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Morphometric Characteristics of the Bone Tissue Structure in White Volga Guineafoes

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Abstract

This article presents the results of the analysis of the morphometric structure of white Volga guineafoes' bone tissue. Histoarchitectonics of guineafoes' compact tissue is presented by circulatory-parallel structure; mixed, osteon, chaotic, and radial structure has been noted in many sectors and areas of bone. There are 4 ways of osteon arrangement in long bone's compact tissue of guineafoes: as zones, islands, scattered, and interspersed with circulatory-parallel structures. The walls of deep-lying osteons in the bones of birds under research (regardless of their age) are thicker than those of superficial osteons. The cross-section dimension of large Haversian canals in perimedullary zone in all long bones is bigger than in subperiosteal zone.

Keywords

Guineafoes; Skeleton; Histological structure; Bone tissue

Introduction

Bone tissue is the basis of birds' and animals' skeleton, providing "the body physical fitness"; it performs mechanical, hematopoietic, trophic, and electrolytic functions [1–5]. Functional load on the bird's bone varies during the body growth and development; therefore, there may be a structural and functional alteration of bone tissue. In this regard, the knowledge of tissues' anatomical structures and the patterns of long bones' histostructural development is important for understanding the pathology and its prevention.

The purpose of the study was to analyze the morphometric features of the bone tissue structure of white Volga guineafoes.

Materials and Methods

The materials for the research were white Volga guineafoes ($n = 264$) aged 1, 60, 90, 180, 270, and 365 days. The materials for the histological research were skeleton bones. The bone samples were fixed in 10–15% neutral formalin solution for 48 h. Decalcification was performed by 5% nitric acid solution at room temperature (18–22°C) for 7–14 days. Slices of 8–10 μm thick were made using a microtome. The slices were stained with hematoxylin-eosin; the staining method by Van Gieson was used for differentiation of connective tissue. The microslides' research was performed using a light microscope with 150 \times and 400 \times magnification. The data were treated using variation statistics method with Student's t-test, Microsoft Excel XP, Statistika 5.0, and MatLab 6.5 software package.

Results and Discussion

The study of the skeleton's long bones has shown that under the influence of external factors, they are characterized by a slight change in structure and reflect the bones' histological structure characteristics, although their development and structure are strongly influenced by age factors and a lack of calcium, phosphorus, and vitamins, especially D, in the diet [6–10]. By measuring the thickness of the compact tissue and external and internal general plates and the number of osteons and their measurements, we have identified certain age morphological differences in bone tissue histoarchitectonics. These differences are as follows: the analysis of the research data of a one-day-old guineafoes'

bones shows that at this age there are more lumens and lacunas than bone tissue in the bones. The leading histoarchitectonics of the bone tissue is circulatory layers of vascular canals in the bone tissue. Bones grow intensively in this period, which is associated with brittle histoarchitectonics. Vascular canals mostly anastomose with each other and sometimes open in the medullary cavity, creating the bone's uneven surface.

At the age of 60 days, the bone tissue is still being intensively redeveloped, with an increase in bone tissue due to the vascular canals' thinning. The compact tissue close to medullary cavity is smoother compared with the previous age. The number of lacunae, anastomosing with each other, is considerably smaller. The edges of vascular canals at this age are smoother as compared with the one-day old. It should be noted that the main feature of the guineafoes' histoarchitectonics is their large porosity. Circulatory-parallel histoarchitectonics is observed. In addition, there are radial vascular canals, indicating the increased blood supply of the bone tissue. At the age of 60 days, radial vascular canals that go separately, that is, without individual plates, are already noted. Mixed structures are also observed. Osteons have up to 6–7 plates, indicating greater bone strength. At the age of 90 days, there is a further intensive redevelopment of the bone tissue's histoarchitectonics, and a new morphological pattern is noted. The plates had already been formed by this period, but they are not pronounced. The leading histoarchitectonics of this age is a circularly parallel structure of vascular canals and bone plates and cells in the majority of regions and sectors of the studied guineafoes' bones, and in addition to this leading structure, other structures including osteons are observed too.

One of the structures of guineafoes' long-bone compact tissue is mixed histoarchitectonics, which is observed in the humerus bone

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(in the lateral, dorsal, and volar sectors of the bone's proximal part; in the diaphysis of the volar and dorsal sector and from the middle of the superficial edge to the middle of the compact tissue of the medial sector; in the bone's distal part of the lateral sector and in the dorsal sector at the perimedullary edge). It is also observed in the ulna (in the proximal and distal part of the bone of the lateral, dorsal, volar, and medial sectors); in the metacarpal bone in the medial and volar sector; in the femoral bone (in the bone's proximal part of the lateral and medial sector and junction of the plantar and lateral sector); and in the diaphysis of the lateral and dorsal sectors and in the distal part of the sector. In the tibia it is observed in the diaphysis of the dorsal, medial, and distal part of the plantar sector bone. Circulatory-parallel structures are observed in the humerus bone in the lateral sector diaphysis; in the medial sector (perimedullary) and the medial sector of the distal bone sector; in the ulna (in all sectors' diaphysis); in the metacarpal bone (in the volar sector and in the dorsal sector at the junction with the medial); in the femoral bone (in the proximal part of the bone in the medial sector at the junction with plantar and in the distal bone's diaphysis of the dorsal sector); and in the tibia (at the junction of the medial sector with plantar). Radial vascular canals are observed in the proximal part of the humerus bone in the dorsal and medial sector at this age; in the middle of the lateral diaphysis; in the medial and in the middle of volar sectors; in the ulna (volar sector's diaphysis, in the distal part of the dorsal sector bone, in the medial sector); and in the femoral bone (in the diaphysis at the junction of the dorsal sector with the medial). Meshwork structure, observed in the femoral bone's diaphysis of the medial sector, is also noted. There is an increase in bone tissue due to the developing general bone plates. Bones grow intensively in this period due to enhanced blood supply to the bone tissue, which has numerous vascular canals. There are a large number of lumens and lacunas in the tibia, with osteons in between. Osteons of most bones have 2-3 plates at this age with the exception of tibia and metatarsal bones, where there are osteons with 3-4 plates as well.

The compact bone tissue of guinea fowls aged 180 days is still being intensively redeveloped, changing its histoarchitectonics. The latter leading type is a circularly parallel structure of vascular canals and bone plates and cells that is observed in all the bones. However, there are other histoarchitectonics. Thus, the radial vascular canals are observed in the medial sector of the humerus distal part; in the femoral bone (in the medial sector of the diaphysis middle); in the tibia (in the middle of diaphysis of the lateral and dorsal sectors); and in the metatarsus (in the proximal part of the dorsal, at the junction of the dorsal with the medial, in the medial, at the junction of the medial with the plantar, and in the plantar sectors; in the middle of the diaphysis at the junction of the lateral with the dorsal and the junction of the medial and plantar). There are net branchings of vascular canals in the dorsal sector of the humerus' proximal part and in the femoral bone (plantar sector of the bone's proximal part, in the dorsal and lateral sectors of the distal part of the bone). There are chaotic branchings of vascular canals in the lateral sector of the proximal femoral bone (in the middle of lateral diaphysis, at the junction of the lateral with the dorsal section, in the medial and plantar sectors, and in the plantar sector of the bone dorsal); in the tibia (at the junction of the lateral with the dorsal sector, at the junction with the medial portion of the bone's proximal part, in the diaphysis of the medial sector, in the bone's distal part, in the lateral and medial sectors); and in the metatarsal (at the junction of the lateral plantar sector with the plantar, in the bone's proximal part, in the middle of the diaphysis in the dorsal, plantar sectors). In histoarchitectonics lumens and lacunas are observed, most of them located perimedullarily to the bone tissue.

Younger (small) osteons are mostly located in the superficial zone of the bone tissue, but they are located in other regions too. Large osteons (old) are located from the middle of the bone tissue to the perimedullary edge. Osteons have 3-4 plates, while in metatarsus and tibia, they have up to 4-5 plates.

During the period of 270-365 days of age, histoarchitectonics of the bone's compact tissue continues to redevelop, but earlier developed basic-support structures stay yet. As in previous cases, the bones leading histoarchitectonics is the circulatory-parallel structure of vascular canals and bone plates and cells. However, the structure mentioned above is not very typical. The net structure is observed in the humerus (in all sectors of the bone's distal part, at the junction of the lateral sector with the volar) and in the femoral bone (in the diaphysis of the lateral and dorsal, at the junction of the lateral sector with the dorsal, in the medial, at the junction of the dorsal sector with the medial). Mixed structure occurs in the humerus in the middle of the diaphysis in most sectors (in the lateral, dorsal, medial, and volar sector and at the junction of the volar sector with the lateral); in the ulna it is marked in the bone's proximal part (lateral, volar sectors); in the diaphysis (lateral); and in the distal part of the bone volar sector. In the femoral bone, it is observed in the bone's proximal part (dorsal, medial, at the junction of the medial with the plantar); in the diaphysis (medial, plantar sectors); in the tibia (in the proximal part of the bone's medial sector); in the diaphysis (dorsal and medial sectors); in the distal part of the bone at the junction of the dorsal with the medial; and in the medial and plantar sectors. In the metatarsus it is marked in the lateral sector of the bone's distal part and of the dorsal, plantar sectors and in the diaphysis (lateral, dorsal, plantar, and medial sectors).

In the birds' age groups under review, large osteons are located primarily in the tibia and femoral bone, while in humerus they are always less in number. In all long bones, the cross-sectional diameter of large osteons located in the perimedullary zone is always bigger compared with those in the subperiosteal zone. In 60-day-old guinea fowl rooster, large osteons located in areas of perimedullary zones of the humerus, by 5.3%, and in hen guinea fowls, by 2.3%; in 90-day-old guinea fowl rooster, by 3.9%, in hen guinea fowls, by 5.6%; in 180-day-old guinea fowl rooster, by 9.2%, in hen guinea fowls, by 5.2%; and in 365-day-old guinea fowl rooster, by 7.7%, and in hen guinea fowls, by 9.7%, exceed the same measurements of the homonymous osteons, which are in subperiosteal zones ($p < 0.05$). The walls of deep-lying osteons in the bones of birds under research (regardless of their age) are thicker than those of superficial osteons. In 60-day-old guinea fowl roosters, large osteons located in the tibial perimedullary zones by the thickness of their walls by 5.9%, in hen guinea fowls by 5.7%; in 90-day-old guinea fowl roosters by 5.8%, in hen guinea fowls by 5.8%; in 180-day-old guinea fowl roosters by 7.1%, in hen guinea fowls by 6.4%; and in 365-day-old guinea fowl roosters by 9.4% and in hen guinea fowls by 7.1% exceed the number of osteons located in the subperiosteal zone ($p < 0.05$). Cross-section dimension of large Haversian canals of perimedullary zone in all long bones is bigger than in subperiosteal zone. The cross-section dimension of the Haversian canals of the femoral bones in perimedullary zone in 60-day-old guinea fowl roosters are on average by 1.8%, in hen guinea fowls by 2.8%; in 90-day-old guinea fowl roosters by 3.2%, in hen guinea fowls by 2.8%; in 180-day-old guinea fowl roosters by 3.8%, in hen guinea fowls by 3.1%; and in 365-day-old guinea fowl roosters by 5.8% and in hen guinea fowls by 3.7% bigger compared with the lumen diameter of homonymous canals of the subperiosteal zone ($p < 0.05$). Regardless of age, the total thickness of the exterior general plates of all long bones exceeds the thickness of the interior. Besides,

the differentiation of the latter is weaker. The internal general plates are sometimes missing or invading into perimedullary zone. The thinnest general plates are observed in humerus and the thickest in the tibia.

While studying the bone tissue, we drew attention to the osteons' shape, their location, the shape of the Haversian canals' transection, osteons' transformation, and other issues, the description of which, undoubtedly, is important in the comparative histology. Generalizing the information, we found out that the vast majority of long bones' osteons are oval and round, occasionally triangular- or rhomboid-shaped. Herewith most of the major osteons have an oval shape, and small ones are rounded. It is known [7,8,10] that Haversian plates are located at the same distance from each other, but our research has shown that this is not always the case. The difference between the osteon's wall thicknesses says that Haversian plates are located in those places where the osteons' walls are thinner. It is impossible to count the number of plates, as they are too thin and are very close to each other, and in some places they are seen as a single thick lamella. In rare cases Haversian plates do not form a complete ring around the Haversian-canal lumen; that is why the osteon wall at discontinuous sections has fewer Haversian plates. This is most clearly evident in the large osteons and Haversian spaces.

We have found out that those osteons which localize in perimedullary bone tissue, especially large ones, are bound from the adjacent bone structures by a clear-cut bounding line. Most of them are straight, and some are wave-shaped. There were occasions when two osteons were surrounded by a common boundary line. However, it does not mean that the osteons that are in subperiosteal zone have a shaded bounding line. It is also noted in large osteons of the indicated region but is much weaker compared with the ones in perimedullary zone. The osteons' boundary line gradually reveals more clearly from the surface bone layer to the deep. A similar pattern is observed in the osteons' differentiation process. In subperiosteal zone the osteons are distributed more densely than in perimedullary. Osteons in both zones are equally distant from each other. In some cases, osteon cluster has been seen, and enclosing of two and sometimes three osteons with common Haversian plates has been noted. The allegation [4,10] that osteons localize only in the space between the external and internal general plates has not been confirmed by our research. At the same time, it has been found that osteons may occur at the depth of external and internal general plates, and they more often protrude into the inside general-plate space than the external one. In such cases, these osteons are distributed less frequently and have a very pronounced Haversian plates and a bounding line.

According to the scientific literature [4,5,9], a vascular canal occupies a central position in osteons in all cases. Our study has shown that in rare cases Haversian canal has an eccentric position. In addition, there have been rare cases where a Haversian system had three canals of the same diameter at different distances. The histostructural analysis of the diaphysis' bone tissue of long bones made by us has shown that secondary and tertiary osteons occur in guinea-fowl hens and roosters with age, which are concentrated in the deep bone-wall layers, contacting the internal general plates. A similar definition can be given to Haversian spaces, with the only difference that they are rarely found in the external general plates. Deep Haversian canals are relatively large and most of them are interconnected. This phenomenon is vividly expressed in adult birds.

When studying histoarchitectonics in the longitudinal section of the diaphysis' bone wall of long bones, our attention was attracted by the

amount of Haversian canals, lumen width in the longitudinal section, width of anastomoses or Volkmann's canaliculi, the anastomoses type and degree, and Haversian canals' location and the distance between them. By the number of Haversian canals, the width of their lumen, and intra-Haversian anastomosis in long bones, rooster guinea-fowls are superior to hen guinea-fowls. There is more distance between the canals in roosters than in hen guinea-fowls. The number of Haversian canals in guinea-fowl hen's long bones is fewer than in roosters. Their greatest number is observed in the femoral, then in humerus, tibial, and tarsal-metatarsal bones, and finally, in the forearm bones.

Conclusion

The undertaken histological and morphometric studies suggest that the bone tissue of one-day-old guinea-fowl bones has a fiber structure with a large number of vascular canals, which, in turn, have different shape and size. At the age of 60-90 days, general bone plates filled with lots of canals are observed. A characteristic feature of this period is also a lamellar structure, the plates of which have poor visibility under the microscope. There are 4 ways of osteon arrangement in a long bone's compact tissue of guinea-fowls: as zones, islands, scattered, and interspersed with circulatory-parallel structures. The distinctive histoarchitectonics of compact bone tissue of guinea-fowls is characterized by a circulatory-parallel structure, but there are other histoarchitectonic features in many sectors and regions (mixed, osteonal, chaotic, radial, etc.). A large number of osteons is noted in the compact bone tissue of guinea-fowls (in the insertion site and the strongest muscles pressure) that indicates their great strength, which is important for bird movement. Guinea-fowls are characterized by a considerable number of lumens and a large diameter of vascular canals. This indicates guinea-fowls' low bone weight, which is especially important for their flight. A large diameter of vascular canal indicates a higher level of oxidation-reduction processes in the bone tissue of guinea-fowls—it places them in close quarters with the Galliformes. Guinea-fowl roosters exceed hens by the thickness of the compact tissue, external and internal general plates, and the number and size of osteons. The number of histological structures and their absolute size increase with the bird's age. Tibia has large osteons with the biggest average cross-sectional diameter; it is followed by metatarsal, femoral, and humerus bones.

By the cross-sectional diameter of large osteons, wall thickness, and cross-sectional diameter of large Haversian canals located in perimedullary zones of the long bones studied, they exceed the same indices in subperiosteal zone.

By the total thickness, the external general plates of long bones exceed the internal ones. The number of large osteons in perimedullary zone is more considerable than in the subperiosteal zone, while the number of small osteons is smaller. The number of large osteons in the bones increases with the bird's age due to the change of small osteons into large ones, while the number of small osteons until the age of 180 days increases and then decreases. The deep-lying osteons in the bones of the studied birds, regardless of their age, have thicker walls than the walls of those lying under the surface. Large osteons in 60-day-old roosters are in perimedullary zones of tibia; their walls' thickness exceeds the same index of osteons located in subperiosteal zone (in roosters by 5.9%, in hen guinea-fowls by 5.7%; in 90-day-old roosters by 5.8%, in hen guinea-fowls by 5.8%; in 180-day-old roosters by 7.1%, in hen guinea-fowls by 6.4%; and in 365-day-old roosters by 9.4% and in hen guinea-fowls by 7.1%). The cross-section dimension of the Haversian canals of the femoral bones in perimedullary zone

in 60-day-old guineafowl roosters are on average by 1.8%, in hen guineafowls by 2.8%; in 90-day-old guineafowl rooster by 3.2%, in hen guineafowls by 2.8%; in 180-day-old guineafowl rooster by 3.8%, in hen guineafowls by 3.1%; and in 365-day-old guineafowl rooster by 5.8% and in hen guineafowls by 3.7% bigger as compared with the lumen diameter of homonymous canals of the subperiosteal zone.

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