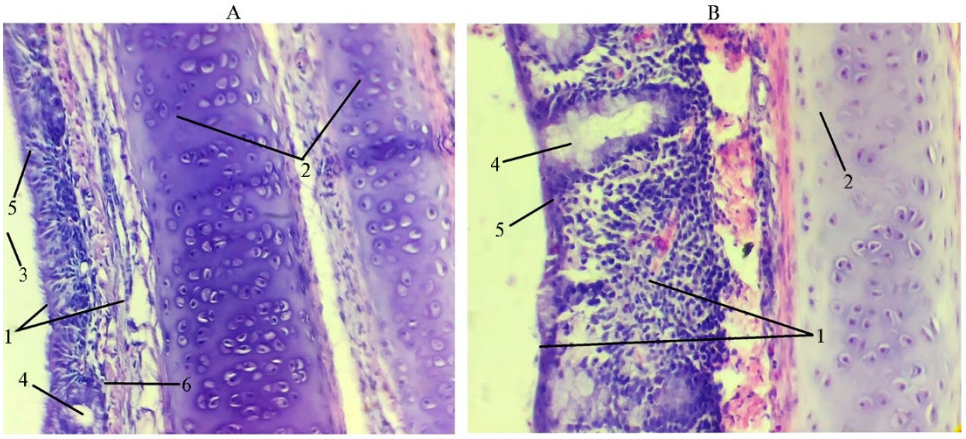

Fig. 1. Histological structure of the tracheal wall in Ross 308 chicks (*Gallus gallus domesticus*) of Group 1 kept in a ventilated facility (A)**, and Group 3 kept in a non-ventilated facility** (B)**:** 1 — the mucous membrane; 2 — the fibrocartilage; 3 — the tracheal lumen; 4 — mucous glands; 5 — epithelium; 6 — lamina propria (poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021). Staining with hematoxylin and eosin, magnification ×15×20, a Biolam M-3 microscope (AO LOMO, Russia).

Complex PAS reaction and Alcian blue staining of sections made it possible to trace the distribution of acidic glycosaminoglycans and PAS-positive glycoproteins in the structures of the organ wall, see Fig. 2.

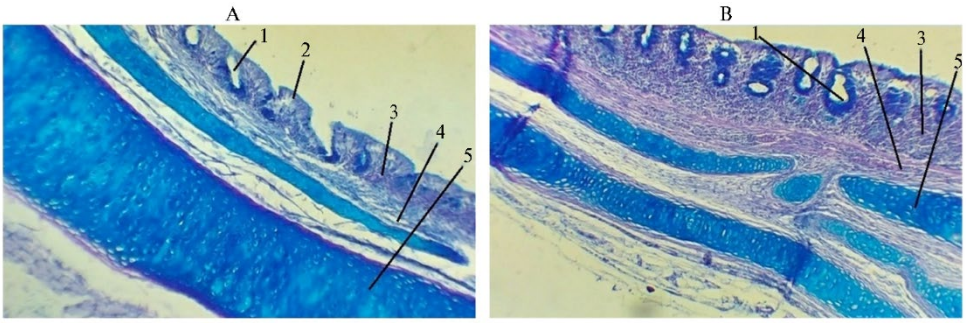

Fig. 2. Histological structure of the tracheal wall in Ross 308 chicks (*Gallus gallus domesticus*) of Group 5 kept in a ventilated facility (A) **and Group 3 kept in a non-ventilated facility** (B)**:** 1 — the glands; 2 — epithelium; 3 — lamina propria; 4 — the submucosa; 5 — the fibrocartilage (poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021). Staining with Alcian blue + Periodic Acid — Schiff (PAS) reaction, magnification ×15×8, a Biolam M-3 microscope (AO LOMO, Russia).

An additional PAS reaction confirmed the localization of neutral mucopolysaccharides, as they would sometimes be overlapped by a brighter Alcian blue stain. Thus, acidic mucopolysaccharides containing sulfated glycosaminoglycans and stained bright blue in the preparation were mainly localized in the cartilage and were part of the mucus secreted by both unicellular and multicellular glands. Additional PAS-enabled identification showed that purple-stained neutral glycoproteins were present in the maximum concentration in the gland secretion yet were much less stained in the cartilage region. In the fibrocartilage, they were mainly localized in the perichondrium and in the young cartilage region. The observed distribution of chemical groups showed the chondromucoid to consist more of sulfated (acidic) mucopolysaccharides rather than their neutral counterparts. Gland-secreted mucus was composed of acidic and neutral mucopolysaccharides in an even distribution. The distribution of these substance categories in

other structures of the wall showed that both acidic glycosaminoglycans and neutral glycoproteins were present in the intercellular substance of loose connective tissue in moderate amounts.

Group 5 birds had no pathological changes in the ciliated epithelium. Lamina propria had visible fibrocyte nuclei, a fibrous component, and an amorphous ground component. Single-layered multirow ciliated epithelium had tall cylindrical tells with cilia on the apical pole, as well as cambial cells that were located near the basal membrane without reaching the edge of the layer. The apical pole of the cells was not destroyed, see Fig. 1. In some places, the epithelial layer had mucus-secreting goblet cells. Beside goblet cells, the epithelial layer also contained simple endoepithelial glands (see Fig. 3) as noted in [9, 12, 13]. Their contents had both a PAS-positive reaction and Alcian blue staining. Goblet cells were found as single cells and clusters alike, see Fig. 4; some studies report the same [4, 12].

Besides, lamina propria had simple tracheal mucous glands that had the same staining. The cilia region had more intense Alcian blue staining whereas the gland-secreted mucus was equally well-stained by both methods. Perhaps the near-cilia mucus, being a more liquid fraction [4], contained more acidic mucopolysaccharides. Mucosal and submucosal connective tissue consisted mainly of acidic mucopolysaccharides, see Fig. 2. Histostructural examination of the trachea in Group 1 birds made findings similar to those of Group 5.

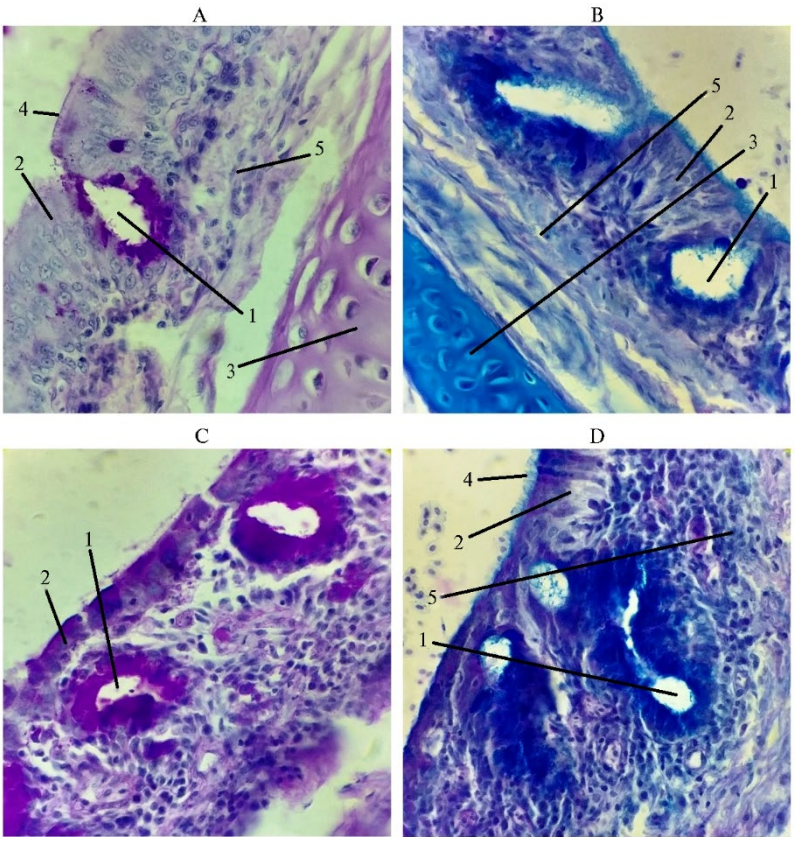

Fig. 3. Histological structure of the tracheal wall in Ross 308 chicks (*Gallus gallus domesticus*) of Group 5 kept in a ventilated facility (A, B) **and Group 3 kept in a non-ventilated facility** (C, D)**:** 1 — the glands; 2 — epithelium; 3 — the cartilage; 4 — the cilia; 5 — lamina propria (poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021). PAS reaction to the left, Alcian blue staining + PAS reaction to the right, magnification ×15×40, a Biolam M-3 microscope (AO LOMO, Russia).

Group 3 birds had noticeably thinner tracheal epithelium, see Fig. 1. The epithelium had local signs of metaplasia; elsewhere, it was significantly lower ($p \leq 0.01$) than in Groups 1 and 5, with local sites of the near-complete absence of cilia. The tracheal lumen sometimes contained cell clusters or singular cells including erythrocytes, macrophages, and leukocytes that had migrated from lamina propria to the epithelial surface. This is in line with the reports on congestion in the avian respiratory systems due to insufficient air circulation [4]. Partial deficit of air exchange results in an excess concentration of harmful gases, as well as in increased dustiness, which may cause inflammation of the tracheal wall. A similar effect was observed when growing broilers from 42 days of age in a poultry house where the air contained 100 ppm NH_3/l (the experiment lasted 1 week) [32], as well as in an experiment where turkeys were kept in a poultry house where the air contained 10 ppm NH_3/l [33]. The experimenters did not report on damage to the ciliated epithelium or goblet cells in the trachea; however, the trachea had suppressed mucociliary apparatus in turkey: the cilia were tangled, and some sites were deciliated [32, 33].

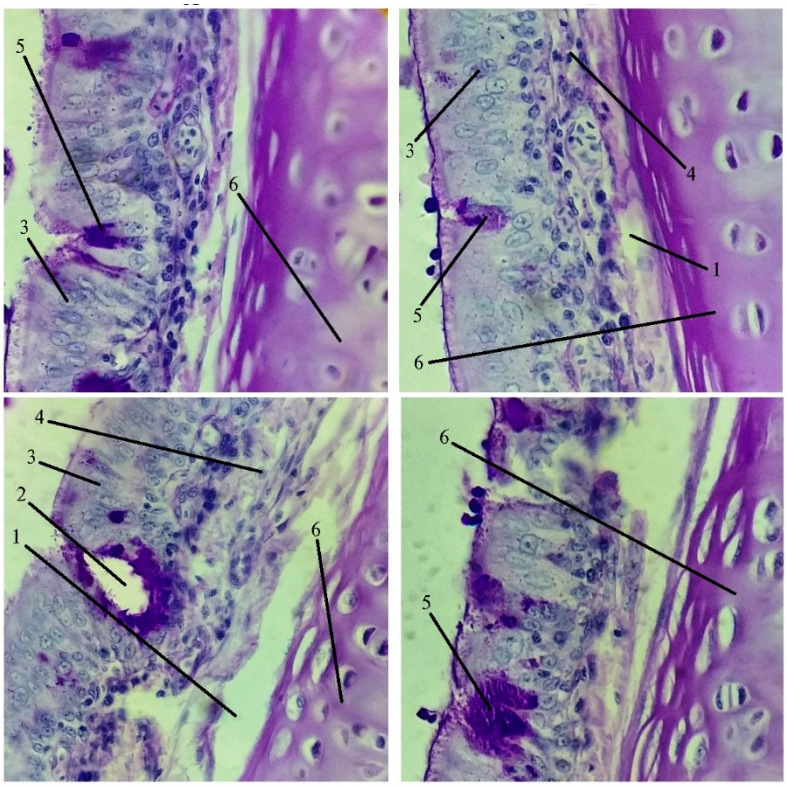

Fig. 4. Endoepithelial glands and goblet cells in trachea of Ross 308 chicks (*Gallus gallus domesticus*) of Group 5 kept in a ventilated facility: 1 — the submucosa; 2 — endoepithelial glands; **3** — epithelium; 4 — lamina propria; 5 — goblet cells; 6 — the cartilage (poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021). PAS reaction, magnification ×15×40, a Biolam M-3 microscope (AO LOMO, Russia).

The data collected in this experiment is partly consistent with the reports on the pathologies that are sometimes encountered in infected chicks. Thus, restructuring of the ciliated epithelium, catarrhal inflammation, and epithelial edema in the mucous membrane due to lymphocyte infiltration are observed in birds affected with an avian influenza virus isolate or with laryngotracheitis [28, 29]. Deciliation, desquamation, and alteration of ciliated epithelium cells in the trachea are observed in chickens infected with the infectious bronchitis virus [26, 27, 35].

Intense PAS reaction and Alcian blue staining of the epithelium were observed when trying to identify mucopolysaccharides in Group 3, which did not happen in Groups 1 or 5. Lamina propria of the tracheal mucosa in Group 3 consisted mainly of the cellular component; fibers were few. Leukocyte (mostly lymphocyte) infiltration of the mucous membrane was observed. The loose connective tissue of the mucosa and submucosa had more glycoproteins in Group 3 than in either Group 1 or 5, see Fig. 2. The epithelium was not intensely multirow; deciliation and metaplasia sites were observed. Groups 2 and 4 had a similar tracheal histostructure to Group 3.

Increased concentrations of carbon dioxide in inhaled air may cause mucosal edema as confirmed earlier in [2]. Mucous membrane layers in the tracheae of chickens infected with infection bronchitis virus strains of various serotypes have moderate lamina propria edema and delamination of tracheal cells with an increased quantity of goblet cells [26, 27].

Goblet cells and tracheal glands are believed to secrete viscous mucus when exposed to local irritants; they are not innervated with adrenergic and cholinergic receptors [35]. Besides, goblet cells are short-lived (2 to 4 days [1]), which causes increased sensitivity to external effects. Thus, various local effects on the mucosa may cause qualitative and qualitative changes in these glands.

The research team discovered the presence of both tracheal glands and goblet cells in the initial segment of the trachea in Groups 1 and 5; however, the balance was shifted towards the glands. These findings are in line with the earlier reports [9, 12, 13] that the glands are dominant in the initial sections whereas the goblet cells dominate the caudal trachea. The goblet cells were found as both isolated and clustered cells. Hystochemical staining revealed cells fully filled with mucous secretion; some rare goblet cells had weak staining, presumably due to generalized production of granule secretion, see Fig. 4. Human goblet cells have been described similarly [1]. Some authors note an increase in goblet cells when the tracheal epithelium is exposed to adverse environmental factors [4, 7, 26, 27].

In this research, Groups 2, 3, and 4, which had insufficient ventilation, had less goblet cells, perhaps due to structural epithelial changes; however, they had more tracheal glands. This was confirmed by the increased amounts of PAS-positive mucopolysaccharides in the loose connective tissue around the glands. Notably, the control groups had significantly more acidic and neutral mucopolysaccharides than the experimental groups. Such histochemical and morphological changes in the tracheal wall of birds whose facilities were not sufficiently ventilated could be a compensatory mechanism for the recovery of mucociliary transport.

2. Live weight (g) **dynamics in Ross 308 broilers (*Gallus gallus domesticus*) as a function of air circulation in the facility** ($M\pm$SEM, poultry house conditions, Chelny-Broiler LLC, Republic of Tatarstan, 2020-2021)

Group (n = 35)	Week 3	Week 4	Week 5
1 test	1047.6±10.6	1712.2±12.4	2366.9±19.2
2 control	1080.9±10.2	1602.0±19.2*	2214.1±29.1*
3 control	1149.3±11.7	1688.2±15.5	2290.7±18.5*
4 control	1110.4±10.3	1591.1±10.4*	2198.2±21.0*
5 test	1159.6±11.2	1690.1±16.5	2317.6±20.0

N o t e. See the description of groups in the "Materials and methods" section.
* Differs statistically significant from Group I at $p \le 0.05$.

Live weight gain in broilers as affected by different air circulation conditions was also studied, see Table 2. At five weeks of age, Group 1 had gained more weight than the birds in Groups 2, 3, and 4 by 6.5%, 3.2%, and 7.1%, respectively ($p \le 0.05$). At four weeks of age, Group 1 differed from Groups 2 and 4 by 6.4% and 7.1% ($p \le 0.05$), whereas no statistically significant differences

had been observed until 4 weeks of age.

These findings confirm the fact that air circulation in enclosed facilities affects the histostructure and the histochemical status of the tracheal wall in Ross 308 broilers. Insufficient air circulation in growing chambers causes destructive changes in the tracheal mucosa, which manifest as the growth of lamina propria, edema, reduced epithelium height, destruction of cilia, and lower productivity. This causes epithelial metaplasia and disrupts mucociliary transport. Air circulation in enclosed facilities reduces the thickness of the mucous membrane and increases the height of the epithelial layer and tracheal cilia. The benefits of air circulation in enclosed poultry houses are reliably evidenced by a 2.6x reduction in tracheal mucosa thickness (by 61.9%), a 2.9x reduction in the lamina propria thickness (by 65.4%), a 1.3x thickening of the epithelial layer (by 23.3%), and a 3.1x increase in cilia height (by 67.5%), as well as an up to 7.1% increase in live weight gain. The obtained histological preparations suggest that the epithelial layer in birds contains simple multicellular endoepithelial glands. The described changes in the tracheal wall histostructure could indicate unfavorable poultry house conditions. Broiler crosses do not take long to grow; thus, investigations of longer-term effects of high gas and dust concentrations in the air need to involve other poultry groups, e.g., the parent flock.

REFERENCES

1. Zavalii M.A. *Rinologiya*, 2014, 1: 38-49 (in Russ.).
2. Belyaeva E.V., Rybakova A.V., Gushchin Ya.A., Vaganova D.S., Koptyaeva K.E., Muzhikyan A.A., Makarova M.N., Makarov V.G. *Laboratornye zhivotnye dlya nauchnykh issledovanii*, 2018, 3: 49-60 (doi: 10.29296/2618723X-2018-03-05) (in Russ.).
3. Pavlov A.V., Esev L.I. *Zhurnal anatomii i gistopatologii*, 2017, 6(2): 62-67 (in Russ.).
4. Tseluiko S.S., Semenov D.A., Gorbunov M.M., Vislobokov N.A., Kazanskii P.R., SHvyndina N.V., Shklover V.Ya. *Byulleten' fiziologii i patologii dykhaniya*, 2012, 45: 52-56 (in Russ.).
5. Ross S.M., Corrsin S. Results of an analytical model of mucociliary pumping. *Journal of Applied Physiology*, 1974, 37(3): 333-340 (doi: 10.1152/jappl.1974.37.3.333).
6. Carvalho O., Gonçalves C. Comparative physiology of the respiratory system in the animal kingdom. *The Open Biology Journal*, 2011, 4: 35-46 (doi: 10.2174/1874196701104010035).
7. Troyanchuk O.V., Levchuk O.K. *Uchenye zapiski uchrezhdeniya obrazovaniya Vitebskaya ordena Znak pocheta gosudarstvennaya akademiya veterinarnoi meditsiny*, 2013, 49(1-1): 73-78 (in Russ.).
8. Maina J.N. Development, structure, and function of a novel respiratory organ, the lung-air sac system of birds: to go where no other vertebrate has gone. *Biological Reviews Cambridge Philosophical Society*, 2006, 81(4): 545-579 (doi: 10.1017/S1464793106007111).
9. Bacha J.W., Bacha M.L. *Color atlas of veterinary histology, 3rd edition*. Lippincott Williams and Willcons Company, USA, 2012.
10. ÇEvik-Demirkan A., Haziroğlu R.M., Kürtül İ. Gross morphological and histological features of larynx, trachea and syrinx in Japanese quail. *Anatomia, Histologia, Embryologia*, 2007, 36(3): 215-219 (doi: 10.1111/j.1439-0264.2007.00758.x).
11. Matveev O.A., Guz'ko A.P., Baimukhambetov R.K. *Izvestiya Orenburgskogo gosudarstvennogo agrarnogo universiteta*, 2018, 6(74): 170-173 (in Russ.).
12. Al-Mussawi A.M., Al-Mehanna A.H., Al-Baghdady F.H. Histological study of the trachea in indigenous male turkey (*Meleagris gallopava*). *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 2012, 11(2): 47-53.
13. Waad S.K. Comparative histological study of trachea in guinea fowl and coot bird. *Basrah Journal of Veterinary research*, 2015, 14(2): 122-128.
14. Samuelson D.A. *Text book of veterinary histology*. Saunders Elsevier, 2007.
15. Pervenetskaya M.V., Fomenko L.V. *Aktual'nye voprosy veterinarnoi biologii*, 2014, 1(21): 10-12 (in Russ.).
16. Vrakin V.F., Sidorova M.V. *Anatomiya i gistologiya domashnei ptitsy* [Anatomy and histology of poultry]. Moscow, 1984 (in Russ.).
17. *Mikroskopicheskaya tekhnika: Rukovodstvo* /Pod redaktsiei D.S. Sarkisova, Yu.P. Perova [Microscopic technique: manual. D.S. Sarkisov, Yu.P. Perov (eds.)]. Moscow, 1996 (in Russ.).
18. Su Y., Wei H., Bi Y., Wang Y., Zhao P., Zhang R., Li X., Li J., Bao J. Pre-cold acclimation improves the immune function of trachea and resistance to cold stress in broilers. *Journal of*

Cellular Physiology, 2019, 234(5): 7198-7212 (doi: 10.1002/jcp.27473).
19. Su Y., Li S., Xin H., Li J., Li X., Zhang R., Li J., Bao J. Proper cold stimulation starting at an earlier age can enhance immunity and improve adaptability to cold stress in broilers. *Poultry Science*, 2020, 99(1): 129-141 (doi: 10.3382/ps/pez570).
20. Fisinin V.I., Trukhachev V.I., Saleeva I.P., Morozov V.Yu., Zhuravchuk E.V., Kolesnikov R.O., Ivanov A.V. Microbiological risks related to the industrial poultry and animal production (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(6): 1120-1130 (doi: 10.15389/agrobiology.2018.6.1120eng).
21. Bakutis B., Monstviliene E., Januskeviciene G. Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. *Acta Vet. Brno*, 2004, 73(2): 283-289 (doi: 10.2754/avb200473020283).
22. Plewa K., Lonc E. Analysis of airborne contamination with bacteria and moulds in poultry farming: a case study. *Pol. J. Environ. Stud.*, 2011, 20(3): 725-731.
23. Radon K., Danuser B., Iversen M., Monso E., Weber C., Hartung J., Donham K., Palmgren U., Nowak D. Air contaminants in different European farming environments. *Ann. Agric. Environ. Med.*, 2002, 9(1): 41-48.
24. Fisinin V.I., Kavtarashvili A.Sh. Heat stress in poultry. I. Danger, related physiological changes, and symptoms (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2015, 50(2): 162-171 (doi: 10.15389/agrobiology.2015.2.162eng).
25. Fedde M.R. Relationship of structure and function of the avian respiratory system to disease susceptibility. *Poultry Science*, 1998, 77(8): 1130-1138 (doi: 10.1093/ps/77.8.1130).
26. Nesterova L.Yu. *Vestnik donskogo gosudarstvennogo agrarnogo universiteta*, 2016, 2-1(20): 16-22 (in Russ.).
27. Hodgson T., Casais R., Dove B., Britton P., Cavanagh D. Recombinant infectious bronchitis coronavirus Baudette with the spike protein gene of the pathogenic M41 strain remains attenuated but induces protective immunity. *Journal of Virology*, 2004, 78(24): 13804-13811 (doi: 10.1128/JVI.78.24.13804-13811.2004).
28. Kirkpatrick N.C., Mahmoudian A., Colson C.A., Devlin J.M., Noormohammadi A.H. Relationship between mortality, clinical signs and tracheal pathology in infectious laryngotracheitis. *Avian Pathology*, 2006, 35(6): 449-453 (doi: 10.1080/03079450601028803).
29. Oldoni I., Rodríguez-Avila A., Riblet S.M., Zavala G., García M. Pathogenicity and growth characteristics of selected infectious laryngotracheitis virus strains from the United States. *Avian Pathology*, 2009, 38(1): 47-53 (doi: 10.1080/03079450802632031).
30. Osmanyan A.K., Malorodov V.V., Cherepanova N.G., Saleeva I.P. *Ptitsevodstvo*, 2020, 12: 42-46 (doi: 10.33845/0033-3239-2020-69-12-42-46) (in Russ.).
31. Osmanyan A.K., Malorodov V.V. *Ptitsa i ptitseprodukty*, 2021, 1: 13-16 (doi: 10.30975/2073-4999-2020-23-1-13-16) (in Russ.).
32. Al-Mashhadani E.H., Beck M.M. Effect of atmospheric ammonia on the surface ultrastructure of the lung and trachea of broiler chicks. *Poultry Science*, 1985, 64(11): 2056-2061 (doi: 10.3382/ps.0642056).
33. Nagaraja K.V., Emery D.A., Jordan K.A., Newman J.A., Pomeroy B.S. Scanning electron microscopic studies of adverse effects of ammonia on tracheal tissues of turkeys. *American Journal of Veterinary Research*, 1983, 44(8): 1530-1536.
34. Hodges R.D. *The histology of the fowl.* CA Academic Press, London, 1974.
35. Naumann H.H. On the defense mechanisms of the respiratory mucosa towards infection. *Acta Oto-Laryngologica*, 1980, 89(3-4): 165-176 (doi: 10.3109/00016488009127124).