



Histo-anatomical and Radiological Study of the Lungs in English White Quail

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Abstract

Background and aim: Due to the importance of the respiratory system in vertebrates, especially in birds, and due to the fact that birds need more oxygen to fly, important and special anatomical changes and adaptations have occurred in these animals. In this study, the histoanatomical and radiological examination of lungs in birds was carried out.

Materials and Methods: For this purpose, 16 Healthy male and female adult white English quail with average weight of 160 ± 5.35 grams was studied. At the beginning, lateral and ventro-dorsal radiographs of the birds were prepared. Then the birds were examined anatomically, and parameters related to the weight, length, width and thickness of the lungs were obtained. Finally, the lungs were removed from the coelomic cavity of the bird and fixed in 10% formalin solution. Tissue sections of the samples were prepared and subjected to histomorphometric evaluation.

Results: Radiological examination shows that the right and left lungs in English white quail are located in the first intercostal space to the sixth intercostal space. Anatomical examination, while confirming the radiological results, shows that the average of lung weight, ratio of lung weight to total body weight, lung length, lung width and the number of lung incisions in the male birds are 0.981 ± 0.065 grams, 0.00623 ± 0.00045 , 2.57 ± 0.030 cm, 1.53 ± 0.021 cm, and 5.00 ± 0.00 respectively, and in the female birds, are 0.935 ± 0.028 grams, 0.0587 ± 0.00023 , 2.50 ± 0.033 cm, 1.34 ± 0.024 cm, and 5.00 ± 0.00 respectively. According to histological results, primary and secondary and Para bronchioles (tertiary bronchioles) have ciliated stratified columnar, simple cuboidal and simple squamous to simple cuboidal epithelium.

Conclusion: The lungs are pyramidal and unlobated in shape and light pink in color, located in the 1st to 6th intervertebral space. There were no significant differences in the radiological, anatomical and histological structure of the lungs in male and female English white quails.

Keywords: Lung, Histology, Anatomy, Radiology, Quail

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Introduction

The respiratory system of birds is structurally and functionally very different from the respiratory system of mammals, and for this reason, it is of interest to biological science researchers. The respiratory system of birds is highly advanced and provides a model of gas exchange that is theoretically more efficient than the respiratory system of mammals. Unlike the lungs of mammals, the lungs of birds are small, and the volume of the lungs does not change much during inhalation and exhalation. Gas exchange does not take place in the alveoli, and instead it occurs in the air capillaries. (Dyce & Sack, 2009).

The changes in the respiratory system of birds and its adaptations caused the lungs to be placed between the airways and the air sacs. The airways in the respiratory system of birds can be presented at three levels of the bronchiole: (1) the primary bronchioles, which are the large passage leading from the trachea to the air sacs; (2) Secondary bronchioles that branch off from the primary bronchiole. These include (a) cranio-medial secondary bronchioles that connect to the cranial air sacs, and (b) caudal-dorsal secondary bronchioles that connect to the lung parenchyma. (3) Tertiary bronchioles (parabronchioles) are the branches that gas exchange with blood takes place. The air capillaries surrounding them and they form the major part of the lung parenchyma. Air capillaries continue into the respiratory atria and have simple squamous epithelium. The epithelium of the gas exchanging parts in the lungs in the birds has type one and two pneumocytes (Eurell & Frappier, 2013).

In addition, there are recurrent connections (recurrent bronchioles) of air sacs that lead into the lung and connect to tertiary bronchioles (Claessens, 2009). After the syrinx, the primary right and left bronchi are seen entering the lung tissue and finally entering the abdominal air sac of the bird. Primary bronchioles are histologically similar to the trachea with a ciliated pseudo stratified columnar epithelium with goblet cells (Muthukrishnan *et al.*, 2008). Epithelium of secondary bronchioles is simple cuboidal to simple columnar. Tertiary bronchioles had simple cuboid to simple squamous epithelium (Eurell & Frappier, 2013). In the lung, smooth muscle cells surround the bronchiole tree and play an important role in the structure and function of the airways (Stephens, 2011). These cells form layers

surrounds the bronchial tree, which have different organizations in the upper and lower airways (Amrani & Panettieri, 2003). Like other vascular contractile cells, smooth muscle cells of pulmonary arteries play an important role in physiological and pathological vascular remodeling (Marettova & Maretta, 2022).

There are approximately 18 quail breeds worldwide, most of which are valuable for commercial meat production and some for egg production. Some quail breeds such as the English white breed, Koturnix, Pharaoh, Tuxedo and Golden Manchurian breeds are valuable in the egg production industry (Arya *et al.*, 2018). Due to the rapid growth of industrial quail breeding and the resistance of this bird to many diseases and the high nutritional value of its meat and eggs, this bird has received attention (Owen & Dike, 2013). Because of its physiological similarities to humans, quail is used as an animal model for biological science studies and the investigation of many developmental diseases, diabetes, etc. (Huss *et al.*, 2008). Considering that many poultry diseases cause casualties due to respiratory complications, studying and investigating the anatomical, radiological and histological structure of the lungs in this bird can be important in identifying diseases and pathogens, evaluating the effectiveness of drugs, and improving the basic research process. (Rundfeldt *et al.*, 2013).

Materials and Methods

Radiological study

Sixteen adult and healthy white English quails with an average weight of 160 ± 5.35 grams (8 males and 8 females) were purchased from the bird market of Urmia city. At first, the birds were examined to ensure that they were healthy and had no abnormalities. To ensure the absence of disease in the Commune period, the birds were placed in standard breeding conditions for two weeks in the same and standard conditions in terms of diet, temperature, humidity and light. The humidity of the storage environment was around 70%, the light was 13 hours of light and 11 hours of darkness, and the temperature of the storage environment was $25-27^{\circ}$ Celsius. At the beginning, lateral and ventro-dorsal radiographs of birds were prepared. The type of device used to prepare radiographs was Dean44 X-ray machine, and the applied kVp and mAs were 40 and 6.3, respectively (Dadashpour *et al.*, 2019).

Anatomical study

After weighing, the animals euthanized with minimal pain and stress by 13 mg/kg ketamine, intramuscular (IM) (Udumoh, 2020). Then the anatomical structure and topography of the lungs were examined. At the same time, the length, width and thickness of the lungs were measured using a digital caliper with an accuracy of 0.01 mm. All stages of transportation, storage, care, euthanization, and taking samples were ethically and humanely.

Histological study

Both lungs of the birds were completely removed along with some of the upper respiratory tract (to prevent possible lung tissue damage) and were placed in 10% formaldehyde for one week (during this period the formaldehyde was changed twice), after routine stages of tissue passage and molding, Histological sections (5-6 micrometers) were obtained (Shirinsokhan et al., 2022). The sections were stained with hematoxylin and the diameters of primary, secondary and tertiary bronchioles, atria and air capillaries were measured using an ocular

micrometer of light microscopy (Olympus Co., Germany). Then their means were calculated.

Statistical Analysis

Statistical analyses were conducted using SPSS (version 20) software and t-test. The p values less than 0.05 were considered as statistically significant.

Results

Radiological results

The lungs were located in the dorso-cranial part part of the coelomic cavity and were better visible in the lateral view. The lungs were located behind the heart and had a honeycomb appearance due to the presence of Para bronchioles. The trachea was easily visible from the skull to the bifurcation of the trachea (syrinx). The syrinx was free of any abnormal opacity and has soft tissue density (which indicated the health of the birds). The air sacs were full of air and void of any abnormal density. Air sacs were visible in the ventro-dorsal view on the lateral side of the liver mass. The walls of the air sacs could not be identified (Figure 1).



Figure 1. Lateral view (Left) and ventro-dorsal view (Right) of adult female quail. 1. Trachea, 2. Syrinx, 3. Lungs.

Anatomical results

In the macroscopic study of the lungs, these structures were observed unlobated and light pink in color. The lungs were observed with a soft and velvety consistency and in the anterior part of the coelomic cavity. The lungs were located in the upper part and sides, next to the thoracic vertebrae and the

vertebral part of the ribs. The ribs had clearly merged by the lungs and left their impression on the lungs. The back surface of the lungs same as the curvature of the ribs was convex. The visceral surface was concave and towards the esophagus, heart and liver, and was the entry point of the main right and left bronchi. The basal surface of the lungs is located next

to the caudal thoracic air sacs, and both lungs are almost the same size and lack any typical lobes in mammals. The right and left lungs extend from the first intercostal space to the sixth intercostal space. The general shape of the lungs was pyramidal, and the apex of the lungs was directed towards the front and their base towards the back. The dorsal edge of

the lungs was round and thick, and the ventral edge was thin and sharp (Figure 2 and 3).

The results of Table 1 show that there is no significant difference between the lung weight, the ratio of the lung weight to the total body and lung length, lung width and the number of rib incisions in the left and right lung ($P < 0.05$).

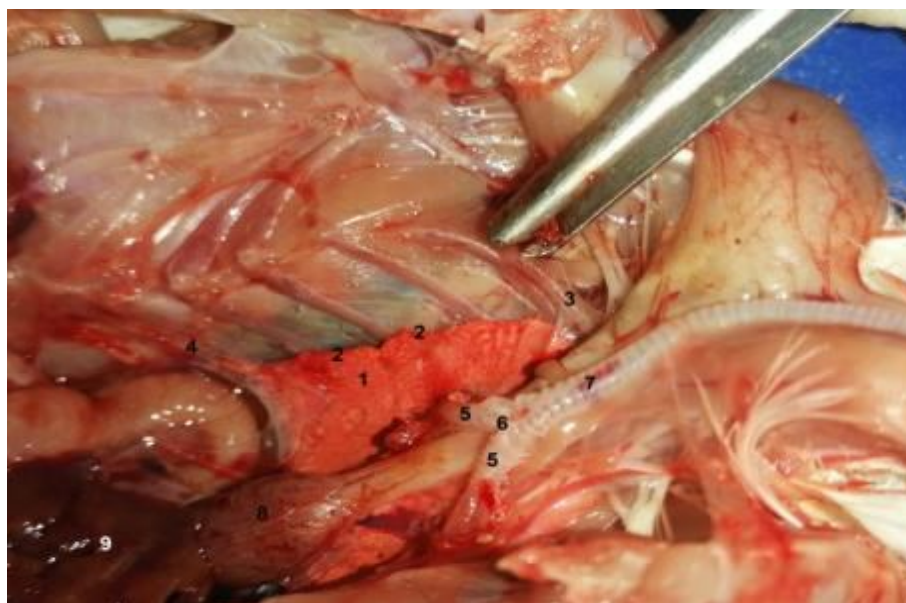


Figure 2. 1. Visceral surface of the lung, 2. Ventral border of the lung, 3. First rib, 4. Sixth rib, 5. principal bronchus, 6. Syrinx, 7. Trachea, 8. proventriculus 9. Liver.

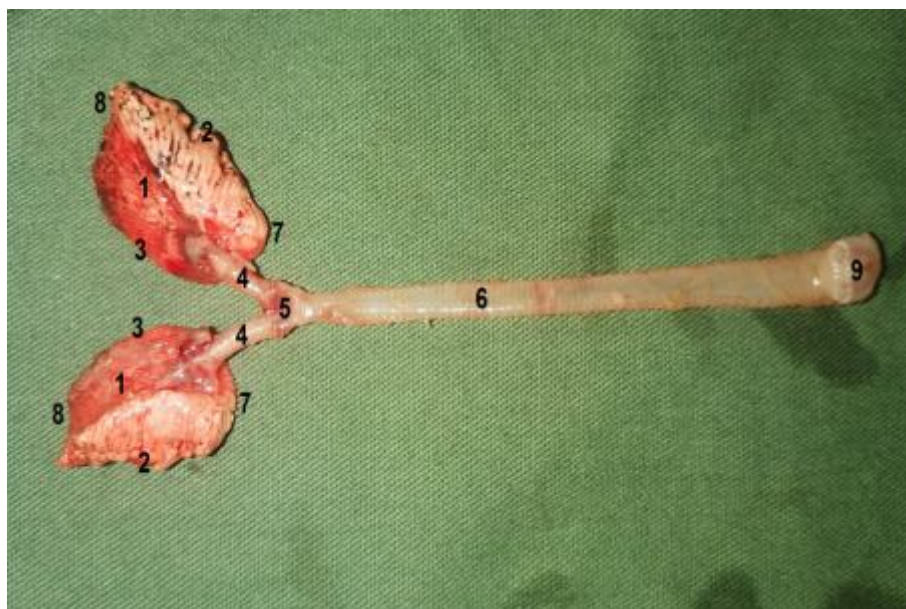


Figure 3. 1. Visceral surface of the lung, 2. Ventral border of the lung, 3. Dorsal border of the lung, 4. Principal bronchus, 5. Syrinx, 6. Trachea, 7. Apex of the lung, 8. Base of the lung, 9. Syrinx.

Gender	Specifics	Lung weight	The ratio of lung weight to total body weight	Lung length	Lung width	Number of rib incisions in the lung (number)
Male	Right lung	(gram)	0.00598±0.00040 ^a	(centimeter)	(centimeter)	5±0.00 ^a
Male	Left lung	0.944±0.017 ^a	0.00648±0.00050 ^a	2.48±0.031 ^a	1.54±0.018 ^a	5±0.00 ^a
Female	Right lung	1.018±0.035 ^a	0.00579±0.00022 ^a	2.67±0.027 ^a	1.53±0.029 ^a	5±0.00 ^a
Female	Left lung	0.912±0.012 ^a	0.00596±0.00038 ^a	2.42±0.032 ^a	1.20±0.016 ^a	5±0.00 ^a

Table 1. Mean±Standard error of the lung weigh, ratio of lung weight to total body weight, length, width and thickness of the lungs and number of rib incisions in the right and left lungs. Different lowercase letters indicate significant differences in columns (P<0.05).

Histological results

In this study, histological findings showed that the lungs contain a connective tissue capsule that is covered by simple squamous epithelium from the outside. Inside the lungs, three types of bronchioles were observed; the walls of the primary bronchioles had deep folds in their wall and were covered by Stratified columnar epithelium. The lamina propria and the sub mucosa are composed of loose connective tissues that are in contact with circular smooth muscles from the outside. Circular smooth muscle tissue was also surrounded by adventitial connective tissue. Primary bronchioles within the lung parenchyma branched into secondary bronchioles, which were covered by cuboidal to columnar epithelium without cilia. In this part, the lamina propria and sub mucosa were also composed of loose connective tissue, and no cartilage was observed,

which was actually replaced by a small layer of smooth muscle bundles (Figure 4 and 5). The epithelium of the tertiary bronchioles was simple cuboid to squamous, and several wide chambers called atrium were opened to the walls of these bronchioles. Atria and air capillaries are covered by epithelium and connective tissue similar to epithelium and connective tissue of tertiary bronchioles. However the smooth muscle fibers and the height of the epithelial cells gradually decrease towards the air capillaries. Secondary and tertiary bronchioles, atria and air capillaries together with connective tissues form the lung lobules (Figure 6). The results of Table 2 show that there is no significant difference between the diameter of the principle bronchi, and tertiary bronchioles, air capillaries and the diameter of the atria in the left and right lungs (P<0.05).

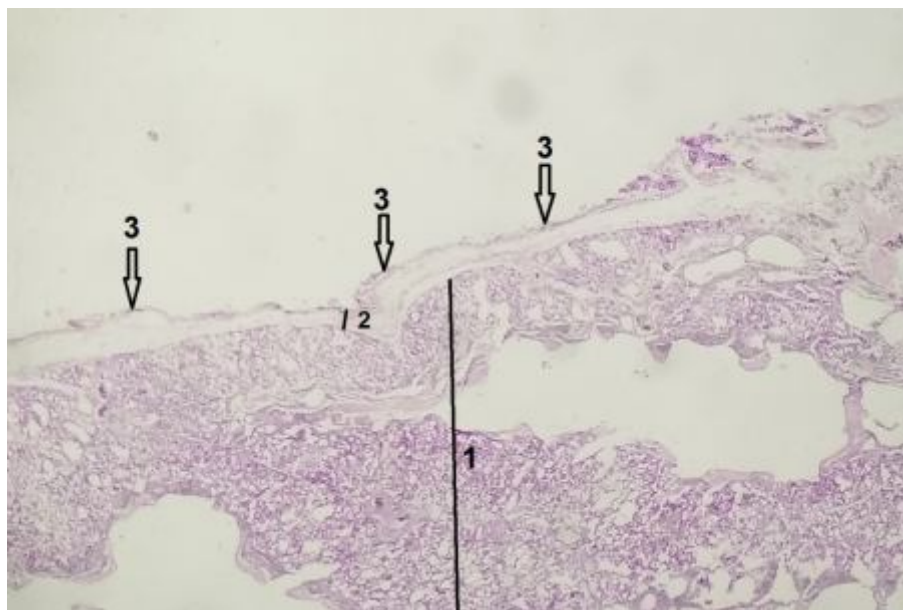


Figure 4. 1. Lung parenchyma, 2. connective capsule (visceral pleura) including; loose connective tissue, and 3. mesothelium cells with simple squamous arrangement. Hematoxylin-eosin staining (250x magnification).

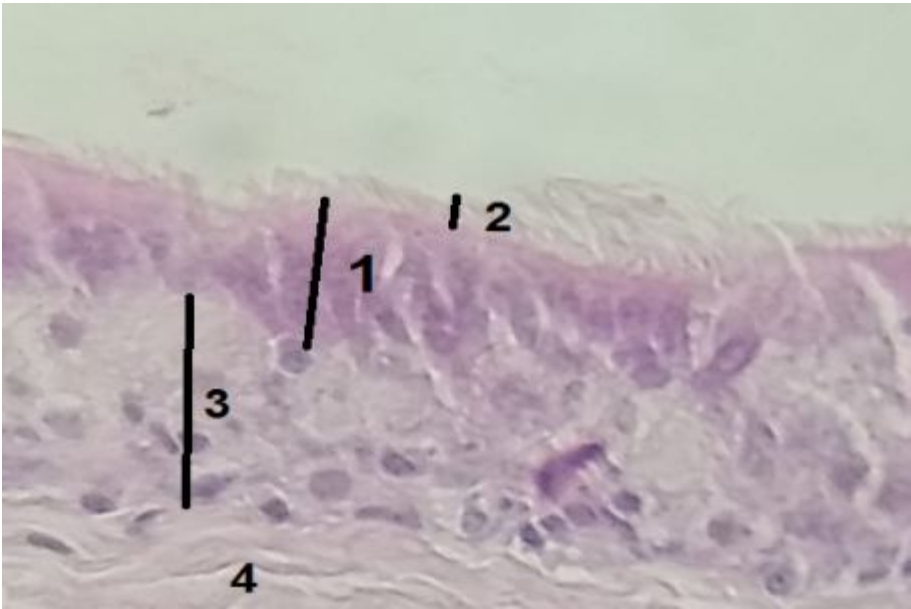


Figure 5. Epithelium of Primary bronchiole; 1. ciliated Stratified columnar epithelium, 2. cilia 3. lamina propria, 4. Submucosa. Hematoxylin-eosin staining (1000x magnification).

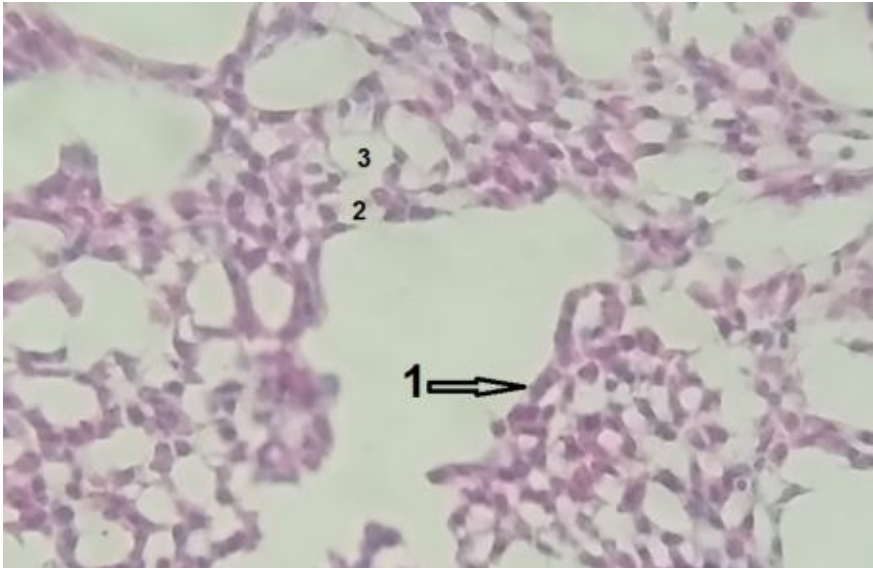


Figure 6. 1. Tertiary bronchiole with simple cuboidal epithelium (arrow), 2. Air capillary, 3. Atrium (where the tertiary bronchiole joins the air capillary). Hematoxylin-eosin staining (400x magnification).

Gender	Specifics	Diameter of principle bronchus (micrometer)	Diameter of tertiary bronchioles (micrometer)	diameter of Air capillary (micrometer)	Diameter of atria (micrometer)
Male	Right lung	2.89±.08 ^a	170.68±4.62 ^a	11.65±.16 ^a	25.05±0.51 ^a
Male	Left lung	3.03±.15 ^a	171.28±3.11 ^a	12.52±0.31 ^a	23.94±0.31 ^a
Female	Right lung	2.86±.09 ^a	159.88±4.11 ^a	11.49±.20 ^a	24.47±0.60 ^a
Female	Left lung	2.81±.11 ^a	173.50±3.70 ^a	11.94±0.25 ^a	23.42±0.27 ^a

Table 2. Mean ± standard error of the diameter of the principle bronchi, Para bronchioles, air capillaries and atria in the right and left lungs. Different lowercase letters indicate significant differences in columns (P<0.05).

Discussion

This study showed that similar to bee-eater bird (Al-Mamoori, 2014), yellow bulbul (Al-Ghakany, 2015), Colombian domestic pigeon (AL-Mahmodi, 2012) and wood pigeon (Alumeri *et al.*, 2013) the lungs of English white quail are pink in color and shaped like pyramidal organs. While, the lungs are trapezoidal and bright red in West African guinea fowl (Lbe *et al.*, 2008) and also trapezoidal, long rhomboidal or wide rectangular in duck, turkey and chicken respectively (Getty, 1975), also triangular in duck and goose (Koenig *et al.*, 2016). Our results were in agreement with the results obtained in the Colombian domestic pigeon, wood pigeon and bee-eater bird. In addition, the lungs of the English white quail extended from the first intercostal space to the sixth intercostal space, which was similar to Colombian domestic chickens and pigeons (Reda & Khaled, 2020) and this condition significantly increased The levels of oxygen exchange compared to a bird like the yellow bulbul, whose lung is stretched from first rib to forth rib (Schachner & Lyson, 2009; Lbe *et al.*, 2008; Al-Ghakany, 2015; Frandson *et al.*, 2009). Our study showed that the lungs of English white quail lacked lobulation, interlobular and lobular notches and also contained 5 incisures on the lung, which is similar to other studies on birds. (Getty, 1975).

The results of this study showed that the lung of English white quail has three (dorsal, visceral and ventral) surfaces. It was similar to the lung surfaces of Japanese quail (Japanese quail lung has dorsal (rib), visceral (middle) and ventral surfaces (Demirkan *et al.*, 2006). The lungs in the domestic Colombia pigeon (AL-Mahmodi, 2012), the domestic turkey and in other bird species (McLelland, 1990; Baumel *et al.*, 1993), have three surfaces: costal, vertebral and ventral surfaces while the lungs of the wood pigeon (Alumeri *et al.*, 2013), the bee-eater bird (Al-Mamoori, 2014) and the yellow bulbul (Al-Ghakany, 2015) have costal and visceral surfaces. In chicken and turkey lungs have a visceral surface (Maina, 2000). The histological investigations of the present study showed that the lungs of the English white quail, like the rest of the birds, have three types of bronchioles. The primary bronchioles in the parenchyma of the lung have several Long folds. They epithelium was ciliated Stratified columnar epithelium. Lamina propria and the sub mucosa is a

thin layer of loose connective tissue without cartilage. In addition, the muscularis mucosae present. These results are in agreement with the results of other investigations on most of the birds (Eurell & Frappier, 2013). In our study, the secondary bronchiole was similar to other birds; without folds, lacking cilia and having cuboidal to simple columnar epithelium, which were consistent with previous studies. (Eurell & Frappier, 2013; Bacha & Bacha, 2012). Tertiary bronchioles of English white quail, similar to other birds, had simple cuboid to simple squamous epithelium, and in the walls of these bronchioles, there were many atria and smooth muscle cells, which finally ended in air capillaries. These results were consistent with previous studies (Bacha & Bacha, 2012; Eurell & Frappier, 2013; Muthukrishnan *et al.*, 2008).

Conclusion

The lungs of English white quail were pyramidal, unlobated, soft and velvety in texture and light pink in color. They were embedded in the 1st to 6th thoracic inter vertebral position. The Para bronchi were surrounded by a dense network of air capillaries. The blood capillaries were intermingled with air capillaries. There were no significant differences in radiological structure, macroscopic and microscopic lung anatomy in male and female English white quail.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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مطالعه هیستوآناتومیکی و رادیولوژیکی ریه ها در بلدرچین سفید انگلیسی

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چکیده

زمینه و هدف: با توجه به اهمیت دستگاه تنفسی در مهره داران بویژه در پرندگان و با توجه به اینکه پرندگان، برای پرواز نیاز بیشتری به مصرف اکسیژن پیدا میکنند لذا تغییرات و سازگاریهای مهم و ویژه ای از لحاظ آناتومیک در این حیوانات به وجود آمده است. در این مطالعه برای بررسی هیستوآناتومیکی و رادیولوژیک ریه ها در بلدرچین سفید انگلیسی انجام شد. برای این منظور، ۱۶ بلدرچین نر و ماده سالم و بالغ سفید انگلیسی با وزن متوسط $160 \pm 5/35$ گرم مورد مطالعه قرار گرفت.

مواد و روش ها: در ابتدای امر تصاویر رادیولوژیک از پرندگان بصورت Lateral و Ventro-dorsal تهیه شد و سپس پرندگان مورد بررسی کالبدشناسی قرار گرفتند و فراسنجه های مربوط به وزن، طول، عرض و ضخامت ریه ها به دست آمد. در نهایت ریه ها از حفره عمومی بدن پرنده خارج و در محلول فرمالین ۱۰ درصد فیکس شدند. مقاطع بافتی از نمونه ها تهیه و مورد ارزیابی هیستومورفومتریک قرار گرفت.

یافته ها: بررسی رادیولوژیک نشان می دهد که ریه های راست و چپ در بلدرچین سفید انگلیسی در فضای بین دنده ای اول تا فضای بین دنده ای ششم قرار دارد. بررسی آناتومیک ضمن تایید نتایج رادیولوژیک، نشان می دهد که میانگین وزن ریه، نسبت وزن ریه به وزن کل بدن، طول ریه، عرض ریه و تعداد بریدگی های ریه در پرنده نر به ترتیب $0/981 \pm 0/065$ گرم، $0/0623 \pm 0/0045$ ، $2/57 \pm 0/030$ سانتیمتر، $1/53 \pm 0/021$ سانتیمتر و $5/00 \pm 0/00$ عدد و در پرنده ماده به ترتیب $0/935 \pm 0/028$ گرم، $0/0587 \pm 0/0023$ ، $2/50 \pm 0/033$ سانتیمتر، $1/34 \pm 0/024$ سانتیمتر و $5/00 \pm 0/00$ عدد می باشد. بر اساس نتایج هیستولوژیک، برونشیول های اولیه و ثانویه به ترتیب دارای اپیتلیوم استوانه ای مطبق مژه دار و اپیتلیوم مکعبی ساده می باشند و پارابرونشیول ها (برونشیول های ثالث) دارای اپی تلیوم سنگفرشی ساده تا مکعبی ساده هستند.

نتیجه گیری: ریه ها هرمی شکل، بدون لوب و به رنگ صورتی روشن در موقعیت بین مهره ای ۱ تا ۶ قرار دارند. هیچ تفاوت مشخصی در ساختار رادیولوژیک، آناتومیک و هیستولوژیک ریه در بلدرچین های سفید انگلیسی نر و ماده وجود نداشت.

واژه های کلیدی: ریه، هیستولوژی، آناتومی، رادیولوژی، بلدرچین

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