Dose and time-dependent effects of glucocorticoid: A morphologic and morphometric study in the broiler lung

RAFIQUL ISLAM
NASRIN SULTANA
RAFIQUL ALAM
ANTORA AKTER
MD. ANISUZZAMAN

See next page for additional authors

Follow this and additional works at: https://journals.tubitak.gov.tr/veterinary

Part of the Animal Sciences Commons, and the Veterinary Medicine Commons

Recommended Citation
ISLAM, RAFIQUL; SULTANA, NASRIN; ALAM, RAFIQUL; AKTER, ANTORA; ANISUZZAMAN, MD.; HAQUE, ZIAUL; and KHAN, MD. ABU HADI NOOR ALI (2022) "Dose and time-dependent effects of glucocorticoid: A morphologic and morphometric study in the broiler lung," Turkish Journal of Veterinary & Animal Sciences: Vol. 46: No. 4, Article 9. https://doi.org/10.55730/1300-0128.4233
Available at: https://journals.tubitak.gov.tr/veterinary/vol46/iss4/9

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Veterinary & Animal Sciences by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.
Dose and time-dependent effects of glucocorticoid: A morphologic and morphometric study in the broiler lung

Authors
RAFIQUL ISLAM, NASRIN SULTANA, RAFIQU ALAM, ANTORA AKTER, MD. ANISUZZAMAN, ZIAUL HAQUE, and MD. ABU HADI NOOR ALI KHAN

This article is available in Turkish Journal of Veterinary & Animal Sciences: https://journals.tubitak.gov.tr/veterinary/vol46/iss4/9
Dose and time-dependent effects of glucocorticoid: A morphologic and morphometric study in the broiler lung

Rafiqul ISLAM1, Nasrin SULTANA1*, Rafiqul ALAM2, Antora AKTER2*, ANISUZZAMAN3, Ziaul HAQUE1, Md. Abu Hadi Noor Ali KHAN4

1Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
2Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
3Department of Parasitology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
4Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract: Glucocorticoids (GCs) play a critical function in neonatal lung growth and maturation though the long-term dose-dependent effects of GCs on lungs are still ambiguous. The purpose of the present study is to explore the morphologic and morphometric changes in broiler lungs in reaction to dietary GC. Day-old chicks (DOCs) were purchased and divided into four groups at random i.e. one control group (C) and three experimental groups (E1, E2, and E3). Commercial broiler feed was provided to both the control and experimental groups where the feed of the experimental groups contained GC, dexamethasone (DEX) at the rate of 3, 5, and 7 mg/kg in E1, E2, and E3 groups, respectively. Lung (left) samples were collected on days 7, 14, 21, and 28. After recording the gross morphologic and morphometric data, the tissue samples were processed for histological investigation. In the gross morphological study, congested lung with reddish to blackish discoloration was seen in experimental broilers. The gross morphometric study revealed a significant decrease in weight, length, and width of the lung. In the histomorphologic study, the lung of experimental broilers revealed vascular and parabronchial lumen congestion. Mild to the severe edematous lesion was also found evident along with the absence of distinguishable inter parabronchial septa. Degeneration of lung parenchyma with the appearance of lipid droplets, inflammatory cell infiltration, and fibrosis was seen to some extent. The histomorphometric investigation revealed a significant decrease in the diameter of the parabronchus as well as the thickness of the parabronchial wall. The study results suggest that dexamethasone may adversely alter the gross and histological characteristics of broiler lungs.

Key words: Glucocorticoid, dexamethasone, broiler, lungs, morphology, morphometry

1. Introduction
Poultry meat production has exploded in recent decades to meet the ever-increasing protein demand of the world's rising population. To meet this increasing demand, accretion of broiler growth rate was a matter of evermore research interest to the breeders. Feed accounts for a large portion of broiler production costs, which is more than 70% of the total expenditure required for broiler production [1]. The growth of broiler is influenced by the genetic characteristics, nutrition, feed conversion efficiency, age, and rearing conditions [2]. Nonetheless, numerous growth promoters are regularly used illegally in livestock singly or in combination with different illicit drugs [3]. Illegal use of steroid growth promoters and their residues in food products can result in detrimental effects on children's mental and physical growth, female fertility and cause cancer, damage of vital organs like brain, liver, kidney, heart, etc. [4]. Long-term use of these drugs may be associated with more serious human health consequences like osteoporosis, aseptic joint necrosis, adrenal insufficiency, gastrointestinal, hepatic, and ophthalmologic effects, hyperlipidemia, growth suppression, and possible congenital malformations [5]. In clinical veterinary medicine, GCs have broad therapeutic applicability. They are frequently used in premature infants for prompt maturation of lungs to avoid neonatal respiratory distress syndrome [6]. However, a great deal of experiments has been done to explore the impacts of exogenous GCs on several aspects of lung biology [6–8].

The fine structures of lung parenchyma are constructed to meet the oxygen demand of the individual. Therefore, any alterations in these structures will lead to diminishing gas exchanging area which will eventually lead to insufficient oxygen saturation in the blood [7].
Glucocorticoid receptors (GRs) are found practically in all organs, including the lungs, and are responsible for the actions of GCs [9]. GCs mainly bind to the dormant cytoplasmic GRs of the target cells and generate complex bonding of GC-GR. These GC-GR complexes are then transferred into the cell nucleus, where they exert their effects either directly or indirectly [10]. In addition to the potent antiinflammatory properties of GCs, previous research findings have also shown that glucocorticoids could ameliorate acute lung injury [8]. Albeit, the results of experimental versus clinical investigations are frequently disputed because animal trials have shown negative effects of GCs on lung development and histomorphology [11]. Reddish hemorrhagic spots on the lung surface in response to DEX treatment were elucidated in the previous study [12]. Side effects can occur at a wide range of doses and depend on how the medication is administered. Long term adverse effects of recurrent or high dose GC treatment include decreased lung growth and changes in lung histomorphometry [13].

For maintaining proper functionality and livability, every cell of the body requires oxygen. By distributing oxygen from inhaled air into the blood, the lung plays a critical role in the animal body. Hence, a healthy and efficiently functioning lung is essential for optimum growth and survival of the broiler. Our recent literature search has revealed that information related to GC-associated side effects was scarce because a majority of the studies were based on the curative roles of GCs rather than the onset and surveillance of side effects. So much research has been done on human and laboratory animals, especially on rats, to explore the role of GC on lung growth and maturation, recovery from severe pulmonary damage, or severe respiratory problems. Impacts of dietary GC (DEX) on the liver, thymus, spleen, and bursa of Fabricius, and blood profile in broilers were previously investigated [14–16]. But the dose-dependent long-term influence of dietary DEX exposure on broiler lungs is not yet clear. So, we inferred that prolonged supplementation of DEX with diet will possibly alter the gross and histological attributes of broiler lungs. To examine this inference, we designed this study to compare different parameters of the lung (i.e. gross morphology, histomorphology, and histomorphometry) in broilers treated with three different doses of DEX with the control group.

2. Materials and methods

2.1. Statement of the study

The experimental broilers were raised in the poultry rearing shed and the rest of the experiment was performed in the molecular lab of the Department of Anatomy and Histology, Bangladesh Agricultural University (BAU), Bangladesh.

2.2. Ethical approval

The present study was performed following the institutional standards for the care and use of animals, commenced by the Animal Welfare and Experimentation Ethics Committee, BAU, Bangladesh [AWEEC/BAU/2021(3)].

2.3. Animal model

A total of 80 DOCs of Cobb-500 strain was collected from Provita Hatchery Limited, Bangladesh. All the DOCs appeared to have good health without any visible abnormalities. To mitigate transportation-induced stress, vitamin-C mixed fresh and cool drinking water was supplied to the DOCs after arrival. Then the DOCs were randomly categorized into four homogenous groups (20 DOCs/group) and housed in individual cages with appropriate bedding materials. The length and width of each cage were 5 feet, and 4 feet, respectively. One group was then designated as the control group (C) and the other three groups were designated as E1, E2, and E3. Brooding temperature (95 °F) was maintained from day 1 to day 3, followed by a continual decrease of 5 °F temperature on each day, and finally, 70° ± 2 °F temperature was maintained up to the 28th day. The relative humidity in the shed was maintained between 50%–60% during the experiment. Proper ventilation was ensured to rule out hypoxic stress.

2.4. Feeding strategy

Antibiotic or growth-promoter-free broiler feed was purchased from Nourish Poultry and Hatchery Limited, Bangladesh. Feed ingredients and pellet size of the broiler diet were selected to meet the nutritional demand at various stages of growth [14]. The composition of the ration is shown in Table 1. Ad libitum feed supply was ensured throughout the experiment.

2.5. Experimental feeding trial

Exogenous GC, dexamethasone (Decason®, BP 0.5 mg, Opsonin Limited) was supplied with feed in three different doses, 0.45 mg (E1), 0.42 mg (E2), and 0.42 mg (E3).

Table 1. Composition of ration.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Starter</th>
<th>Grower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolizable energy (kcal/kg)</td>
<td>3000</td>
<td>3050</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.95</td>
<td>0.9</td>
</tr>
<tr>
<td>Phosphorus (g)</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.05</td>
<td>1</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>Vitamin and mineral</td>
<td>Ad libitum</td>
<td>Ad libitum</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>
doses “i.e. 3 mg/kg in group E1, 5 mg/kg in group E2, and 7 mg/kg in group E3”. Proper care and continuous monitoring were performed to assure that each bird is receiving the intended amount of DEX with feed.

2.6. Sample collection
Specimens were collected at 7, 14, 21, and 28 days of the experiment. Five birds from each group were sacrificed manually on each sampling day by the cervical subluxation method. To study the impacts of dietary DEX on lungs, the left lung was gently removed from the thoracic cavity following immediate dissection. Physiological saline (0.9%) was used to wash the samples. Tissue fixation was done with a 10% solution of buffered formalin.

2.7. Gross study
For the gross morphologic and morphometric study, the color, weight, length, and width of the lung were investigated. The color of the lungs was compared between the control and experimental groups by eye observation. The lung specimens were weighed (gm) using a high precision balance (FGH Series, AND Company Limited, Korea), and length and width (cm) were measured by a graded scale. After careful removal of the lung from the rib cage of the broiler, it was placed on a flat tray and the measurements of lengths and widths were taken.

2.8. Histomorphological study
The collected specimens were processed and stained (Hematoxylin and Eosin; H & E) following the method mentioned in the previous study [15]. To avoid personal variation and biases, all stained tissue sections were labeled anonymously and investigated by one individual. The histomorphological attributes of lungs tissue sections were studied using a light microscope (Leica DMR; Leica Microsystems, Wetzlar, Germany) under low (100X) and high (400X) magnifications.

2.9. Histomorphometric study
For the histomorphometric investigation, the longest and shortest diameter of parabronchi, as well as the thickness of the parabronchial wall were measured from the histological sections by using a calibrated stage micrometer. The sections were placed under the microscopic lens and adjusted in the x and y directions of the scale bars of the eyepiece and then the measurements were taken. A total of 15 parabronchi were considered from each tissue section which was chosen at random. Thus, a total of five tissue sections were evaluated from all the groups. The unit of measurement was a micrometer (µm).

2.10. Photomicrographs
Photomicrographs were taken by photomicroscope (Model: CX41U - LH50HG, Olympus Corp., Tokyo, Japan) from ten different microscopic fields (which were chosen at random) under low (100X) and high (400X) magnifications for a better presentation of the histomorphologic and histomorphometric findings.

2.11. Statistical analyses
Analysis of all the experimental data recorded from the macroscopic and microscopic investigation was performed by IBM SPSS Statistics 22. Shapiro-Wilk test was done to see if the data were normally distributed. In all cases, data were presented as mean ± standard error of the mean (SEM). Comparison between different groups of broilers was done by one-way ANOVA with posthoc Duncan's multiple range test. A probability value (p) of 0.05 or less was considered statistically significant.

3. Results
3.1. Gross morphological and morphometric alterations
The gross morphological alterations and morphometric data of lungs of both control and treatment groups are shown in Figure 1. The lungs of all the groups were located in the craniodorsal part of the thoracic cavity, deeply indented by thoracic vertebrae and ribs. The control group exhibited reddish-pink color lungs whereas the experimental groups revealed dark reddish to blackish color with varying degrees of congestion. Accumulation of adipose tissue was also seen on the surface of the lungs with a glossy appearance (Figure 1A).

On day 7, the weight of the lung was significantly (p < 0.05) less in the DEX treated groups as compared to group C. Though the weight was lower in the higher dose groups than in group C, it was statistically insignificant. The length of the lung was also significantly (p < 0.05) less as compared to group C whereas the width of the lung showed only a numerical decrease. There was no significant variation among the DEX treated groups.

On day 14, all the gross morphometric parameters of the lung were significantly (p < 0.05) decreased in all the DEX treated groups. In case of experimental groups, a significant (p < 0.05) difference in the weight of the lungs was found between the E1 and E3 groups whereas the value was less in group E3 than in group E1. A numerical decrease in the length of the lung was seen from lower dose to higher dose group which was not statistically significant (p < 0.05). The width of the lung was significantly (p < 0.05) higher in group E2 than in group E1.

On day 21, all the gross morphometric parameters of the lung were significantly less (p < 0.05) in the DEX treated groups as compared to group C. No significant variation in the weight and width was seen between the DEX treated groups. The length of the lung was significantly less (p < 0.05) in groups E2 and E3 as compared to group E1.

On day 28, the weight of the lung was significantly (p < 0.05) less in the DEX treated groups as compared to group C but no significant variation was seen between the DEX treated groups. There was a significant decrease (p < 0.05)
in length in the DEX treated groups from group C but the length was numerically increased from lower to higher dose group. The width was also decreased significantly (p < 0.05) in group C but increased in E2 and E3 groups significantly from the E1 group.

3.2. Histomorphological alterations

The histomorphological alterations are shown in Figures 2–5. In group C, the lung parenchyma was arranged into tubular parabronchi. The parabronchi were separated by thin fibrous septa. There were blood vessels in between the parabronchi within the septa. There were shallow atria towards the luminal side of the parabronchi lined by epithelial ridges. A smooth muscle bundle called Muscularis Atriales was seen over the epithelial layer. There were tubule-shaped pneumocapillaries with their complex network-like branches arising from the atria.

Parabronchial lumens were found congested in E1, E2 groups on day 7 and E1 group on day 14. Parabronchial lumens were also dilated in the E1 group on day 28. No distinguishable septum was seen in the DEX treated groups on different days of the experiment. Exfoliation of atrialis muscle was seen in the E1 and E2 groups on day 28. There was serofibrinous exudate within the parabronchial lumen in the E1, E2 groups on day 7 and the E3 group on day 28. Serofibrinous exudates were also seen in the empty spaces created due to degenerated lung parenchyma surrounded by fibrous connective tissue in E1, E3 groups on day 14. Congested arterioles and veins with thickened venous walls were seen in all the DEX treated groups on different days of the experiment. Congestion of the pneumocapillaries was also found. There was extravasation of blood into the parabronchial lumen in the E2 group on day 28.

Mild to severe edema in the parenchymal region as well as in the parabronchial lumen was seen in all the experimental groups on days 7, 14, 21, and 28. Excessive edematous areas were seen in the E2 group on days 14, 21, and E2, E3 groups on day 28. A small amount of fibrous connective tissue proliferation was seen in the E3 group on days 7, 14, and 21 which was found more extensive on day 28. Lipid droplets were found within the lung parenchyma.

Figure 1. A. Gross view of broiler left lung during collection of samples at day 28. A1–Lung of the control group, C; A2–Lung of the experimental group 1, E1; A3–Lung of the experimental group 2, E2; A4–Lung of the experimental group 3, E3. C–Congestion in the lung, Ad–Adipose tissue accumulation on the surface of the lung. Effects of dietary glucocorticoid on the weight (gm), length (cm) and width (cm) of left lung in DEX treated broiler are shown in figure B, C, and D, respectively. Data were expressed as mean ± SEM. Columns with different alphabetic superscripts are significantly (p < 0.05) different from each other.
Figure 2. Representative photomicrographs of transverse section (H & E stained) of lungs from 7-day old broiler of group C (A1, A2), E1 (B1, B2), E2 (C1, C2) and E3 (D1, D2). PW- Parabronchial wall, PL-Parabronchial lumen, PC-Pneumocapillaries, PS-Interparabronchial septum, A-Atria, MA-Muscularis Atriales, RBC-Red blood cells, VC-Venous congestion, CA-Congested arterioles, CPL-Congested parabronchial lumen, SFE-Serofibrinous exudate, Ed-Edema, EB-Extravasation of blood, LD-Lipid droplets, F-Fibrosis, G-Granuloma, DA- Degenerative area.
Figure 3. Representative photomicrographs of transverse section (H & E stained) of lungs from 14-day old broiler of group C (A1, A2), E1 (B1, B2), E2 (C1, C2) and E3 (D1, D2). PW-Parabronchial wall, PL-Parabronchial lumen, PC-Pneumocapillaries, PS-Interparabronchial septum, A-Atria, MA-Muscularis Atriales, BV-Blood vessels, C-Congestion, VC-Venous congestion, CA-Congested arterioles, CPL-Congested parabronchial lumen, SFE-Serofibrinous exudate, Ed-Edema, EB-Extravasation of blood, LD-Lipid droplets, F-Fibrosis, IC-Inflammatory cells, G-Granuloma, DA-Degenerative area.
Figure 4. Representative photomicrographs of transverse section (H & E stained) of lungs from 21-day old broiler of group C (A1, A2), E1 (B1, B2), E2 (C1, C2) and E3 (D1, D2). PW-Parabronchial wall, PL-Parabronchial lumen, PC-Pneumocapillaries, PS-Interparabronchial septum, A-Atria, MA-Muscularis Atriales, AMA-Adhesion of Muscularis Atriales, C-Congestion, VC-Venous congestion, CA-Congested arterioles, EBPL-Extravasation of blood into parabronchial lumen, SFE-Serofibrinous exudate, Ed-Edema, EB-Extravasation of blood, LD-Lipid droplets, F-Fibrosis, IC-Inflammatory cells, DA-Degenerative area.
Figure 5. Representative photomicrographs of transverse section (H & E stained) of lungs from 28-day old broiler of group C (A1, A2), E1 (B1, B2), E2 (C1, C2) and E3 (D1, D2). PW-Parabronchial wall, PL-Parabronchial lumen, PC-Pneumocapillaries, PS- Interparabronchial septum, A-Atria, MA-Muscularis Atriales, EMA-Exfoliation of Muscularis Atriales, BV-Blood vessels, C-Congestion, CB-Capillary blood, VC-Venous congestion, CA-Congested arterioles, CPL-Congested parabronchial lumen, dPL-Dilated parabronchial lumen, EBPL-Extravasation of blood into parabronchial lumen, SFE-Serofibrinous exudate, Ed-Edema, EB-Extravasation of blood, LD-Lipid droplets, F- Fibrosis, IC-Inflammatory cells, DA-Degenerative area.
days 7, 14, 21, and 28. Area of parenchymal degeneration was seen on day 7 and found more explicitly later on, especially in the E3 group on day 28. The formation of granuloma with centrally located hemolyzed blood was seen in the E3 group on days 7 and 14. A mild degree of inflammation was seen in the E1 group on day 14 and the E3 group on day 28.

3.3. Histomorphometric alterations

The histomorphometric data on the diameter of the parabronchial lumen and the thickness of the parabronchial wall are shown in Table 2. In the histomorphometric study, both the diameter of the parabronchus and the thickness of the parabronchial wall decreased gradually.

On day 7, the longest diameter of the parabronchus was significantly less (p < 0.05) in all the DEX treated groups than the group C. The longest diameter was also significantly (p < 0.05) less in group E2 and E3 than in group E1. The shortest diameter of parabronchus also decreased numerically in all the DEX treated groups but a significant (p < 0.05) decrease was seen in E2 and E3 groups. The thickness of the parabronchial wall was decreased numerically in all the experimental groups which was not statistically significant.

On day 14, both the diameter of the parabronchus and the thickness of the parabronchial wall were significantly (p < 0.05) decreased as compared to group C. In case of DEX treated groups, only a significant (p < 0.05) difference in the longest diameter was found between E2 and E3 groups.

On days 21 and 28, the results were consistent with day 14 but no significant dose-dependent difference was observed.

4. Discussion

Both endogenous and exogenous GC based therapeutics like DEX play a major role in the development and maturation of lungs [6]. However, the adverse effects of GC on broiler lungs in relation to dosing rate and duration are not well documented. The current study was designed to investigate the roles of different doses of exogenous GC, DEX on the gross morphology and morphometry as well as the histomorphology and histomorphometry of broiler lungs. The lung of the control group displayed normal gross attributes and histomorphological architecture as mentioned in the previous studies [17,18]. On the contrary, the findings of our study indicate that dietary DEX causes salient gross and histological changes in broiler lungs.

4.1. Gross alterations

In the gross morphological investigation, congested lung with darkish discoloration was seen in the broilers of the experimental groups which is in line with the previous findings [12]. Deposition of fat on the surface of the lungs was seen on day 28. GC up-regulates hepatic lipogenesis and causes fat deposition in visceral organs [19]. The fat deposition amplifies the incidence of pulmonary hypertension which may alter pulmonary functionality [20]. Despite the role of GC in postnatal growth, development, and maturation of the lungs, the weight of the lung was also decreased significantly as compared to group C. The length and width of the lung were also found significantly lesser than the group C. Decreased lung volume in response to DEX treatment was also mentioned in previous study report [11]. These findings are rational because GC does not improve the overall growth performance or bodyweight of broilers [7,16].

4.2. Histomorphological alterations

In the histomorphological investigation, vascular congestion was seen in all the experimental groups. Previous reports suggest that GC is responsible for increased sodium retention which leads to hypertension [21]. This increased blood pressure may be the underlying cause of pulmonary vascular congestion [22]. The lung parenchyma as well as the parabronchial lumen were also found congested. This may be due to endothelial cell injury as endothelial cells play a fundamental role in maintaining the fluid barrier. They regulate the permeability of the pulmonary blood vessels through a potent mechanism known as the 'fail-safe' mechanism [23]. In case of increased vascular permeability induced by endothelial cell damage, there may be extravasation of blood into the lung parenchyma as well as parabronchial lumen which also provides a clear explanation of our research findings. The appearance of mild edematous condition in the earlier events with severe pulmonary edema, later on, was seen in the experimental groups. Pulmonary edema is mainly caused by a breakdown of the epithelial barrier which leads to increased pulmonary permeability [24]. Increased hydrostatic pressure in the pulmonary capillaries modulated by hypertension, may also act in concert with the mechanism mentioned earlier. Increased pulmonary permeability not only drains edematous fluid into the lung parenchyma but also drains macromolecules or plasma proteins [25]. This mechanism justifies the presence of extravasation of serofibrinous exudates within the parabronchial lumen and lung parenchyma.

In the experimental groups, no distinguishable septum was seen in between the parabronchi as in group C. This finding is in line with the previous findings where a noticeable reduction of septal volume in reaction to GC treatment was mentioned [11]. This may be due to the GC-induced precocious microvascular maturation of parenchymal septa that arrests septal development [7]. For the development of gas exchanging areas, existing airspaces are needed to be subdivided by the formation of septa. Degeneration of lung parenchyma was seen to some extent though an increase in lung parenchymal volume in
The response to GC treatment was described in the previous study [11]. There was also a mild degree of fibrosis with thickening of the vascular wall and inflammation. The vascular wall thickening may be due to the fibrosis induced by endothelial damage. The presence of inflammatory cells within the parenchyma also suggests cellular damage and degeneration in those areas. Exfoliation of atrialis muscle was seen in some events which may be due to the degeneration of the underlying epithelial lining.

A varying degree of fatty change marked by the presence of lipid droplets throughout the parenchyma was observed in the DEX treated groups, especially in the lower dose group. Long-term exposure to GC at a lower dose promotes lipogenesis whereas exposure to GC at a higher dose induces lipolysis [26]. This mechanism clearly justifies our findings. Accumulation of excess lipid in the lungs reduces overall respiratory function, enhances pulmonary resistance resulting in hypventilation syndrome, affects the diffusion of gases through lung parenchyma, and reduces the strength of the respiratory muscle [27]. Formation of granuloma was seen in the high dose group on days 7 and 14. This may be due to the severe inflammation induced by cellular damage and extravasated hemolyzed blood into the lung parenchyma. However, GC is supposed to have a role in preventing granuloma formation [28].

**4.3. Histomorphometric alterations**

In the histomorphometric investigation, the diameter of the parabronchi of DEX treated lungs showed a significant difference from group C. Significant differences between the experimental groups were seen only on days 7 and 14. This indicates that lower doses can possess similar deleterious effects as higher doses in case of long-term exposure. These findings mismatch the findings mentioned in the previous study report where improvement of lung morphology and function was reported in case of low-dose GC treatment [29]. Nonetheless, these findings comply with the histolomorphogical findings described earlier. The parabronchial wall thickness was also significantly less in the DEX treated groups as compared to group C which is in line with the previous findings [13]. The decrease in parabronchial diameter and parabronchial wall thickness points out the decrease in gas exchange area in the lung. In the earlier study, a 19.13% reduction in oxygen uptake was reported in the DEX treated rats (0.093 mL O$_2$/min/mmHg) compared to the control group (0.115 mL O$_2$/min/mmHg) after four days of treatment [11]. Reduced gas exchange in the lungs will cause hypoxemia and hypercapnia which may lead to brain damage or death [30].

Looking at the summary of the current study’s findings, it is obvious that DEX therapy alters the macroscopic and microscopic characteristics of broiler lungs. Congestion in the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDG</td>
<td>SDG</td>
</tr>
<tr>
<td>C</td>
<td>562.6 ± 36.73$^a$</td>
<td>403.1 ± 8.45$^a$</td>
</tr>
<tr>
<td>E1</td>
<td>472.7 ± 9.83$^b$</td>
<td>356.7 ± 24.95$^b$</td>
</tr>
<tr>
<td>E2</td>
<td>394.4 ± 14.24$^b$</td>
<td>316.1 ± 12.47$^b$</td>
</tr>
<tr>
<td>E3</td>
<td>388.6 ± 18.57$^b$</td>
<td>274.4 ± 15.48$^b$</td>
</tr>
</tbody>
</table>

**Table 2A.** Histomorphometric data on parabronchi of broiler lungs on day 7 and 14.

$^a,b$ Values with different superscripts in the same column indicate significant (p < 0.05) difference between each other.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDG</td>
<td>SDG</td>
</tr>
<tr>
<td>C</td>
<td>819.25 ± 22.93$^a$</td>
<td>646.7 ± 26.18$^a$</td>
</tr>
<tr>
<td>E1</td>
<td>471.25 ± 27.51$^b$</td>
<td>402.45 ± 21.17$^b$</td>
</tr>
<tr>
<td>E2</td>
<td>520.55 ± 46.56$^b$</td>
<td>392.95 ± 67.56$^b$</td>
</tr>
<tr>
<td>E3</td>
<td>443.7 ± 43.16$^b$</td>
<td>369.75 ± 28.81$^b$</td>
</tr>
</tbody>
</table>

**Table 2B.** Histomorphometric data on parabronchi of broiler lungs on day 21 and 28.

$^a,b$ Values with different superscripts in the same column indicate significant (p < 0.05) difference between each other.
lungs with discoloration was seen in the gross study. The gross morphometric parameters were also reduced significantly in the DEX treated groups. Vascular and parabronchial lumen congestion along with varying degrees of the edematous lesion was evident in the histomorphological study. Degenerated lung parenchyma, lipid droplets, inflammatory cell infiltration, and fibrosis were also seen to some extent. Both the diameter of parabronchus and parabrachial wall thickness was also significantly decreased in the treatment groups. These adverse impacts of DEX on lung parabronchus and parenchyma may lead to diminished gaseous exchange, hypoxia, and ultimately respiratory failure and death. However, further study is recommended to picture the pulmonary functionality in response to DEX treatment.

References


12. Salmin RJ, Hassooni ZA. Morphological and Histological effect induced by each of Cefotaxime, Dexamethazon and mixture of them to the stomach, liver, kidney and lung in rats. Drug Invention Today 2020; 11 (12): 3313-3319.


