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Micromorphometric characteristics of the adrenal gland in birds

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Abstract. The adrenal gland is a peripheral organ of the endocrine system that directly affects the formation of bird productivity indicators, which is an important characteristic for the development of industrial poultry farming. The purpose of this study was a morphometric assessment of the microstructural components of the adrenal gland of birds of the order Galliformes (domestic quail, chicken, turkey), Anseriformes (Muscovy duck, domestic duck, and goose) and Columbiformes (common pigeon). Anatomical, histological, morphometric, and statistical research methods were used in this study. It was found that the thickness of the adrenal capsule of birds directly depends on their body weight and varies from 10.82 ± 0.56 μm (domestic quail) to 28.53 ± 1.36 μm (domestic turkey). Interrenal tissue compared to suprarenal tissue in Muscovy ducks, common pigeons, domestic ducks, and geese occupies a larger ($P < 0.001$) area of the central zone (3.50, 2.77, 3.10, and 3.11 times, respectively) and peripheral zone (1.27, 2.71, 1.38, and 1.55 times, respectively) of the adrenal gland, which indicates its morphofunctional activity. The area of the venous sinuses in the central zone compared to the peripheral zone of the adrenal gland is larger in domestic quail by 2.80 times ($P<0.05$), domestic chicken – by 3.62 times ($P < 0.05$), domestic turkey – by 3.68 times ($P < 0.05$), domestic ducks – by 5 times ($P < 0.01$), domestic ducks – by 3 times ($P < 0.05$), domestic geese – by 2 times ($P < 0.05$). The common pigeon is characterized by a uniform placement of venous sinuses along the entire periphery of the adrenal gland and, as a result, similar indicators of their area in the peripheral and central zones. The index of the nuclear-cytoplasmic ratio of endocrinocytes of the adrenal gland of birds varies. It is the lowest in the cells of the second type of interrenal tissue (from 0.052 ± 0.004 in the common pigeon to 0.092 ± 0.016 in the domestic quail), slightly higher in the cells of the first type of interrenal tissue (from 0.065 ± 0.004 in the common pigeon to 0.111 ± 0.012 in the domestic turkey) and the largest in chromaffin cells of the supranarial tissue (from 0.102 ± 0.015 in the domestic chicken to 0.166 ± 0.018 in the common pigeon). It is recommended to use the established features of the morphometric indicators of the microstructural components of the adrenal gland of birds to create a base for its normal morphological characteristics. This allows assessing the morphological and functional state of the adrenal gland under several factors and pathology

Keywords: adrenal zones, interrenal and suprarenal tissues, venous sinuses, endocrinocytes, nuclear-cytoplasmic ratio

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Introduction

Birds are a large class of vertebrates. Presently, about 10 thousand bird species from 27 genera and 170 families are known. Among them, over 5 thousand birds are songbirds. The total number of birds reaches 100 billion specimens. The largest number of species is recorded in countries such as Colombia (1,700), Ecuador (1,357), and Brazil (1,440). A much smaller number of bird species is typical for Cameroon (670), the USA and Canada (775), and very few – for Portugal (315) and Greece (339) (Batool *et al.*, 2020; Lees *et al.*, 2022).

Over many centuries, humans have domesticated several Galliformes – turkeys, guineafowl, chickens, quails, pheasants, peacocks; of Anseriformes – ducks, geese, mute swan; of Columbiformes – common pigeon. Man has learned to breed canaries, ostriches, cormorants, parrots, and other birds in captivity (Domyan & Shapiro, 2017).

Industrial poultry farming occupies a leading position among livestock industries (Hafez & Attia, 2020). At the beginning of the 21st century, the production of eggs and poultry meat increased fivefold (OECD-FAO Agricultural..., 2016). According to the OECD-FAO agricultural forecast for 2019-2028, poultry is the most consumed animal protein in the world (OECD-FAO Agricultural..., 2019).

The main area of development of modern poultry farming and veterinary medicine is to improve the productive qualities of birds and prevent diseases of various origins (Rudik *et al.*, 2021). Organizational and economic activities measures require an in-depth study of the bird body in general and the morphology of organs and systems in particular. Morphometric studies of endocrine organs in clinically healthy birds at the tissue and cellular levels are an urgent issue, since they can be used as morphological criteria for diagnosing diseases in humans and animals. The high efficiency of morphometric assessment of the state of the animal body at the organ, tissue, and cellular levels has been proven (Shevchenko, 2018).

The endocrine system, together with the nervous system, coordinates the activity of the entire body (Lotfi, 2018). According to the modern classification, the organs of the endocrine system are divided into three groups: central, peripheral, and mixed. They also include single endocrinocytes. They are located in non-endocrine organs, forming a dissociated endocrine system (Scanes, 2015).

An essential role in the functioning of the endocrine system of birds belongs to the adrenal gland. Its hormones affect tissue differentiation and growth, the development of reproductive organs, and the course of the sexual cycle (Banerjee, 2018; Kot *et al.*, 2021; Gjuen, 2022). They regulate water, protein, carbohydrate, fat and mineral metabolism (Lotfi *et al.*, 2018; Di Lorenzo, 2020). The adrenal gland is sensitive to changes in the internal and external environment. Stress, hypothermia, and diseases change the mass, size, vascularization of the adrenal glands, the ratio of cortical and cerebral zones, and their cytophysiological characteristics (Lotveld *et al.*, 2017; Qureshi *et al.*, 2020).

In birds, compared to mammals, the microscopic structure of the adrenal gland has class features of the histoarchitectonics of its structural components. Externally, the adrenal gland of birds is covered with a connective tissue capsule, under which the parenchyma is located. The latter is formed by suprarenal and interrenal tissues, which are represented by cell cords that are chaotically intertwined. The different configuration of cell cords of interrenal and suprarenal tissues allows conditionally distinguishing two zones – peripheral and central – on the section of the adrenal gland of birds. The narrow spaces between the cellular cords of the suprarenal and interrenal tissues are filled with loose fibrous connective tissue with venous sinuses and sinusoidal hemocapillaries (Kober *et al.*, 2012; Matos, 2008; Colcimen & Carmak, 2020; Prokopenko & Kot, 2021).

Morphology uses a variety of research methods: anatomical, pathoanatomical, histological, immunohistochemical, ultramicroscopic, histochemical, and morphometric. The latter method is characterized by high accuracy for assessing the structural and functional state of animals at the levels of organs, tissues, and cells in normal and pathological conditions (Bancroft & Gamble, 2007). Scientific sources mainly present information on organometry of the adrenal gland of birds (Kober *et al.*, 2012; Colcimen & Cakmak, 2020; Prokopenko & Kot, 2021). Information on the morphometry of microstructural components of the adrenal glands in birds is limited. Most authors investigated one to two adrenal micromorphometric parameters in birds of certain species and age groups. Specifically, Fathima & Lucy (2014) established an inter-suprarenal ratio of the adrenal glands in domestic ducks. Matos (2008) determined the height of endocrinocytes and the diameter of their nuclei in this gland in domestic chicken. Moghadam & Mohammadpour (2017) specified the relative volume of the interrenal and suprarenal tissues of the adrenal gland in domestic guineafowls. El-Desoky & El-Zahraa (2021) noted the area of interrenal and suprarenal adrenal tissues in domestic quail. There are no data from a comprehensive morphometric study of the microstructural components of the adrenal gland of birds in the comparative-species aspect. The issue of cytomentric parameters of adrenal endocrinocytes in birds is not covered.

Therefore, the purpose of this study was a comprehensive morphometric assessment of the microstructural components of the adrenal gland of birds of various species, the indicators of which are morphological criteria of physiological and pathological changes in the endocrine system and can be used during the diagnosis of diseases of various aetiology.

To fulfil the purpose of the study, it was necessary to solve the following tasks after anatomical and histological examination of the adrenal gland: to establish the thickness of the adrenal capsule; to find the area of suprarenal, interrenal tissues, and venous sinuses in the peripheral and central zones of the adrenal gland; to conduct morphometric analysis of adrenal endocrinocytes.

Literature Review

The specialized literature of the last five years presents information on the morphology of the adrenal gland of fish, amphibians, reptiles (Gaber & Abdel-maksoud, 2019; Di Lorenzo *et al.*, 2020) and mammals (Kigata & Shibata, 2018; Zakrevska & Tybinka, 2019). Regarding birds, most of the studies by ornithologists and morphologists provide data on the anatomical and microscopic structure of the adrenal gland of various bird species (Qureshi *et al.*, 2020; Prokopenko & Kot, 2021). The adrenal gland is a parenchymatous organ, the microscopic structure of which corresponds to the regularities of the structure and function of endocrine

organs. There are no excretory ducts in the endocrine organs. The products of their activity (hormones) are released directly into the lymph and blood. In terms of the structure, endocrine organs consist of connective tissue stroma and parenchyma. The latter forms follicles, rods, islands, the cells of which are in close contact with the vessels of the microcirculatory channel. There are many blood capillaries between the structural components of the adrenal parenchyma, mainly of the sinusoid type (Scanes, 2015; Kigata & Shibata, 2018; Zakrevska & Tybinka, 2019). Externally, the adrenal gland of birds is covered with a capsule, which is formed by loose fibrous connective tissue, contains blood vessels and clusters of nerve cells. In the common pigeon, nodes of the autonomic nervous system are also registered in it, in the domestic chicken – supplementary adrenal glands.

In birds of the Anseriformes order, the outside of the capsule of the adrenal gland contains a layer of adipose tissue, in Muscovy duck – a group of nodes of the autonomic nervous system (Kot *et al*., 2021; Prokopenko & Kot, 2021). The results of the morphometric study of the macro- and microscopic components of the adrenal gland of birds are incomplete, scattered, and refer to individual species. There is evidence that the mass, length, thickness, and width of the right and left adrenal glands of birds are not the same. In domestic birds, the left adrenal gland has a larger mass (Prokopenko & Kot, 2021). The absolute mass index of the right and left adrenal glands of domestic chicken is 97.2 ± 1.2 and 104.1 ± 1.4 mg; length – 0.8 ± 0.0 and 0.9 ± 0.0 cm; width – 0.6 ± 0.0 and 0.5 ± 0.0 cm; thickness – 0.5 ± 0.0 and 0.4 ± 0.0 cm, respectively (Kober *et al*., 2012).

According to Fathima *et al*. (2014), adrenal gland mass is more correlated with bird age than with body weight. In common pigeon (*Columba livia*), house sparrow (*Passer domesticus*), house crow (*Corvus splendens*), common myna (*Acridotheres tristis*), bank myna (*Acridotheres ginginianus*), black kite (*Milvus migrans*), grey francolin (*Francolinus pondicerianus*), and in western cattle egret (*Bubulcus ibis*), the mass of adrenal glands increases during the period of their sexual activity (Vyas & Jacob, 1976). In addition, the organometric indicators of the adrenal gland of birds are affected by their growing conditions, stress, and the effect of pharmacological preparations (Qureshi *et al*., 2020; Rudik *et al*., 2021).

In terms of the morphometric parameters of the microstructural components of the adrenal gland of birds, the ratio between the interrenal and suprarenal tissues is determined by the species, age, and sex of the birds. According to Kober *et al*. (2012), in the adrenal gland of domestic chicken, the proportion of supraranal tissue in the central zone (49.7%) is twice as high as in the peripheral zone (24.8%). In general, the inter-supraranal ratio is 1.6:1. In the domestic duck, the inter-suprarenal ratio of the adrenal gland changes from 1.15:1 (one day old) to 2:1 (24 weeks old) due to an increase in the proportion of interrenal tissue in the gland (Fathima & Lucy, 2014). In domestic chicken, the inter-suprarenal ratio in males and females is not the same – 1.9:1 and 1.43:1, respectively. In a 35-day-old domestic quail, this indicator is 1:1, and by the 55th day it changes to 6:1 (Matos, 2008).

Morphological analysis of the cells of the adrenal parenchyma of birds shows that the endocrinocytes of the suprarenal tissue have a basophilic cytoplasm, a polygonal shape, and a rounded nucleus located in the centre (Vyas & Jacob, 1976; Prokopenko & Kot, 2021). In domestic chickens, the height of cells and the diameter of their nuclei in the adrenal glands of males (8.72 ± 0.231 μm and 4.39 ± 0.359 μm, respectively) are greater than in females (8.67 ± 0.218 μm and 3.84±0.326 μm, respectively) (Matos, 2008). Cells of the interrenal adrenal tissue of birds have an eosinophilic stained cytoplasm, columnar or cubic shape, rounded or oval nucleus, which is located eccentrically (Fathima & Lucy, 2014; Prokopenko & Kot, 2021). In domestic chickens, the height of cells and the diameter of their nuclei in the adrenal glands of males (8.96 ± 0.159 μm and 4.26 ± 0.166 μm, respectively) are smaller than in females (10.57 ± 0.628 μm and 3.69 ± 0.087 μm, respectively) (Matos, 2008).

Materials and Methods

The study was conducted during 2019-2022 in the Educational and Scientific Clinical and Diagnostic Laboratory of the Faculty of Veterinary Medicine of the Polissia National University (Zhytomyr). The study performed is a fragment of the research "Morphology of the adrenal gland of birds" (State Registration No. 0120U101089). The adrenal gland was collected from sexually mature birds of such species as domestic quail (*Coturnix coturnix. var. domesticus*), domestic chicken (*Gallus gallus. var. domesticus*), domestic turkey (*Meleagris gallopavo. var. domesticus*), domestic duck (*Anas platyrhynchos. var. domesticus*), Muscovy duck (*Cairina moschata*), domestic goose (*Anser anser. var. domesticus*) and common pigeon (*Columba livia*).

The test birds were clinically healthy and showed no signs of disease. All intervention and slaughter of birds was carried out in compliance with the recommendations of Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010 "On the Protection of animals used for Scientific Purposes" (Directive 2010/63/EU..., 2010) and accordingly to the Law of Ukraine No. 692 "On the Protection of Animals from Cruel Treatment" (3447-IV) dated February 21, 2006 (On the Protection..., 2006).

Morphometric studies of the adrenal glands of birds were preceded by anatomical and histological studies. Anatomical studies included weighing of birds, their slaughter and exsanguination, dissection of the chest cavity, preparation of the adrenal gland and its subsequent removal (Reavill & Schmidt, 2019). Avian euthanasia included an inhaled overdose of chloroform followed by acute exsanguination due to subclavian artery incision (Brooks Brownlie & Munro, 2016). Body weight of birds (233.17 ± 3.84 g – domestic quail, 1,703.33 ± 37.74 g – domestic chicken, 4,330.00 ± 67.18 g – domestic turkey, 2,145.17 ± 16.86 g – Muscovy duck, 2,492.50 ± 12.23 g – domestic duck, 3,147.50 ± 26.13 g – domestic goose, 327.00±13.28 g – common pigeon) was determined by weighing on a PS6000/C/2 scale (Poland). For histological examination, the adrenal glands of birds were fixed in a 10% aqueous neutral solution of formalin, gradually dehydrated in 40°, 70°, 96° and 100° ethyl alcohol, compacted in one portion of alcohol-xylene in a 1:1 ratio and two servings of xylene, poured into paraffin at a temperature not exceeding 60°C. Histological sections 5-8 μm thick were made from paraffin blocks on a sled microtome MC-2 (Ukraine). They were placed on slides and stained with hematoxylin and eosin (Mulisch & Welsch 2015; Bancroft & Gamble, 2019).

Morphometric methods were used to obtain objective morphometric parameters of the microstructural components of the adrenal glands in birds. For this, the authors of this study used the Aperio ImageScope software (Leica Biosystem Inc. USA, 2021). The following morphometric parameters were determined: capsule thickness, area of supreranal and interrenal tissues, venous sinuses, volume of endocrinocytes and their nuclei, nuclear-cytoplasmic ratio. These morphometric parameters were determined on 5 preparations from each bird, in 10 fields of view of a Primo Star microscope (Carl Zeiss, Germany).

Since the endocrinocytes of the adrenal gland of birds have a shape close to an ellipsoid, the Equation 1 of W. Jacobi was used to determine the volume of these cells and their nuclei (Bancroft & Gamble, 2018).

$$V = \frac{\pi}{6} \times A \times B^2 \quad (1)$$

where V is the cell volume;
π is 3.14; A – large diameter;
B – small diameter.

The nuclear-cytoplasmic ratio is the main cytomorphometric indicator of the level of cell differentiation and metabolism in animals, depending on their living conditions (Gaber & Abdel-maksoud, 2019). Therefore, thanks to this indicator, it is possible to establish the functional activity of adrenal endocrinocytes. The nuclear-cytoplasmic ratio was determined according to the Equation 2:

$$NCR = \frac{Vnucleus}{Vcells - Vnucleus} \quad (2).$$

Digital data of morphometric studies were processed using the variational statistical method using the Statistica 6 software package (Stat Soft Inc., USA). The analysis of the obtained data was based on indicators of descriptive statistics, specifically the arithmetic mean (M) and the standard error of the mean (m). The reliability of the results obtained was evaluated according to the Fischer F-criterion. The difference between the two values was considered significant at $P < 0.05$; $P < 0.01$; $P < 0.001$.

This study is a continuation of a series of works investigating the features of the morphology of the adrenal glands of sexually mature birds in the species aspect (Kot *et al.*, 2021; Prokopenko & Kot, 2021). Preliminary studies were conducted on the topography, shape, consistency, and colour of the adrenal gland of birds; determination of the mass, length, thickness, and width of the adrenal gland of birds; clarification of the features of the microscopic structure of the adrenal gland of birds.

Results and Discussion

Analysis of morphometric studies has proved that the thickness of the adrenal capsule of chickens directly depends on their body weight. The greatest absolute mass of the adrenal gland is typical for the domestic turkey (28.53 ± 1.36 μm), slightly smaller – for the domestic chicken (20.12 ± 1.41 μm), and the smallest – for the domestic quail (10.82 ± 0.56 μm). An analogous dependence of this indicator on body weight was recorded in birds of the Anseriformes order. The thickness of the capsule of the adrenal gland of the domestic goose (27.0 ± 0.89 μm) is the largest, the duck (21.30 ± 1.09 μm) is the smallest, and the domestic duck (24.82 ± 0.51 μm) is intermediate. In the common pigeon (Columbidae order), the thickness of the adrenal capsule occupies an intermediate position among all the birds under study and is 13.46 ± 0.67 μm (Fig. 1).

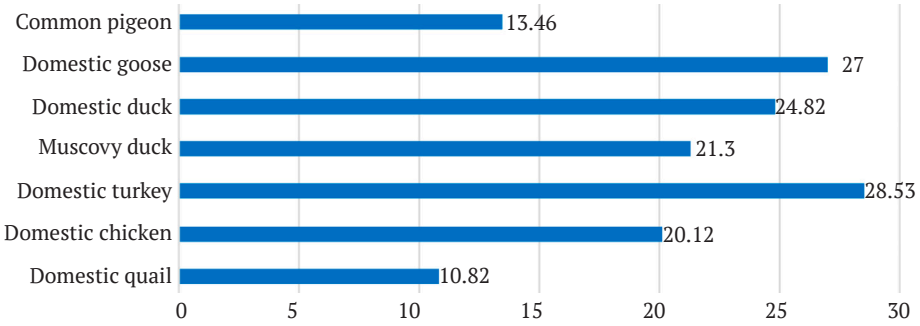

Figure 1. Capsule thickness (μm) of the adrenal gland of birds

The parenchyma of the adrenal gland of birds is formed by cell cords of suprarenal and interrenal tissues that are closely intertwined. Between the cords of these tissues, layers of loose fibrous connective tissue with venous sinuses and sinusoidal hemocapillaries are observed (Prokopenko & Kot, 2021; Erdem *et al.*, 2021). The specific features of the location and configuration of these cell cords are determined by the formation of two zones of the adrenal gland parenchyma of the birds under study – peripheral and central, which confirms the research of other authors who investigated the adrenal gland of the domestic goose (Ye *et al.*, 2018; Grymak *et al.*, 2020). H. Kober *et al.* (2012), investigating the adrenal gland of a domestic chicken, recorded the peripheral and central zones, as well as the subcapsular layer, which corresponds to the research by Fathima and Lucy (2014) in the domestic duck. In the adrenal gland of the ostrich, the central and peripheral zones are distinguished. The latter consists of the outer (subcapsular zone) and inner parts (Ye *et al.*, 2018).

The morphometric study allowed establishing that in the peripheral zone of the adrenal gland, the area of the interrenal tissue compared to the area of the suprarenal tissue is significantly larger in all species of birds under study. Specifically, in domestic quail – by 2.65 times, $P < 0.001$ (71.67 ± 2.93 vs. 27.0 ± 3.17%); domestic chickens – by 2.66 times, $P<0.001$ (71.87 ± 3.62 vs. 27.01 ± 2.64%); turkeys – by 2.85 times, $P < 0.001$ (73.22 ± 5.12 vs. 25.67 ± 4.18%); Muscovy duck – by 3.5 times, $P < 0.001$ (77.0 ± 4.95 vs.

22.0 ± 3.17%); domestic duck – 3.10 times, P < 0.001 (74.5 ± ± 3.54 vs. 24.0 ± 3.65%); domestic goose – 3.11 times, P < 0.001 (74.17 ± 4.09 vs. 23.83 ± 4.35%); grey pigeon – 2.77 times, P < 0.001 (71.5 ± 3.46 vs. 25.83 ± 3.51%). Such ambiguous morphometric indicators of the interrenal and suprarenal tissues of the adrenal gland of experimental birds are consistent with the results of the study of its inter-suprarenal ratio by other researchers (Vyas & Jacob, 1976; Matos, 2008; Grymak *et al*., 2020). According to (Matos, 2008), the inter-suprarenal ratio of the adrenal gland of birds can vary widely from 2:1 (domestic duck) to 6:1 (domestic quail). Venous sinuses occupy the smallest part of the peripheral zone of the adrenal gland of the birds under study. The indicator of their area varies in the birds of the Galliformes order – from 1.12 ± 0.47% (domestic chicken) to 1.33±0.33% (domestic quail), in the birds of the Anseriformes order – from 1.0 ± 0.26% (Muscovy duck) to 2.0 ± 0.63% (domestic goose). In common pigeon, the value of this indicator is the highest among the birds under study and is 2.67 ± 0.33% (Fig. 2).

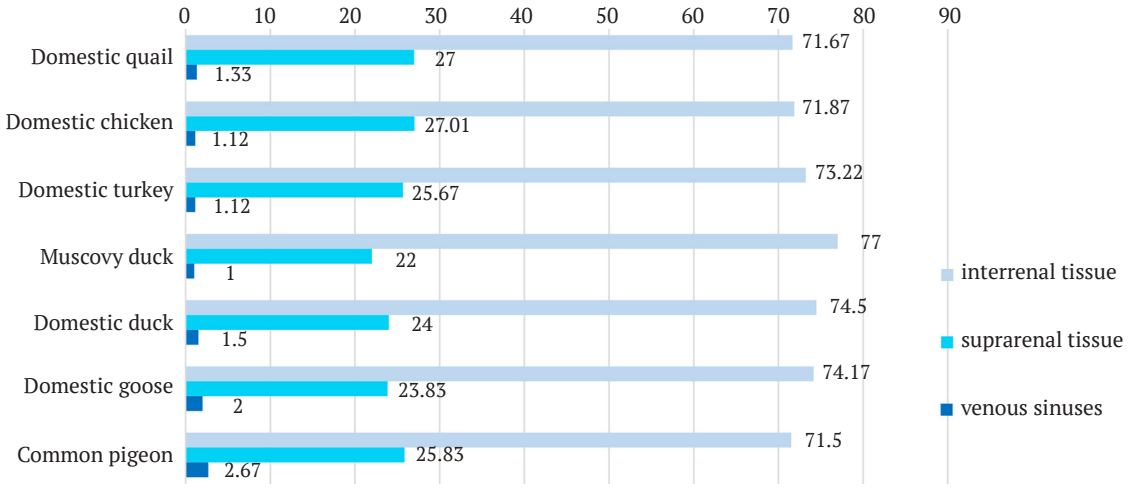

Figure 2. Area (%) of structural components of the peripheral zone of the adrenal gland of birds

In the central zone of the adrenal gland compared to the peripheral zone of domestic quail, chicken, turkey, duck, goose, and Muscovy duck, a significant (P < 0.001) decrease in the area of the interrenal tissue was registered by 1.43, 1.50, 1.44, 1.35, 1.35, and 1.45 times, respectively, to 50.21 ± 1.85%, 48.09 ± 2.03, 50.83 ± 2.30, 55.0 ± 4.68, 58.33 ± 3.07, and 53.08 ± 3.82%, respectively. The indicator of the area of suprarenal tissue of birds of these species, on the contrary, significantly increased (P < 0.001) to 46.88 ± 4.75%, 47.86 ± 2.83, 45.05 ± 2.40, 40.5 ± 5.03, 37.67 ± 2.86 and 41.92 ± 3.37%, respectively. As a result, in birds of the Galliformes order, interrenal and suprarenal tissues occupy a relatively equal share of the central zone of the adrenal gland.

In Anseriformes birds, the area of interrenal tissue is significantly (P < 0.001) larger than the area of suprarenal tissue by 1.27 times (Muscovy duck), 1.38 times (domestic duck), and 1.55 times (domestic goose), which indicates a greater morphofunctional activity of interrenal tissue in this area of the adrenal gland. In common pigeon, the area of the interrenal and suprarenal adrenal tissues in the central zone (71.0 ± 3.50 and 26.17 ± 3.56%, respectively) does not significantly differ from that in the peripheral zone (71.50 ± 3.46 and 25.83 ± 3.51%, respectively) (Fig. 3).

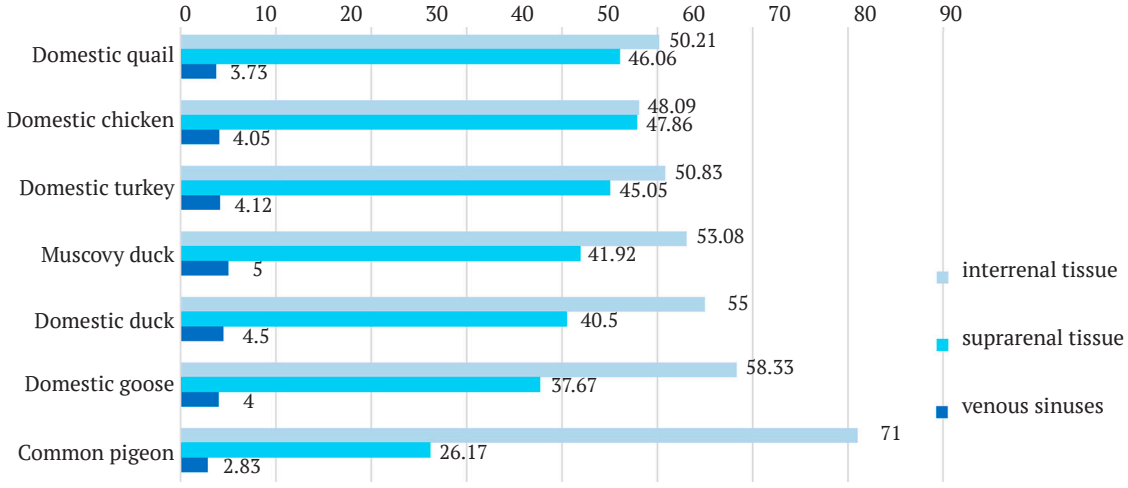

Figure 3. Area (%) of structural components of the central zone of the adrenal gland of birds

In terms of venous sinuses, the indicator of their area in the central zone compared to the peripheral adrenal gland increases in most of the species of birds under study. Specifically, in domestic quail – by 2.80 times (3.73 ± 0.25%, $P < 0.05$), in domestic chickens – by 3.62 times (4.05 ± 0.12%, $P < 0.05$), turkeys – 3.68 times (4.12 ± 0.51%, $P < 0.05$), Muscovy ducks – 5 times (5.0 ± 0.52%, $P < 0.01$), domestic ducks – 3 times (4.5 ± 0.46%, $P < 0.05$), domestic goose – 2 times (4.0 ± 0.26%, $P < 0.05$). In common pigeon, there was no significant difference between the area of the venous sinuses of the central (2.83 ± 0.48%) and peripheral (2.67 ± 0.33%) zones of the adrenal gland (Figs. 2, 3), which indicates the uniform placement of this microstructure in the adrenal parenchyma (Kot *et al.*, 2021; Erdem *et al.*, 2021).

According to the special literature on morphological characteristics of adrenal parenchyma cells in domestic goose (Prokopenko & Kot, 2021), endocrinocytes of suprarenal and interrenal tissues differ in shape, location of the nucleus, and colour of the cytoplasm. The former have a basophilic cytoplasm, while the latter have an acidophilic one. The same two types of cells (basophilic and acidophilic, respectively) were differentiated in domestic ducks by other researchers (Fathima & Lucy, 2014), which contradicts the data of Matos (2008) on four types of cells of the interrenal adrenal tissue of domestic ducks and guineafowl.

To assess the morphological and functional state of the cellular component of the adrenal parenchyma of birds, the volume of endocrinocytes of interrenal and supraranal tissues was found (Figs. 4, 5). According to the obtained digital data, interrenal tissue cells can be divided into two types. Cells of the first type were placed in the peripheral zone, cells of the second type – in the central zone of the adrenal gland of birds. The volume of cell nuclei of these two types probably did not differ and ranged within 11.83 ± 0.65-14.0 ± 1.03 μm³ (domestic quail), within 18.50 ± 1.12-18.66 ± 1.5 μm³ (domestic chicken), within 20 ± 1.69-22 ± 1.13 μm³ (domestic turkey), within 19 ± 1.86-20.0 ± 1.29 μm³ (Muscovy duck), within 19.83 ± 1.08-20.17 ± 1.05 μm³ (domestic duck), within 22 ± 2.35-23.17 ± 1.94 μm³ (domestic goose), within 20.17 ± 1.25-21.33 ± 1.82 μm³ (common pigeon). However, the indicator of the volume of cells of the second type compared to the volume of cells of the first type was significantly greater in all birds under study. Specifically, in domestic quail – by 1.31 times, $P < 0.001$ (171.82 ± 15.12 vs. 131.0 ± 16.02 μm³); domestic chicken – by 1.19 times, $P < 0.01$ (249.5 ± 16.53 vs. 209.67 ± 24.11 μm³); turkey – by 1.43 times, $P < 0.001$ (286.0 ± 13.65 vs. 200.17 ± 13.34 μm³), Muscovy duck – by 1.32 times, $P < 0.001$ (265.67 ± 33.7 vs. 200.67 ± 18.92 μm³), domestic duck – by 1.14 times, $P < 0.01$ (299.83 ± 25.08 vs. 262.67 ± 24.06 μm³), domestic goose – by 1.22 times, $P < 0.001$ (367.67 ± 38.72 vs. 300.33 ± 23.33 μm³), common pigeon – 1.17 times, $P < 0.001$ (401.17 ± 22.15 vs. 342.33 ± 13.80 μm³).

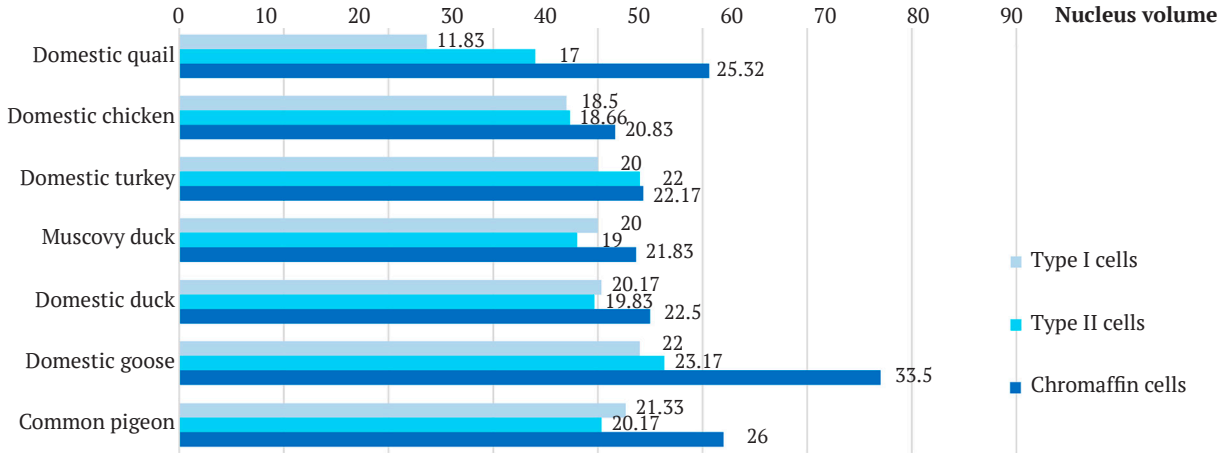

Figure 4. The volume of the nucleus (μm³) of endocrinocytes of the adrenal gland of birds

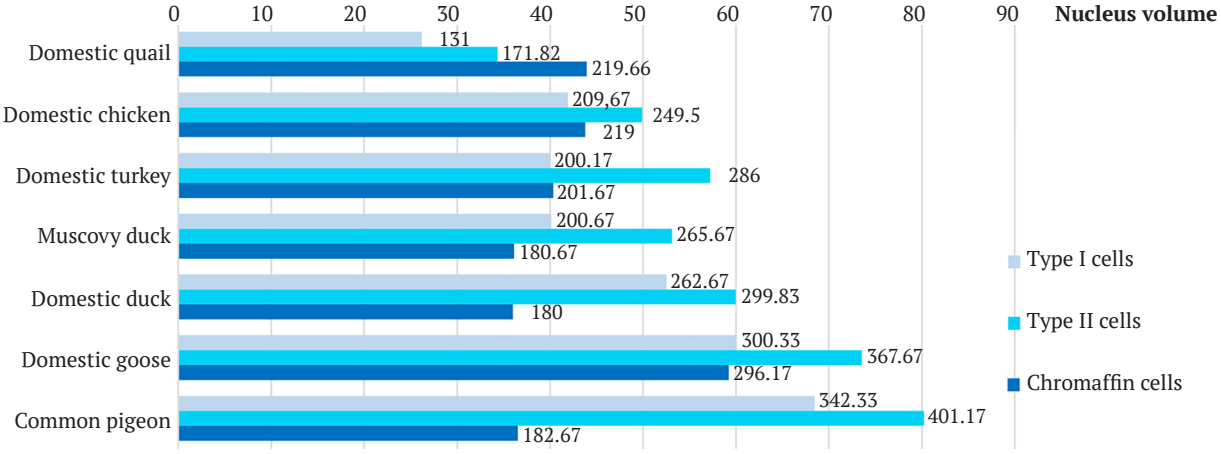

Figure 5. Cytoplasm volume (μm³) of endocrinocytes of the adrenal gland of birds

Consequently, the cells of the second type of interrenal tissue located in the central adrenal zone of the birds under study are the largest, and probably the most functionally active. They possibly correspond to zona fasciculi cells in the adrenal gland of mammals, which are also characterized by large sizes (Kigata & Shibata, 2018; Zakrevska & Tybinka, 2019). Endocrinocytes of the zona fasciculi of the adrenal gland synthesize cortisone, hydrocortisone, and corticosterone. These hormones regulate the metabolism of carbohydrates, proteins, and lipids, suppress inflammatory processes, and stimulate energy metabolism (Scanes, 2015). According to U. Moawad *et al*. (2017), the cytoplasm of acidophilic cells of the domestic chicken adrenal gland contains many mitochondria, ribosomes, lipid droplets, as well as agranular and granular endoplasmic reticulum.

As noted above, basophilic cells form the suprarenal tissue of the adrenal glands. Individual authors (Fathima & Lucy 2014; Moghadam, & Mohammadpour 2017; El-Desoky & El-Zahraa, 2021) call these cells chromaffin cells because of their ability to reduce chromium, silver, and osmium oxides. The cytoplasm of chromaffin cells contains mitochondria with tubular crystals, ribosomes, endoplasmic reticulum, lipid droplets, and secretory granules. Depending on the shape of the secretory granules, they are divided into two types: norepinephrine cells containing secretory granules with an electron-dense nucleus limited by a light border and adrenaline cells containing homogeneous polymorphic electron-dense secretory granules (Moawad & Hassan, 2017). It is proved that hormones of adrenal chromaffin cells and mediators of the sympatho-adrenal system belong to the main regulators of adaptive responses of the body and to exo- and endogenous factors. The biological effects of catecholamines contribute to the transition of the body from a state of rest to a state of arousal, and also ensure its stay in this state for a long time (Scanes, 2015).

In the specialized literature, information on the morphometry of chromaffin cells of the adrenal gland is contradictory and incomplete (Matos, 2008; Kober *et al*., 2012; El-Desoky & El-Zahraa, 2021). According to the results of morphometric studies, it was found that the chromaffin cells of the adrenal glands of birds were characterized by a large nucleus. Its volume exceeded that of cells of the second type of interrenal tissue and was 25.32 ± 1.05 μm³ (domestic quail), 20.83 ± 2.06 μm³ (domestic chicken), 22.17 ± 1.22 μm³ (domestic turkey), 21.83±1.42 μm³ (Muscovy duck), 22.50 ± 1.02 μm³ (domestic duck), 33.50 ± 3.68 μm³ (domestic goose), 26 ± 1.69 μm³ (common pigeon). Therewith, the significant ($P < 0.05$) difference between these indicators was recorded in domestic quail, domestic goose, and common pigeon. In terms of the volume of chromaffin cells, this indicator is probably smaller compared to the volume of cells of the second type of interrenal tissue of the domestic chicken by 1.13 times, $P < 0.01$ (219 ± 24.09 μm³); domestic turkey – by 1.42 times, $P < 0.001$ (201.67 ± 16.59 μm³); Muscovy ducks – by 1.47 times, $P < 0.001$ (180.67 ± 13.16 μm³); domestic ducks – by 1.67 times, $P < 0.001$ (180 ± 20.22 μm³), domestic geese – by 1.24 times, $P < 0.001$ (269.17±26.15 μm³), common pigeon – by 2.2 times (182.67 ± 17.60 μm³). Compared to the volume of cells of the first type, the volume of chromaffin cells decreased by 1.11 times ($P < 0.05$) in Muscovy duck and by 1.46 times ($P < 0.05$) in domestic duck. In domestic quail, on the contrary, the volume of chromaffin cells significantly ($P < 0.001$) exceeded the volume of cells of the first and second type of interrenal tissue by 1.67 and 1.28 times, respectively, and amounted to 219.66 ± 19.33 μm³ (Figs. 4, 5).

The volume of the nucleus and cytoplasm of somatic cells are unstable values. Their average values vary with the age of animals, biological rhythms, gender, etc. (Gyorfy *et al*., 2021). Therefore, during the analysis of the morphological and functional activity of cells, according to the results of cytometric indicators, it is advisable to consider the nuclear-cytoplasmic ratio of endocrinocytes, which, undoubtedly, is a criterion for evaluating the endocrine function of the adrenal gland (Zakrevska & Tybinka, 2019). It was found that domestic quail, chicken, turkey, duck, goose, Muscovy duck, and blue pigeon have the lowest nuclear-cytoplasmic ratio inherent in cells of the second type of interrenal tissue (respectively: 0.092 ± 0.016, 0.082 ± 0.009, 0.083 ± 0.010, 0.072 ± 0.010, 0.066 ± 0.005, 0.076 ± 0.007, and 0.052 ± 0.004), slightly larger – for cells of the first type of interrenal tissue (respectively: 0.109 ± 0.009, 0.097 ± 0.012, 0.111 ± 0.012, 0.081 ± 0.008, 0.079 ± 0.007, 0.110 ± 0.015, and 0.065 ± 0.004), and the largest – for chromaffin cells of the suprarenal tissue (respectively: 0.113 ± 0.014, 0.102 ± 0.015, 0.112 ± 0.010, 0.138 ± 0.007, 0.126 ± 0.006, 0.137 ± 0.003, and 0.166 ± 0.018) (Fig. 6). Such ambiguous cytometric parameters of endocrinocytes may be associated with their various morphofunctional activities, specifically with the development of their hormone-synthesizing apparatus and the state of metabolism.

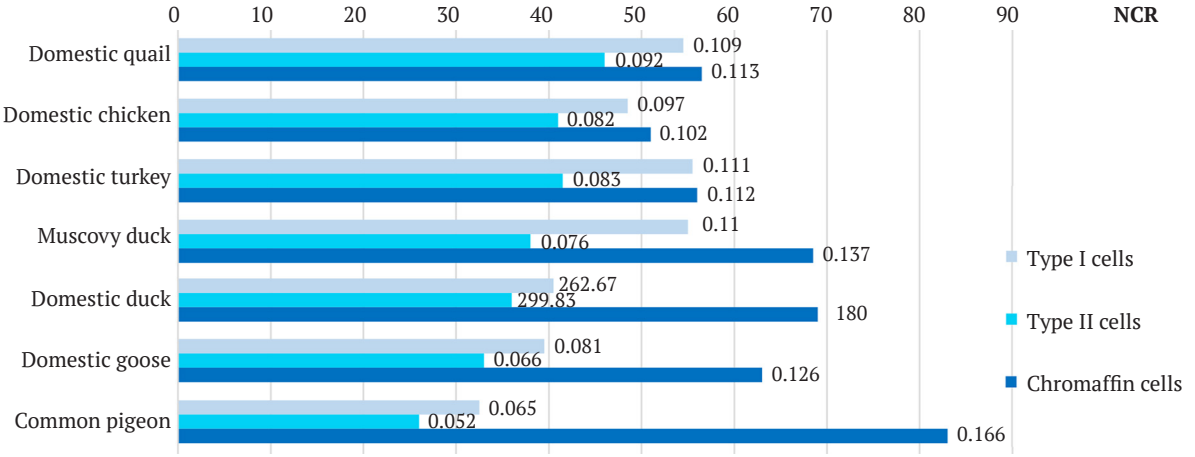

Figure 6. The nuclear-cytoplasmic relationship of endocrinocytes of the adrenal gland of birds

Thus, the adrenal gland of the birds under study has certain differences in morphometric parameters of microstructural components. They can be used to create a base for the normal morphological characteristics of the adrenal gland. This allows assessing the morphofunctional state of the adrenal gland of birds of individual species under a range of factors and pathology.

Conclusions

Morphometric parameters of the microstructural components of the adrenal gland of birds of the Galliformes order (domestic quail, domestic chicken, domestic turkey), Anseriformes (Muscovy duck, domestic duck, domestic goose) and Columbidae (common pigeon) are not the same. They also differ in individual species within the same order. The thickness of the adrenal capsule of birds directly depends on their body weight. Values of indicators of the area of interrenal and suprarenal tissues testify to different morphofunctional activity of these tissues, specifically to more intensive development of interrenal tissue in the peripheral zone of the adrenal gland of all birds under study. In the central zone of the adrenal gland of domestic quail, chicken, and turkey, the indicators of the area of interrenal and suprarenal tissues probably do not differ, which is an inherent morphometric feature of this microstructure of the adrenal gland of chickens.

Indicators of the area of venous sinuses in the central and peripheral zone of the adrenal gland of the common pigeon (Columbidae order) do not differ significantly, which corresponds to the uniform placement of venous sinuses in the parenchyma of the organ under study. In the adrenal gland of birds of the Galliformes and Anseriformes orders, the central zone, in contrast to the peripheral one, is characterized by greater vascularization, which was reflected in the values of the venous sinus area indicator. The nuclear-cytoplasmic ratio is the smallest in the cells of the second type of interrenal tissue (from 0.052 ± 0.004 in common pigeon to 0.092 ± 0.016 in domestic quail), slightly higher in the cells of the first type of interrenal tissue (from 0.065 ± 0.004 in common pigeon to 0.111 ± 0.012 in domestic turkey) and the largest in the chromaffin cells of the suprarenal tissue (from 0.102 ± 0.015 in domestic chicken to 0.166 ± 0.018 in common pigeon), which is associated with the morphological and functional activity of endocrinocytes, namely with the development of their hormone-synthesizing apparatus and the state of metabolism.

Further research intends to involve the histochemical study of the structural components of the adrenal glands in birds at the tissue and cellular levels.

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Мікроморфометрична характеристика надниркової залози птахів

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Анотація. Надниркова залоза – периферичний орган ендокринної системи, який має безпосередній вплив на формування показників продуктивності птахів, що є важливою характеристикою для розвитку промислового птахівництва. Мета роботи полягала у проведенні морфометричної оцінки мікроструктурних компонентів надниркової залози птахів ряду Куроподібні (свійські перепел, курка, індик), Гусеподібні (індокачка, свійські качка і гуска) та Голубоподібні (голуб сизий). Використано анатомічні, гістологічні, морфометричні та статистичні методи досліджень. Встановлено, що товщина капсули надниркової залози птахів прямо залежить від маси їх тіла і варіює від 10,82 ± 0,56 мкм (свійський перепел) до 28,53 ± 1,36 мкм (свійський індик). Інтерреналова тканина порівняно з супрареналовою тканиною в індокачки, голуба сизого, свійських качки і гуски займає більшу ($P < 0,001$) площу центральної зони (відповідно в 3,50, 2,77, 3,10 і 3,11 раза) і периферичної зони (відповідно в 1,27, 2,71, 1,38 і 1,55 раза) надниркової залози, що свідчить про її морфофункціональну активність. Площа венозних синусів у центральній зоні порівняно з периферичною зоною надниркової залози більша у свійського перепела – в 2,80 раза ($P < 0,05$), свійської курки – в 3,62 раза ($P < 0,05$), свійського індика в 3,68 раза ($P < 0,05$), індокачки в 5 разів ($P < 0,01$), свійської качки в 3 рази ($P < 0,05$), свійської гуски в 2 рази ($P < 0,05$). Для голуба сизого характерне рівномірне розміщення венозних синусів по всій периферії надниркової залози і як наслідок близькі за значенням показники їх площі у периферичній та центральній зонах. Показник ядерно-цитоплазматичного відношення ендокриноцитів надниркової залози птахів різниться, зокрема найменший він у клітинах другого типу інтерреналової тканини (від 0,052 ± 0,004 у голуба сизого до 0,092 ± 0,016 у свійського перепела), дещо більший – у клітинах першого типу інтерреналової тканини (від 0,065 ± 0,004 у голуба сизого до 0,111 ± 0,012 у свійського індика) і найбільший – у хромафінних клітинах супрарналової тканини (від 0,102 ± 0,015 у свійської курки до 0,166 ± 0,018 у голуба сизого). Встановлені особливості морфометричних показників мікроструктурних компонентів надниркової залози птахів рекомендовано використовувати для створення бази її нормальної морфологічної характеристики. Це дасть можливість робити оцінку морфо-функціонального стану надниркової залози в умовах впливу різних факторів і за патології

Ключові слова: зони надниркової залози, інтерреналова і супрареналова тканини, венозні синуси, ендокриноцити, ядерно-цитоплазматичне відношення