UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2022. Volume 13, No. 2. P. 35-45

Received: 03.02.2022 Revised: 12.04.2022 Accepted:16.05.2022

UDC 591.471.36/.37:636.597
DOI: 10.31548/ujvs.13(2).2022.35-45

# Comparative Morphology of Tubular Bones of Blagovarsky Cross Ducks in the Postnatal Period of Ontogenesis

**Oleksandra Pasnichenko[1*], Zbihniev Sobek[2], Svitlana Tkachuk[3], Ivan Yatsenko[4], Roman Bokotko[3]**

[1]Odesa State Agrarian University
65000, 13 Panteleimonovska Str., Odesa, Ukraine

[2]Poznan University of Life Sciences
60-637, 33 Wołyńska Str., Poznań, Poland

[3]National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

[4]State Biotechnological University
61002, 44 Alchevskikh, Str., Kharkiv, Ukraine

**Abstract.** The microstructure of limb skeletal bones is closely related to ontogenetic age, localised skeletal growth dynamics, biomechanical modes of bone load, and possible taxonomic differences. This is important for the investigation of the problematic issues of ontogenetic changes in the compact bone tissue of domestic ducks. The purpose of the study was to compare the microstructure of the mid-diaphysis of the humerus and femur bones of Blagovarsky cross ducks, depending on age and gender. The material for research was the tubular bones of the thoracic (humerus) (n = 72) and pelvic (femur) (n = 72) limbs of Blagovarsky cross ducks aged 1 day, 10, 20, 30, 90, 196, 268, 341 and 483 days of postnatal ontogenesis of both sexual groups (females and males of 36 ducks each), a total of 72 ducks. The timing of the selection of ducks coincided with the technological cycle of their cultivation. Histological sections with a thickness of 5-10 microns were obtained, which were dyed with hematoxylin Karatsi and eosin, and according to Van Gieson for connective tissue differentiation. Morphometry determined the quantitative indicators of compact bone tissue in the middle of the diaphysis of tubular bones: the diameter of the diaphysis, the thickness of the periosteum, compact bone tissue, the diameter of osteons and central channels of osteons (Havers channels). It was discovered that the growth of the humerus and femur bones in length and thickness is completed on the 196$^{th}$ day of the postnatal period of ontogenesis in both female and male ducks. It was established that medullary (cerebral) bone tissue (1.01 ± 0.10 mm) appears from the femoral endostus of females, which is formed on the 196$^{th}$ day of the postnatal period of ontogenesis (the beginning of sexual maturity of females) and is further observed in intensive periods of their egg production on 268 and 341 days (2.43 ± 0.56 and 2.55 ± 0.62 mm, respectively), and disappears on the 483$^{rd}$ day (0.20 ± 0.03 mm) when the duck is not laying eggs. This study for the first time determined the age dynamics of morphometric parameters of microstructures of compact bone tissue of the humerus and femur and established their sex differences in ducks of the Blagovarsky cross. The results of comparative morphology studies are necessary for determining the age, sex, and species of birds based on the microstructure of compact bone tissue in the middle of the diaphysis of tubular bones, and for the ability to differentiate changes in the occurrence of limb pathologies in poultry

**Keywords:** humerus and femur, age groups, female, male, microstructure, diaphysis

***Suggested Citation:***
Pasnichenko, O., Sobek, Z., Tkachuk, S., Yatsenko, I., & Bokotko, R. (2022). Comparative morphology of tubular bones of Blagovarsky cross ducks in the postnatal period of ontogenesis. *Ukrainian Journal of Veterinary Sciences*, 13(2), 35-45.

*Corresponding author

## Introduction

Ontogenetic adaptations regarding the shape of limb bones in birds during the postnatal period of ontogenesis are associated with the distribution of static resistance and dynamic load on the musculoskeletal system. Constant load on the skeleton of body weight is an important factor in the formation of the structure, development and strength of tubular bones [1-3].

The microstructure of the limb skeleton bones is closely related to the age of the bird, the dynamics of localised growth of skeletal elements, biomechanical modes of bone load, and possible taxonomic differences. The variability of the microstructure of bones from different parts of the skeleton indicates differences in the rate of its growth in response to functional loads and the possible development of pathology [4-6].

Microstructural adaptations are most pronounced in the lamellar bone. However, compact bone tissue consists of units known as the Havers system, but some bird species may not have the typical Havers system described in mammals.

Some studies have shown that long bones, namely their structure and location of the Havers system, differ depending on the type of animal and the position of the bone in the skeleton of the limbs. Therewith, histological variations observed in the compact bone tissue of the mammalian and avian species under study can be associated with both the mechanical load acting on long bones and their different lifestyle [7; 8].

Medullary bone is an ephemeral type of bone tissue that occurs only in sexually mature female birds and is associated with reproductive function. This tissue is a rapidly mobilised source of calcium and phosphorus for egg shell formation [9]. The systemic distribution of medullary bone in the avian skeleton is used to identify reproductive tissues in tubular bones [10; 11].

Therefore, all of the above is of great importance for a better understanding of the micro - and macrostructure, and functional purpose of tubular bones in the postnatal period of ontogenesis of various bird species, depending on age and gender characteristics.

*The purpose of the study* was to determine age and sex differences in the microscopic structure in the middle of the bone diaphysis of the stylopodia of ducks of the Blagovarsky cross.

## Literature Review

The dynamics of morphological, geometric, densitometric, and mechanical parameters of tubular bones vary depending on the type of animal with different growth rates, and the position of the bone in the skeleton of the limbs [12-14].

Thus, different microscopic structures and the absence of the typical Havers system observed in mammals were discovered in the compact bone tissue of tubular bones in different bird species [7]. The compact femoral bone tissue of the sparrow has one side vascular, and the other – avascular. Vascularisation is represented by primary vascular channels surrounded by several misplaced osteocytes inside their lacunae, which form primary osteons. Reduced vascular supply and the appearance of secondary osteons are associated with bone rearrangement in birds such as sparrows. On the other hand, in ducks and geese, compact bone tissue was similar and consisted mainly of dense Havers tissue, while in some parts of it Primary osteons were discovered. Morphological variations in the tubular bone observed in sparrows compared to ducks and geese in terms of microstructure and its different density can be explained by the fact that the biomechanical load on the tubular bone occurs differently in each of the investigated bird species [7].

In the postnatal period of ontogenesis, the tubular bones of chickens are characterised by a rough surface with numerous grooves (depressions) that contain small holes. On the other hand, the bones of chicks, which are as large as those of adults, but more slender, are characterised by the presence of single depressions and holes. According to the histological examination, the author confirmed that these rough surface textures belong to fibrolamellar bone tissue. Instead, the smooth textured surface of the tubular limb bones in adult birds is formed after the growth of bones in width stops and coincides with their plumage and reaching sexual maturity [15].

On the example of the growth and development of Avialans – *Cretaceous enantiornithine,* those who lived in the Mesozoic Era can trace the presence of intra-skeletal osteohistovariability, which identifies complex pre-structures in tubular bones. Their osteohistological data indicate a unique strategy for the growth of the middle diaphysis of tubular bones, characterised by a high rate in *Mirarce eatoni* (terrestrial Late Cretaceous bird). The radius consists of parallel fibrous bone, similar to histological descriptions of other enantiornithines. Other elements of bones, on the contrary, are noticeably different from other representatives of these bird species. The humerus consists of a parallel fibrous bone with a middle layer of the original fibrolamellar bone and several growth lines in the outer surrounding layer and at the border with the endosteum. The structure of the endosteal and periosteal layers of bone indicates cyclical changes in the rate of its growth. The femur is represented by sections of coarse compact spongy and parallel-fibrous bone with numerous secondary osteons, and only one growth line. Therewith, tarsometatarsus is predominantly fibrolamellar in texture with several asymmetric growth lines located behind the entire compact bone tissue, which forms a strong cortical drift. Growth lines in both the endosteal and periosteal parts of compact bone indicate that, as in the humerus, the growth rate of this bone changed cyclically. Therewith, the two phalanges of the fingers investigated consist of parallel fibrous bone and remodelling in areas of rough compact spongy bone [8].

In addition, variability in the evolution of *Avialans* is confirmed by changes in the evolution of the three main bone layers behind the entire compact bone, both between species and within the same species of extinct Avialans. However, the most noTable is the adaptive change from the general lamellar (parallel-fibrous) bone tissue to the fibrolamellar complex in the middle part of the compact bone, both in representatives of the *Pygostylia,* and *Jeholornithiformes*. This change is usually associated with an increase in the density of compact bone tissue and complexity in the structure of the neurovascular network [13].

Changes in the condition of the skeleton of the limbs of poultry depend on the speed of its growth. More body weight, and in particular, the pectoral muscles, play an important role in the development of pathologies of the pelvic limb skeleton. Such pathologies can be detected by the method of geometric morphometry, which is a tool for analysing the dynamics of bone shape and its correlation with changes in the skeletal movements of broiler chickens. Weight gain is positively correlated with the curvature of the anterior-posterior tibial axis; tibial shape and active behaviour during movement have a positive correlation by which the genetic lines of broiler chickens can be distinguished; the predominance of static behaviour also correlated with the curvature of the anterior-posterior tibial axis [16].

The movements of poultry (laying hens) that are raised in enclosures that differ in structure depend on the type of locomotion (the duration of standing, sitting, walking, running, flying, and the indicators of jumping, flying, group running, and walking), the occurrence of genetic stress associated with the musculoskeletal system and on breed characteristics. Thus, white-feathered young birds had proportionally stronger shins and femurs compared to brown ones. Therewith, the type of locomotion of poultry did not affect the strength of tubular bones and their deformation indicators. However, differences in the design of enclosures for raising chickens can affect the types of movement of birds and, in turn, the development of their skeleton, which is confirmed by the appearing deformities [17].

In addition, the condition of the limb skeleton can be assessed by comparative analysis of bird bone tissue, depending on age, breed, and calcium content in it. Such an assessment is possible by applying the coefficient of uniformity of bone hardness and its correlation with the age of birds and the strength of bone material, which allowed reliable assessment of the qualitative condition of tubular bones, depending on the influence of the internal and external environment. Based on the developed approach, it is easy to trace the features of the growth and development of tubular bones in the skeleton of any animal [4].

In the tubular bones, during egg production of female birds, a medullary bone is formed, which is estrogen-dependent and its deposition is dictated by skeletal distribution patterns, both pneumaticity and bone marrow; two environmental factors (body size, food production). Thus, the presence of medullary bone in the skeleton can be widely represented in small and diving birds, but more limited – in large-bodied bird species and those that fly intensively [10; 18].

The microscopic structure of tubular bones, namely compact bone tissue, is used to differentiate bone fragments from different animal species. Currently, a large number of histological studies devoted to the dynamics of compact mammalian bone tissue are covered in scientific Ukrainian and foreign literature, but there are insufficient papers on the microstructure of tubular bones in poultry.

## Materials and Methods

The object of the study in 2018 was ducks of the Blagovarsky cross in the postnatal period of ontogenesis. Ducks were raised in private production (village Tsebrykove, Velykomykhailivskyi district, Odesa region). According to the technology of breeding, the duck population is divided into the following age groups: rearing young birds aged 1 day, 10, 20, 30 and 90 days, and breeding stock aged 196 days (the beginning of egg production). Therewith, 268 and 341 days are intensive periods of egg production, and on 483 days egg production stops.

Keeping ducks is outdoor. The ducks were fed balanced feeds according to their age: wheat, corn, sunflower, and soy meal, fish meal, vegeTable oil, melusa (shell rock), monocalcium phosphate, Table salt, baking soda, premix (vitamins: retinol (a), cholecalciferol (D3), tocopherol (E), menadiol (K3), thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid (B9), cyancobalamin (B12), magnesium, iron, copper, cobalt, iodine, selenium, methionine, lysine) [19]. Up to 32 days of the postnatal period of ontogenesis, a complete diet with the addition of premix was used (the duck is intensively gaining body weight); after 32 days, a diet without the addition of premix was used.

On the farm, ducks were vaccinated against pasteurellosis at the age of 40 days and hepatitis at the age of 60 days.

When working with experimental poultry, the "General Ethical Principles of Animal Experiments" adopted at the first National Congress on Bioethics (Kyiv, 2001) were adhered, which is consistent with the provisions "On the protection of animals from cruelty" [20] and "European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [21].

Before slaughter, the ducks were weighed on VLKT-500 g-M scales (at the age of 1, 10 days) and electronic scales of the company "Nova" (at the age of 20, 30, 90, 196, 268, 341, and 483 days) and removed the humerus and femur.

The material for research was the tubular bones of the thoracic (humerus) (n = 72) and pelvic (femur) (n = 72) limbs of Blagovarsky cross ducks. Female and male ducks (36 heads each) were selected for the study at the age of 1 day, 10, 20, 30, 90, 196, 268, 341, and 483 days of postnatal ontogenesis.

Later, after slaughter, duck carcasses were dissected and the tubular bones of the thoracic and pelvic limbs were removed. The left tubular bones were fixed in a 10% neutral aqueous formalin solution. Then, columns were cut from the middle of the diaphysis (0.5 cm) and decalcified in an 8% nitric acid solution, and degreased and dehydrated in 96% and 100% alcohol. After that, the alcohol-ether was poured into celloidin. Using a Microtome (Easily ERM3000), histological sections with a thickness of 5-10 microns were obtained, which were dyed with Karatsi hematoxylin and Van Gieson eosin for connective tissue differentiation [22].

Light microscopy and photographing of histological preparations were performed using a MICROmed XS 5520 microscope and a MICROmed – 5.0 Mega CMOS camera. Subsequently, using the method of histomorphometry, quantitative indicators of bone tissue were obtained by optical-visual method. When describing the structure of tissues, histological nomenclature was observed [23].

The obtained digital results of the study were subjected to statistical processing using the "Microsoft Excel" software package. Therewith, the arithmetic mean and its error (M ± m), the probability level (P) were calculated according to the Student's Table ($P < 0.05$; $P < 0.01$; $P < 0.001$) [24].

## Results and Discussion

As a result of the study of tubular bones of ducks of the Blagovarsky cross in the postnatal period of ontogenesis, the microscopic structure of the middle of the diaphysis was established and morphometric indicators of microstructures of compact bone tissue were obtained (Table 1).

**Table 1.** Dynamics of morphometric parameters of compact bone tissue in the middle of the humerus diaphysis, mm, M ± m, n = 8

| Age, days | Sex ♀♂ | Diaphysis diameter | Periosteum thickness | Thickness of compact bone tissue | Osteones diameter | Central osteones canals (Havers channels) diameter |
|---|---|---|---|---|---|---|
| 1 | ♀ | 1.10 ± 0.22 | 0.04 ± 0.01 | 0.15 ± 0.06 | 0.04 ± 0.01 | 0.03 ± 0.01 |
| | ♂ | 1.19 ± 0.15 | 0.05 ± 0.01 | 0.16 ± 0.04 | 0.05 ± 0.01 | 0.04 ± 0.01 |
| 10 | ♀ | 1.71 ± 0.15 | 0.06 ± 0.01° | 0.21 ± 0.03 | 0.07 ± 0.01°° | 0.05 ± 0.01° |
| | ♂ | 1.65 ± 0.04° | 0.06 ± 0.01 | 0.21 ± 0.02 | 0.08 ± 0.01° | 0.06 ± 0.01° |
| 20 | ♀ | 3.12 ± 0.20°° | 0.08 ± 0.02°°° | 0.42 ± 0.06° | 0.14 ± 0.06 | 0.05 ± 0.00* |
| | ♂ | 3.22 ± 0.21°°° | 0.06 ± 0.01 | 0.59 ± 0.10° | 0.07 ± 0.01 | 0.04 ± 0.00° |
| 30 | ♀ | 4.61 ±0.34°° | 0.11 ± 0.023 | 0.54 ± 0.10 | 0.08 ± 0.01 | 0.06 ± 0.00° |
| | ♂ | 4.82 ± 0.34°° | 0.08 ± 0.01°° | 0.55 ± 0.10 | 0.07 ± 0.01 | 0.05 ± 0.01 |
| 90 | ♀ | 7.76 ± 0.23°°° | 0.04 ± 0.01° | 0.79 ± 0.03 | 0.17 ± 0.05 | 0.09 ± 0.07 |
| | ♂ | 8.10 ± 0.44°° | 0.04 ± 0.01°° | 0.75 ± 0.03 | 0.09 ± 0.01 | 0.80 ± 0.26 |
| 196 | ♀ | 7.69 ± 0.33 | 0.03 ± 0.01 | 0.60 ± 0.05° | 1.96 ± 0.41°°° | 0.61 ± 0.21 |
| | ♂ | 7.25 ± 0.30 | 0.03 ± 0.01 | 0.68 ± 0.07 | 2.33 ± 0.27°°° | 1.23 ± 0.23° |
| 268 | ♀ | 6.44 ± 0.28° | 0.03 ± 0.02 | 0.62 ± 0.05 | 2.05 ± 0.17 | 0.88 ± 0.20* |
| | ♂ | 7.07 ± 0.19 | 0.01 ± 0.01 | 0.59 ± 0.02 | 1.23 ± 0.39 | 0.19 ± 0.17° |
| 341 | ♀ | 6.95 ± 0.44 | 0.04 ± 0.01 | 0.49 ± 0.02° | 0.07 ± 0.01°°° | 0.01 ± 0.00°° |
| | ♂ | 6.69 ± 0.66 | 0.03 ± 0.01°°° | 0.59 ± 0.06 | 0.07 ± 0.01° | 0.02 ± 0.00* |
| 483 | ♀ | 6.95 ± 0.51 | 0.02 ± 0.01° | 0.58 ± 0.02 | 0.07 ± 0.01 | 0.01 ± 0.00 |
| | ♂ | 7.05 ± 0.46 | 0.03 ± 0.01 | 0.65 ± 0.04 | 0.06 ± 0.01 | 0.01 ± 0.00°° |

**Note:** °P < 0.05; °°P < 0.01; °°°P < 0.001, compared to the previous age;
* P < 0.05, compared to the female and male of the previous age

Based on the results obtained, presented in Table 1 it follows that on day 1 of the postnatal period of duck ontogenesis, there was no statistically substantial difference in the dynamics of morphometric parameters of compact bone tissue in the middle of the humerus diaphysis. On the 10th day, compact bone tissue continues to rebuild and its size increases. Thus, the diameter of the humerus diaphysis of females substantially increases by 1.6 times ($P < 0.05$), and males – by 1.4 times ($P < 0.05$), compared to the previous age. Therewith, the diameter of the femoral diaphysis of females increases by 1.7 times ($P < 0.05$) (Fig. 1, c), and males – 1.6 times ($P < 0.05$) compared to the previous age (see Table 2).

**Table 2.** Dynamics of morphometric parameters of compact bone tissue of the middle of the femoral diaphysis, mm, M ± m n = 8

| Age, days | Sex ♀♂ | Diaphysis diameter | Periosteum thickness | Thickness of compact bone tissue | Diaphysis diameter | Central osteones canals (Havers channels) diameter |
|---|---|---|---|---|---|---|
| 1 | ♀ | 1.64 ± 0.04 | 0.04 ± 0.01 | 0.23 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.00 |
| | ♂ | 2.04 ± 0.34 | 0.03 ± 0.01 | 0.36 ± 0.12 | 0.05 ± 0.01 | 0.03 ± 0.01 |
| 10 | ♀ | 2.73 ± 0.37° | 0.05 ± 0.01 | 0.30 ± 0.05 | 0.06 ± 0.01° | 0.04 ± 0.00° |
| | ♂ | 3.19 ± 0.17° | 0.06 ± 0.01°° | 0.43 ± 0.04 | 0.08 ± 0.01 | 0.05 ± 0.01 |
| 20 | ♀ | 4.84 ± 0.07°° | 0.05 ± 0.01 | 0.53 ± 0.03°° | 0.07 ± 0.01 | 0.03 ± 0.00 |
| | ♂ | 5.26 ± 0.08°°°** | 0.07 ± 0.01 | 0.69 ± 0.04°°* | 0.07 ± 0.01 | 0.03 ± 0.00 |
| 30 | ♀ | 5.87 ± 0.24°° | 0.07 ± 0.01 | 0.70 ± 0.06° | 0.07 ± 0.01 | 0.02 ± 0.00 |
| | ♂ | 6,11 ± 0.19°° | 0.07 ± 0.01 | 0.60 ± 0.02 | 0.08 ± 0.01 | 0.03 ± 0.01 |
| 90 | ♀ | 6.00 ± 0.19 | 0.02 ± 0.01°° | 0.70 ± 0.04 | 0.15 ± 0.03° | 0.39 ± 0.37 |
| | ♂ | 6.86 ± 0.29 | 0.06 ± 0.01°* | 0.59 ± 0.05 | 0.14 ± 0.03 | 0.27 ± 0.22 |
| 196 | ♀ | 6.29 ± 0.31 | 0.04 ± 0.01°° | 0.59 ± 0.03 | 2.44 ± 0.11°°°* | 0.54 ± 0.32 |
| | ♂ | 6.91 ± 0.33 | 0.02 ± 0.01° | 0.65 ± 0.06 | 1.30 ± 0.45° | 0.47 ± 0.29 |
| 268 | ♀ | 5.95 ± 0.13 | 0.03 ± 0.02 | 0.56 ± 0.02 | 1.72 ± 0.09°°° | 0.46 ± 0.27 |
| | ♂ | 5.93 ± 0.34 | 0.02 ± 0.01 | 0.63 ± 0.05 | 2.02 ± 0.10 | 0.44 ± 0.24 |

Table 2, Continued

| Age, days | Sex ♀♂ | Diaphysis diameter | Periosteum thickness | Thickness of compact bone tissue | Diaphysis diameter | Central osteones canals (Havers channels) diameter |
|---|---|---|---|---|---|---|
| 341 | ♀ | 6.36 ± 0.28 | 0.06 ± 0.03 | 0.54 ± 0.03 | 0.08 ± 0.01°° | 0.02 ± 0.01 |
| | ♂ | 6.59 ± 0.21 | 0.03 ± 0.01 | 0.66 ± 0.02* | 0.07 ± 0.01°°° | 0.02 ± 0.01 |
| 483 | ♀ | 5.98 ± 0.42 | 0.02 ± 0.01 | 0.65 ± 0.05 | 0.08 ± 0.01 | 0.02 ± 0.01 |
| | ♂ | 6.64 ± 0.13 | 0.02 ± 0.01 | 0.74 ± 0.08 | 0.23 ± 0.17 | 0.01 ± 0.00° |

**Note:** °P < 0.05; °°P < 0.01; °°°P < 0.001, compared to the previous age;
* P < 0.05; * * P < 0.01, compared to the female and male of the previous age

In the humerus of females, osteons of irregular shape with radially directed central channels were discovered, which increase by 1.8 times (P < 0.01), and in males – by 1.6 times (P < 0.05); in the femur of females, osteons increase by 1.5 times (P < 0.05), and in males – by 1.6 times compared to the previous age (see Table 1; 2).

A study of the humerus of ducks discovered that on day 1, the bone tissue of the middle of the diaphysis was formed, but uneven in thickness. There is an osteonic type of structure of compact bone tissue. Osteons of irregular elongated circular shape with a large number of longitudinal expanded central channels, the concentric bone plates of which are in the stage of formation (Fig. 1). However, the density of osteons is uneven. The outer and inner surrounding plates are not formed. The outer and inner surfaces of the osteon layer of the diaphysis are uneven, and the proliferation of cells of the osteogenic layer of the periosteum is present in its cavities. The bone marrow in the humerus of females is predominantly red and red-yellow in males, while in the femur it is red-yellown both females and males.

On the 10th day of the postnatal period of duck ontogenesis, proliferation of cells of the osteogenic layer of the periosteum was detected (Fig. 1, a-d, 1), the formation of deep lacunae and wide cavities (Fig. 1, a-d, 2).

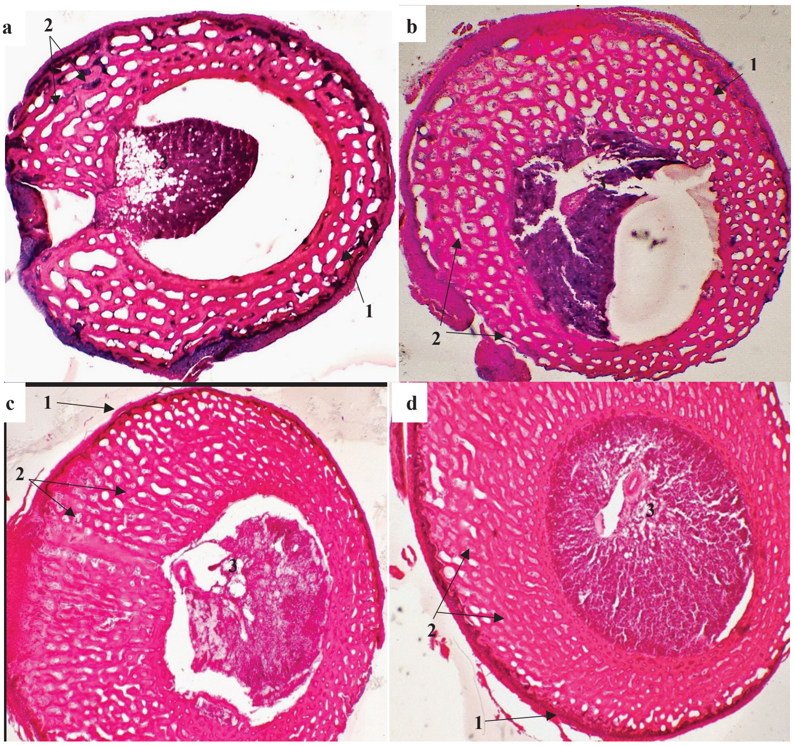


**Figure 1.** Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 10 days (female – a, c; male – b, d): 1 – proliferation of cells of the osteogenic layer of the periosteum; 2 – central channels of osteons of various shapes; 3 – bone marrow. Staining with hematoxylin Karatsi and eosin. Magnification ×32 (a-d)

The formation of the outer and inner surrounding plates begins. The bone marrow in the humerus of females is red-yellow and red in males, and in the femur of females and males – red (Fig. 1, a-d, 3).

On the 20th day of the postnatal period of duck ontogenesis, the diameter of the humerus diaphysis of females increases 1.8 times (P < 0.01), males – 2.0 times (P < 0.001); femur of females – 1.8 times (P < 0.01), males – 1.7 times (P < 0.001) compared to the previous age. Therewith, the density of osteons increased compared to that of birds of the previous age, and the diameter of the central osteon channels (Havers channels) decreased (see Table 1, 2).

On the 30th day of the postnatal period of duck ontogenesis, the compact bone tissue of the humerus and femur is represented by densely arranged osteons of various diameters and shapes (see Table 1). The diameter of the humerus diaphysis of females increases 1.5 times ($P < 0.01$), and males – 1.5 times ($P < 0.01$); femur of females – 1.2 times ($P < 0.01$) and males – 1.2 times ($P < 0.01$), compared to the previous age (see Table 1, 2).

In ducks aged 90 days (see Table 1) the cross-sectional diameter of the humerus diaphysis of females increased by 1.7 times ($P < 0.001$), and males – by 1.7 times ($P < 0.01$) compared to the previous age. Therewith, there is no statistically substantial difference in the diameter of the cross-section of the femoral diaphysis of females and males with such indicators of the previous age (see Table 2), (Fig. 2, a-d). Osteons are densely arranged, have a longitudinal and transverse course in the periosteal, mesostal and endostal zones (Fig. 2, a-d 1), and the spaces between osteons are filled with insertion (interstitial) bone plates (Fig. 2, a-d, 2).

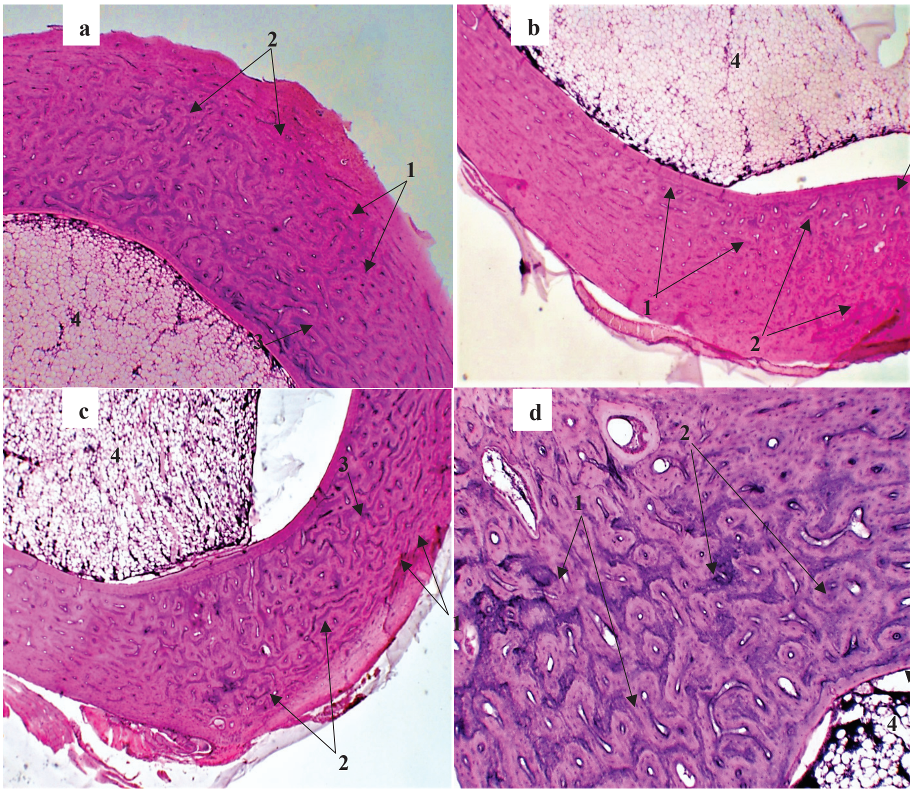


**Figure 2.** Microstructure of compact bone tissue of cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 90 days (female – a,c; male – b, d):
1 – osteons with longitudinally and transversely directed central channels; 2 – insertion (interstitial) bone plates;
3 – formed internal surrounding system of bone plates with Cementation lines; 4 – bone marrow.
Karatsi hematoxylin and eosin staining, ×30 (a-b), ×75 (g)

The diameter of the central osteon channels (Havers channels) increased slightly during this age period (see Table 1, 2). External surrounding system of bone plates in the process of formation. The internal surrounding system of bone plates is formed and clearly separated by Cementation lines, it borders the endoost (Fig. 2, a-d, 3). Bone marrow in the humerus and femur of females and males, mostly yellow (Fig. 2, a-d, 4). The compact bone tissue of the middle part of the humerus diaphysis on the 196$^{th}$ day of the postnatal period of duck ontogenesis is formed due to densely located osteons in the peri-, meso-, and endostal zones, which have mainly longitudinal and transverse directions (Fig. 3, a-d, 1).

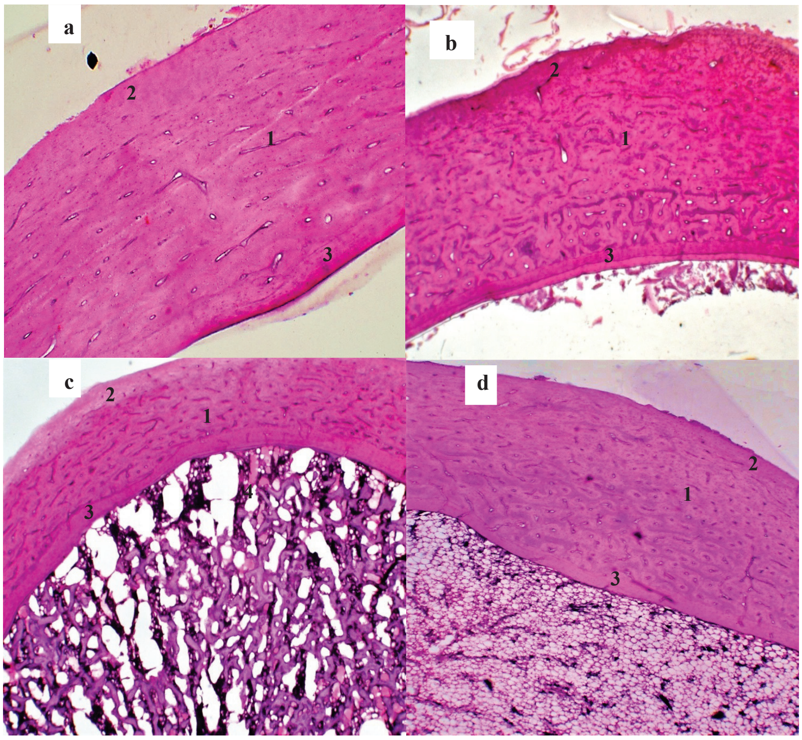


**Figure 3.** Definitive microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 196 days (female – a, c; male – b, d): 1 – osteons of compact bone tissue; 2 – formed external and 3 – Internal surrounding systems of bone plates; 4 – medullary (brain) bone tissue. Karatsi hematoxylin and eosin staining, ×30 (b-d), ×75 (a)

On day 196 of the postnatal period of duck ontogenesis, there was no statistically substantial difference in the diameter of the humerus and femoral diaphysis, both with those of the previous age and between the female and male. Diameter of osteons in the humerus of females (see Table 1) increases by 1.5 times ($P < 0.001$) and males – by 25.9 times compared to the previous age. Therewith, the diameter of osteons in the femur of females increases by 16.3 times ($P < 0.001$) and males – by 9.3 times ($P < 0.05$) compared to the previous age (see Table 2). Therewith, the diameter of osteons in the femur of females increased by 1.9 times ($P < 0.05$), compared with this indicator in males of this age.

During this age period, external (Fig. 3, a-d, 2) and internal (Fig. 3, a-d, 3) the surrounding systems of bone plates are formed, their surface is mostly smooth. Medullary bone tissue is absent in the humerus. The bone marrow in the humerus of female and male ducks is yellow. This structure corresponds to the definitive stage of tubular bone development.

In females of this age period, medullary (cerebral) bone tissue appears in the bone marrow cavity of the femur, its thickness is 1.01 ± 0.10 mm, which displaced and replaced part of the bone marrow. Medullary tissue is represented by bone junctions, and the cavities between them are filled with oxyphilic protein fluid (Figs. 3, b, 4). This is due to the onset of sexual maturity and preparation for the egg production period of the breeding stock of ducks. The bone marrow in the tubular bones of both females and males is predominantly yellow.

The microstructure of the cross-section of the middle of the duck humerus diaphysis on day 268 of the postnatal period of ontogenesis is characterised by the presence of an external surrounding system of bone plates, an osteon layer that includes osteons and insertion systems of bone plates (Fig. 4, a-d, 1) and internal surrounding systems of bone plates.

The diameter of the humerus diaphysis of females decreases by 1.2 times ($P < 0.05$) compared to the previous age. Therewith, the diameter of osteons in the compact femoral bone tissue of females decreases by 1.4 times ($P < 0.001$) compared to the previous age.

External (Fig. 4, a-d, 2) and internal (Fig. 4, a-d, 3) surrounding systems of bone plates on the periosteal and endosteal surfaces of the osteon layer of compact bone are formed. Medullary bone tissue is absent in the humerus of females.

In the femur of females, a layer of medullary bone tissue of uneven thickness in most of the compact bone tissue of the middle of the diaphysis. Therewith, the intertrabecular spaces of medullary bone tissue are filled with protein masses and blood cells (Fig. 4, c, 4). This indicates an intensive period of egg production of the breeding stock of ducks, which corresponds to the technology of raising poultry. The bone marrow in the humerus and femur of females and males is yellow (Fig. 4, c, d, 5).In ducks on 341 days of postnatal ontogenesis, compact bone tissue is represented by densely located osteons in the peri-, meso- and endostal zones (Fig. 5, a-d).

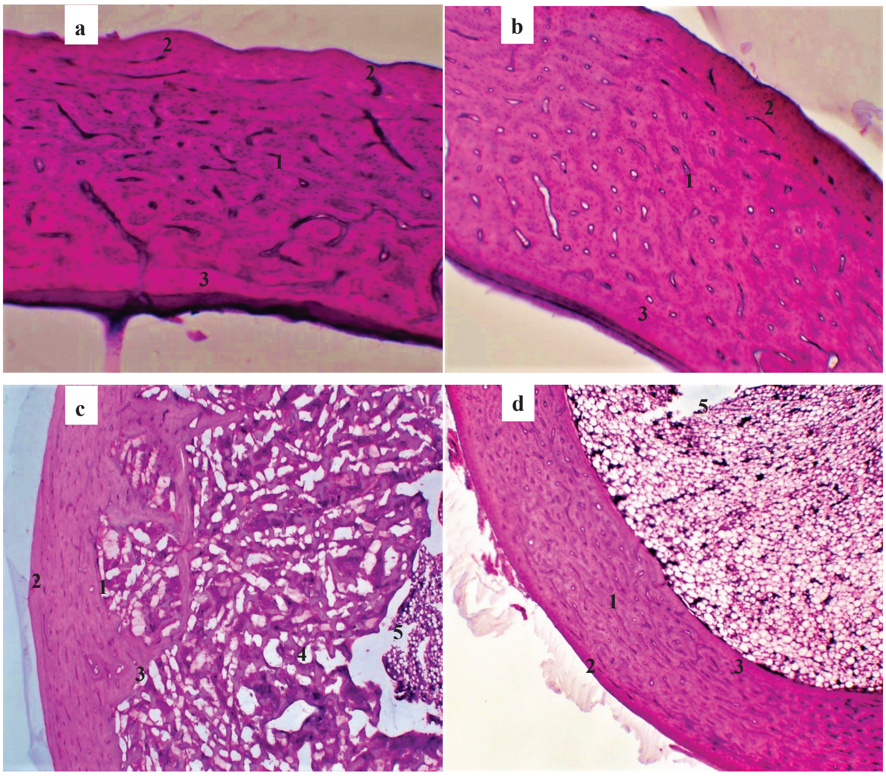


**Figure 4.** Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) bone of ducks aged 268 days (female – a; male – b): 1 – dense osteon Layer; 2 – external, and 3 – iternal surrounding systems of bone plates. Karatsi hematoxylin and eosin staining, ×30 (c, d); ×75 (a, b)
Microstructure of the cross-section of the middle of the femoral diaphysis (b, d) of ducks aged 268 days (female – c; male – d): 1 – dense osteon Layer; 2 – external, and 3 – internal surrounding systems of bone plates; 4 – layer of medullary bone tissue; 5 – bone marrow.
Karatsi hematoxylin and eosin staining, ×30 (c, d); ×75 (a, b)

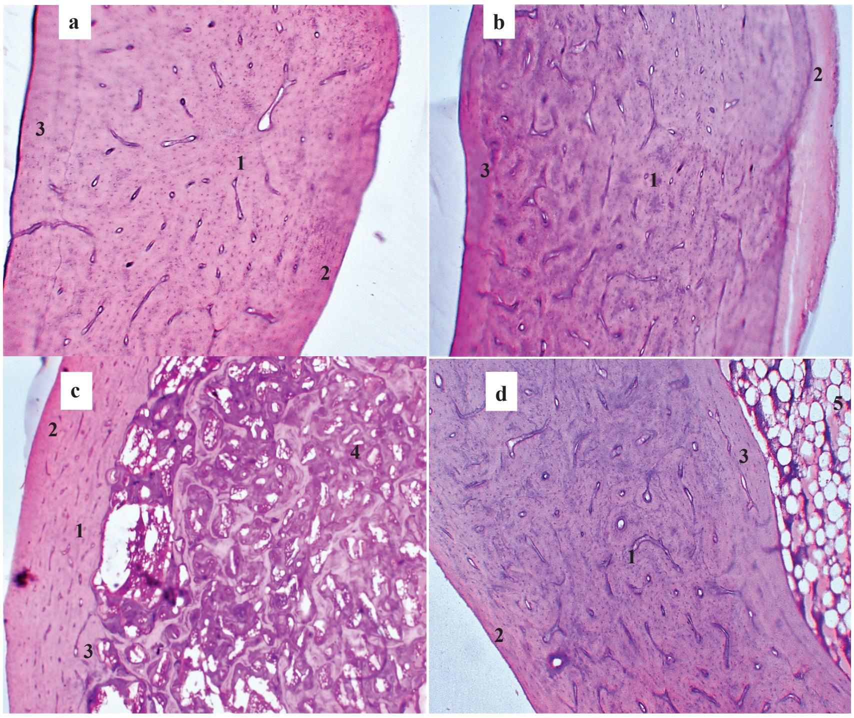


**Figure 5.** Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 341 days (female – a, c; male – b, d): 1 – dense osteon Layer; 2 – external, and 3 – internal surrounding systems of bone plates; 4 – layer of medullary bone tissue; 5 – bone marrow. Staining with hematoxylin Karatsi and eosin, ×30 (a, b); ×75 (c, d)

Insertion (interstitial) systems of bone plates were discovered, which are parts of previously formed osteons that were preserved during bone reconstruction. The thickness of the compact bone tissue of the humerus of females decreases by 1.3 times (P < 0.05) compared to the previous age. Therewith, in the femur of males, the thickness of compact bone tissue is 1.2 times greater (P < 0.05) than in females (see Table 1, 2).

The diameter of osteons in the compact bone tissue of the humerus of females decreases by 29.3 times (P < 0.001), and in males – by 17.6 times (P < 0.05) compared to the previous age. The diameter of osteons in the compact femoral bone tissue of females decreases by 21.5 times (P < 0.01), and males – by 28.9 times (P < 0.001) compared to the previous age.

The diameter of the central osteon channels (Havers channels) in the compact humerus bone tissue of females decreases by 88.0 times (P < 0.01) compared to the previous age. Instead, the diameter of the central osteon channels (Havers channels) in the compact humerus bone tissue of males is 2.0 times larger (P < 0.05) than that of females (see Table 1).

External (see Fig. 5, a-d, 2) and internal (Fig. 5, a-d, 3) surrounding systems of bone plates are formed. Medullary bone tissue is absent in the humerus of female ducks. During this period, the medullary bone layer of the femur of females has the greatest thickness (2.55 ± 0.62 mm), the cavities between its bone layers are filled with oxyphilic protein fluid (see Fig. 5, c, 4). The bone marrow in the humerus of females and males is yellow, and in the femur – red-yellow (see Fig. 5, d, 5).

The middle of the duck humerus diaphysis is represented by compact bone tissue on day 483 of the postnatal period of ontogenesis. It is formed by the outer surrounding system of bone plates, the osteon layer, insertion (interstitial) bone plates, and the inner surrounding system of bone plates. In terms of diaphysis diameter, compact bone thickness, and osteon diameter, there was no statistically substantial difference with the previous age of ducks (see Table 1, 2). Instead, the thickness of the periosteum in the humerus of females decreases by 2.0 times (P < 0.05) compared to previous ages. Therewith, the diameter of the central channels of osteons (Havers channels) in the compact bone tissue of the humerus (P < 0.01) and femur (P < 0.05) of males decreases by 2.0 times compared to previous ages (see Table 1, 2).

Medullary bone tissue is absent in the humerus of females. The medullary bone layer of the femur of females almost disappears, its thickness is 0.20 ± 0.03 mm. The bone marrow is yellow, both in the tubular bones of females and males.

Thus, according to the conducted studies, it was established that the compact bone tissue of ducks of the Blagovarsky cross was represented by a formed, but uneven in thickness layer of bone tissue with a large number of expanded central channels of osteons. With age, the osteon layer of compact bone tissue increases, the osteons are denser, and the Havers channels decrease in diameter. Another study reported that compact bone tissue *Muscovy ducks* and *Egyptian geese*was similar and consisted of dense Havers tissue with a large number of rounded clusters of osteons, separated from each other by thick dark lines [7].

Other osteohistological studies have established a unique strategy for the growth of the middle of the diaphysis of tubular bones in *Mirarce eatoni* (terrestrial Late Cretaceous bird). The humerus consists of parallel-fibrous bone (with extremely high vascular porosity for a mature skeleton of *Enantiornithes*) with the middle layer of the initial fibrolamellar bone and several growth lines in the outer surrounding layer and near the endoost. The endosteal and periosteal layers in slow-growing bone can be followed by cyclical changes in the growth rate of the limb skeleton. In the femur, there are areas of rough compact, spongy, and parallel-fibrous bone with numerous secondary osteons, and only one growth line. Therewith, to achieve the appropriate size and structure characteristic of an adult, various remodeling processes occurred in the tubular bone, including cycles of slow and rapid growth, asymmetric growth within a single bone element [5; 8; 12].

Morphological variations and dynamics of osteon density observed in the tubular bones of the above-mentioned bird species can be explained by different biomechanical loads on these bones, which depend on body weight, habitat conditions of wild and domestic birds [16-18].

Therewith, the presence of medullary bone tissue in the middle of the femoral diaphysis of female ducks of the "Blagovarsky" cross, which appears during egg production of the breeding stock, is confirmed by data from other studies on the formation of the brain bone in the medullary cavity of long bones of domestic and wild birds, considering their body weight, ecological (terrestrial, semi-aquatic, aquatic, migration) and reproductive status (stage in the egg cycle) [9; 11; 18].

## Conclusions

A study of the tubular bones of Blagovarsky cross ducks using morphological methods demonstrated that the results obtained vary depending on their age and gender.

From 1 to 10 days of the postnatal period of ontogenesis, microscopic structure the middle of the diaphysis of tubular bones of ducks is represented by formed, uneven in thickness bone tissue, which consists of osteons, whose vascular channels are directed longitudinally, irregular elongated circular shape, do not have formed concentric bone plates.

By 20-30 days of the postnatal period of duck ontogenesis, due to the proliferation of cells of the osteogenic layer of the periosteum, resorption of osteon endoost cells and bone tissue from the brain cavity, tubular bones grow in thickness. This process slows down from the 30$^{th}$ day of the postnatal period of duck ontogenesis, the osteon layer of bone tissue is compacted and from the 90$^{th}$ day the internal surrounding system of bone plates with cementation lines is formed. Therewith, the external surrounding system of bone plates is also formed by the 196$^{th}$ day of the postnatal period of ontogenesis.

The compact bone tissue of the investigated tubular bones acquires a definitive state on the 196$^{th}$ day of the postnatal period of ontogenesis of female and male ducks of the Blagovarsky cross.

On day 196, female ducks form medullary bone tissue from the femoral endoost. The process of formation of medullary bone tissue coincides with the beginning of sexual maturity and the period of egg production of the breeding stock of ducks (196 days) and intensive periods of egg production on 268 and 341 days, and on 483 days when the duck does not lay eggs – completely disappears. In turn, medullary bone tissue is absent in the humerus of females.

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# Порівняльна морфологія трубчастих кісток качок кросу «Благоварський» у постнатальному періоді онтогенезу

**Олександра Сергіївна Пасніченко[1], Збігнєв Собек[2], Світлана Алімівна Ткачук[3], Іван Володимирович Яценко[4], Роман Романович Бокотько[3]**

[1]Одеський державний аграрний університет
65000, вул. Пантелеймонівська, 13, м. Одеса, Україна

[2]Познанський університет природничих наук
60-637, вул. Волинська, 33, м. Познань, Польща

[3]Національний університет біоресурсів і природокористування
03041, вул. Героїв Оборони, 15, м. Київ, Україна

[4]Державний біотехнологічний університет
61002, вул. Алчевських, 44, м. Харків, Україна

**Анотація.** Мікроструктура кісток скелета кінцівок тісно пов'язана з онтогенетичним віком, локалізованою динамікою росту скелета, біомеханічними режимами навантаження на кістку та можливими таксономічними відмінностями. Це має важливе значення для вивчення проблемних питань онтогенетичних змін компактної кісткової тканини свійської качки. Мета дослідження полягала в проведенні порівняння мікроструктури середини діафіза плечової та стегнової кісток качок кросу «Благоварський» залежно від віку та статі. Матеріалом для досліджень слугували трубчасті кістки грудної (плечові кістки) (n = 72) та тазової (стегнова кістка) (n = 72) кінцівок качок кросу «Благоварський» у віці 1 доби, 10, 20, 30, 90, 196, 268, 341 і 483 доби постнатального періоду онтогенезу обох статевих груп (самок і самців по 36 качок), всього 72 качки. Терміни відбору качок збігалися із технологічним циклом їх вирощування. Виготовляли гістологічні зрізи товщиною 5-10 мкм, які фарбували гематоксиліном Караці та еозином, і за Ван Гізон для диференціювання сполучних тканин. Морфометрією визначено кількісні показники компактної кісткової тканини середини діафіза трубчастих кісток: діаметр діафіза, товщину окістя та компактної кісткової тканини, діаметр остеонів і центральних каналів остеонів (Гаверсові канали). Встановлено, що ріст плечової і стегнової кісток в довжину та товщину завершується на 196 добу постнатального періоду онтогенезу як у самок, так і в самців качок. Встановлено, що з боку ендоосту стегнової кістки самок з'являється медулярна (мозкова) кісткова тканина (1,01 ± 0,10 мм), що формується на 196 добу постнатального періоду онтогенезу (початок статевої зрілості самок) і у подальшому спостерігається в інтенсивні періоди їх несучості на 268 і 341 добу (відповідно, 2,43 ± 0,56 і 2,55 ± 0,62 мм), і зникає на 483 добу (0,20 ± 0,03 мм), коли качка не несеться. Цим дослідженням вперше визначено вікову динаміку морфометричних показників мікроструктур компактної кісткової тканини плечової та стегнової кісток і встановлено їх статеві відмінності у качок кросу «Благоварський». Результати досліджень порівняльної морфології необхідні для визначення віку, статі та виду птахів за мікроструктурою компактної кісткової тканини середини діафіза трубчастих кісток, а також для вміння диференціювати зміни за виникнення патологій кінцівок у свійської птиці

**Ключові слова:** плечова та стегнова кістки, вікові групи, самка, самець, мікроструктура, діафіз