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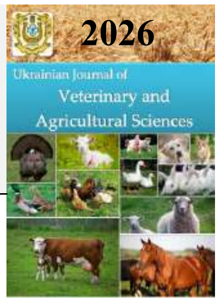

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Reference morphometric parameters of cerebellar cortical neurons in domestic birds: a comparative histomorphometric study

L. P. Horalskyi[1], I. M. Sokulskiy[2], N. L. Kolesnik[2], O. F. Dunaievska[2], O. V. Pavliuchenko[1], S. Y. Shevchuk[1], Z. A. Guta[3]

[1]Zhytomyr Ivan Franko State University, V. Berdychivska Str., 40, Zhytomyr, 10002, Ukraine
[2]Polissia National University, Staryj Boulevard, 7, Zhytomyr, 10002, Ukraine
[3]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Pekarska Str., 50, Lviv, 79010, Ukraine

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Correspondence author
Leonid Horalskyi
Tel.: +38-098-878-58-66
E- mail: goralsky@ukr.net

Contents

Abstract

Domestic birds are characterized by a highly developed functional organization of the central nervous system, which has evolved as an adaptation to complex sensorimotor activity, spatial orientation, and diverse locomotor patterns. One of the principal integrative centers of the brain is the cerebellum, whose morphofunctional status is determined by the cytoarchitectonic organization of its cortex and the quantitative characteristics of its neuronal populations. Despite the considerable number of studies devoted to cerebellar morphology, comparative histomorphometric characteristics of cerebellar cortical neurons in domestic birds remain insufficiently investigated, highlighting the relevance of the present study. The aim of this study was to establish reference morphometric parameters of cerebellar cortical neurons in domestic birds and to determine their species-specific characteristics based on a comparative histomorphometric analysis. The study was conducted on the cerebella of clinically healthy sexually mature domestic chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), domestic ducks (*Anas platyrhynchos domesticus*), and domestic geese (*Anser anser domesticus*). Histological, neurohistological, morphometric, and statistical methods were employed for morphological evaluation. The cerebellar cortex of all examined species exhibited the typical trilaminar organization; however, significant interspecies differences were observed in the morphometric characteristics of neurons within the ganglionic (Purkinje cell) layer. The largest neuronal perikaryon volume was recorded in turkeys (1423.86 ± 81.60 μm³), followed by geese (1315.22 ± 88.53 μm³) and ducks (1139.49 ± 88.79 μm³), whereas the smallest value was observed in chickens (713.95 ± 68.58 μm³). Nuclear volume varied to a lesser extent and showed no statistically significant interspecies differences ($P > 0.05$). In contrast, the nuclear-to-cytoplasmic ratio demonstrated species-specific variability, with the highest value observed in ducks (0.087 ± 0.013), followed by turkeys (0.083 ± 0.024) and geese (0.079 ± 0.013), while chickens exhibited a significantly lower ratio ($P < 0.001$). The obtained findings expand current knowledge of the structural organization of the cerebellar cortex in domestic birds, provide reference histomorphometric parameters of cerebellar neurons, and may serve as a normative basis for comparative anatomical, histological, experimental morphological, and forensic veterinary investigations.

Keywords: central nervous system; domestic birds; histomorphometry; neurons; Purkinje cells; morphometric parameters; nuclear-to-cytoplasmic ratio; comparative morphology; veterinary anatomy; histology; forensic veterinary medicine.

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1. Introduction

Birds (Aves) represent one of the most diverse, evolutionarily successful, and highly specialized groups of vertebrates, surpassed in species richness only by bony fishes (Brusatte et al., 2014). Their distinctive morphological features, including a feathered body covering, forelimbs modified into wings (Melnyk & Melnyk, 2017), the ability to lay hard-shelled eggs, and the highly specialized organization of organs and physiological systems, have enabled exceptional ecological adaptability and widespread distribution across a broad range of environmental conditions (Brusatte et al., 2015; O'Connor et al., 2024).

Domestic birds play an essential role in modern agriculture (Pustkowiak et al., 2021), contributing substantially to global poultry production while also serving as valuable biological models for both fundamental and applied research in veterinary medicine (Crawshaw, 2003; Dunaievska et al., 2024). Investigation of the structural organization of their nervous system is of considerable theoretical and practical importance because the functional state of the central nervous system determines the efficiency of adaptive mechanisms, coordination of motor activity, behavioral responses, and the maintenance of physiological homeostasis (Horalskyi et al., 2020; Sokulskyi et al., 2021). Comparative analyses of brain morphology among different domestic bird species provide valuable insights into species-specific specialization of neural structures that have evolved in response to diverse ecological and behavioral adaptations (Jarvis et al., 2005; Henriksen et al., 2016).

The cerebellum is one of the most structurally complex and functionally important regions of the brain (Wylie et al., 2018). It is responsible for integrating sensory information, coordinating voluntary movements, maintaining balance, regulating muscle tone, and ensuring the precision of complex motor activities (Cunha et al., 2021). Owing to the highly ordered organization of the cerebellar cortex and its precisely arranged neuronal circuitry, the cerebellum plays a pivotal role in the coordinated functioning of the central nervous system (Pakan & Wylie, 2006; Hall et al., 2013). The cytoarchitectonic organization of the cerebellar cortex is closely associated with the functional activity of its neuronal populations, making detailed investigations of their morphological and morphometric characteristics an important area of contemporary neuroanatomical research (Ashwell, 2020).

The evolutionary development of the class Aves has been accompanied by substantial improvements in the structural organization of the central nervous system, which have become one of the key prerequisites for the emergence of complex behavioral patterns, high locomotor activity, and effective adaptation to diverse environmental conditions. Compared with reptiles, the avian brain exhibits a considerably higher degree of development in several of its regions, reflecting the increasing complexity of sensory integration, motor control, and neural regulation of behavioral responses (Jarvis et al., 2005).

One of the characteristic features of birds is the high level of development of their sensory systems, particularly the visual and auditory systems, which enable rapid perception and processing of environmental information. The efficient functioning of these sensory modalities is closely associated with the specialization of their corresponding brain centers, providing accurate spatial orientation, complex behavioral responses, and rapid adaptation to external stimuli (Gumus et al., 2015).

To date, numerous national and international studies have demonstrated substantial progress in understanding the development, histological structure, and functional organization of the vertebrate cerebellum from ontogenetic, phylogenetic, and experimental perspectives. Particular attention has been devoted to investigating the cellular composition of the cerebellar cortex, the mechanisms underlying the formation of its cortical layers, interneuronal interactions, and the plasticity of nervous tissue (Horalskyi et al., 2026). Nevertheless, most published studies remain primarily descriptive and focus on the general morphological features of the cerebellum, whereas quantitative characterization of its histological organization has received comparatively limited attention.

Current understanding of the functional significance of the cerebellum has expanded considerably. In addition to its well-established role in the coordination of movement, the cerebellum contributes to the regulation of the cardiovascular, respiratory, endocrine, and immune systems through its extensive functional connections with the cerebral cortex, brainstem, hypothalamus, and other integrative centers of the central nervous system (Zhang et al., 2016). The multilayered organization of cerebellar cortical neuronal networks enables the integration of afferent information and the generation of appropriate motor and autonomic responses, thereby playing a fundamental role in maintaining physiological homeostasis (Olkowicz et al., 2016).

Despite the substantial body of available scientific evidence, comparative histomorphometric studies of the cerebellar cortex in domestic birds remain limited and insufficiently systematized. The existing literature lacks comprehensive data on species-specific morphometric characteristics of neurons within the different cortical layers, including neuronal density, linear and areal measurements, the proportional relationships among structural components, and the cytoarchitectonic organization of the cerebellar cortex in domestic chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), domestic ducks (*Anas platyrhynchos domesticus*), and domestic geese (*Anser anser domesticus*). Furthermore, interspecies variability in neuronal morphometric characteristics, the spatial distribution of neurons within individual cortical layers, the density of neuronal populations, and the relationship of these parameters to species-specific morphofunctional adaptations remain largely unexplored. Likewise, species-related differences in the morphometric parameters, morphology, and spatial organization of cerebellar cortical neurons, as well as their morphofunctional significance, have not been comprehensively characterized. The absence of such integrated comparative studies limits the establishment of reference morphometric criteria required for the objective assessment of the physiological state of nervous tissue and for distinguishing normal from pathological changes in veterinary morphology, neuropathology, and forensic veterinary medicine. The establishment of reference morphometric characteristics of cerebellar neurons is therefore of considerable importance for comparative anatomy, histology, neuromorphology, veterinary pathology, and forensic veterinary investigations, as it provides a morphological basis for the objective evaluation of structural alterations in nervous tissue under various pathological conditions.

Accordingly, comprehensive comparative histomorphometric investigation of the cerebellar cortex in domestic birds, with quantitative assessment of neuronal parameters across the different cortical layers, represents an important and timely area of research in contemporary veterinary morphology. The findings of such studies will expand current knowledge of species-specific features of cerebellar structural organization, establish reference morphometric parameters of its histological architecture, and provide a scientific foundation for further investigations in comparative anatomy, histology, neuropathology, and forensic veterinary medicine.

The aim of the present study was to perform a comparative histomorphometric analysis of neurons located in the different layers of the cerebellar cortex of domestic chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), domestic ducks (*Anas platyrhynchos domesticus*), and domestic geese (*Anser anser domesticus*), and to identify species-specific features of their morphological organization.

2. Materials and methods

The study was conducted using cerebella obtained from clinically healthy, sexually mature domestic birds (Class Aves), including domestic chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), domestic ducks (*Anas platyrhynchos domesticus*), and domestic geese (*Anser anser domesticus*). Only clinically healthy birds without clinical signs of central nervous system disorders or gross pathological alterations of the brain were included in the study. The experimental groups were formed according to species and physiological maturity to ensure the validity of the comparative histomorphometric analysis.

Following brain removal, the cerebellum was carefully dissected using a standardized sampling protocol. The absolute cerebellar mass was determined using an electronic analytical balance, whereas its linear dimensions were measured with a digital caliper accurate to 0.1 mm. The collected specimens were subsequently processed for histological examination and histomorphometric evaluation of cerebellar cortical neurons. Comparative analyses were performed among the four domestic bird species by assessing morphometric parameters of neurons located within the molecular, ganglionic (Purkinje cell), and granular layers of the cerebellar cortex.

All experimental procedures were conducted in accordance with internationally accepted principles of bioethics governing the use of animals in scientific research and with current regulations for morphological investigations. The experimental protocol complied with the principles of Good Laboratory Practice (GLP) (1981), the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (Strasbourg, 1986), the Law of Ukraine *"On the Protection of Animals from Cruelty"* (No. 3447-IV, February 21, 2006), and the applicable regulatory documents of the Ministry of Health of Ukraine governing the use of experimental animals in scientific research (Yablons'ka, 2007; Nichiporuk et al., 2022).

For histological examination, cerebellar tissue samples were fixed in 10 % neutral buffered formalin and Carnoy's solution. Following routine histological processing, the tissues were embedded in paraffin, and serial sections 8–10 µm thick were prepared using an MC-2 sliding microtome. After deparaffinization, the sections were stained with hematoxylin and eosin to evaluate the general histological architecture of the cerebellar cortex (Horalskyi et al., 2026).

The cytoarchitectonic organization of the cerebellar cortex, neuronal morphology, neuronal processes, the neurofibrillary apparatus, and interneuronal connections were examined in sections impregnated with silver nitrate according to the Ramón y Cajal and Bielschowsky–Gros staining methods.

Histomorphometric analysis was performed using light microscopy. Measurements included the thickness of the molecular, ganglionic (Purkinje cell), and granular layers of the cerebellar cortex, the linear dimensions and cross-sectional area of neuronal perikarya, nuclear diameter, the nuclear-to-cytoplasmic ratio, and neuronal density within the corresponding cortical layers. Morphometric measurements were obtained using an ocular micrometer, followed by digital processing and statistical analysis of the collected data (Horalskyi et al., 2026).

Statistical analyses were performed using Microsoft Excel and STATISTICA 6.0 software. For each variable, the arithmetic mean (M), standard error of the mean (SE), standard deviation (SD), and correlation coefficient (r) were calculated. Differences between groups were assessed using Student's t-test, and values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

Comprehensive morphological investigation of the central nervous system is essential for establishing reference characteristics of its structural organization and for the objective assessment of the morphofunctional status of the animal organism. Such studies are of particular importance in veterinary morphology (Ragulya et al., 2023), where quantitative characteristics of nervous tissue provide the basis for comparative analyses, identification of species-specific features, and differentiation between physiological and pathological changes. The present findings represent a logical continuation of our comprehensive morphological investigation of the cerebellum in domestic birds. While gross anatomical and histological examinations provide information on the general structural organization of the organ and its cytoarchitectonic features, histomorphometric analysis enables a quantitative evaluation of the structural components of the cerebellar cortex. Determination of neuronal morphometric parameters (Arendt et al., 2019), their spatial distribution, and the relative proportions of neuronal populations within the different cortical layers provides an objective basis for identifying species-specific patterns of cerebellar organization and enhances our understanding of its morphofunctional specialization.

Domestic birds exhibit a highly specialized nervous system that has evolved through long-term adaptation to the specific demands of locomotion, spatial orientation, and complex sensory integration (Jarvis et al., 2005). Despite considerable interspecies differences in body size, locomotor behavior, and lifestyle, the avian central nervous system demonstrates a high degree of structural differentiation, with the cerebellum representing one of its most highly organized regions. Its development is closely associated with the requirements for precise motor coordination, maintenance of balance, regulation of muscle tone, and integration of sensorimotor functions (Sultan, 2005; Kang, 2021).

Contemporary neuromorphological studies have demonstrated that the avian brain is characterized not only by a high neuronal density but also by a remarkably high concentration of neurons compared with other vertebrate groups possessing a similar brain mass. This organizational feature reflects the high efficiency of avian nervous tissue and provides substantial capacity for rapid information processing and coordination of complex behavioral responses (Herculano-Houzel et al., 2005; Olkowicz et al., 2016). Nevertheless, the spatial organization of cerebellar neurons, their morphometric characteristics, and the distribution of neuronal populations across the different cortical layers remain insufficiently investigated in domestic bird species. Therefore, comprehensive comparative histomorphometric analysis is essential for identifying species-specific patterns of cerebellar cytoarchitecture and expanding current knowledge of its morphofunctional specialization.

Histological examination demonstrated that the cerebellar cortex of all examined bird species retained the typical trilaminar organization. However, pronounced interspecies differences were observed in the thickness of the individual cortical layers, the morphometric characteristics of neurons, and their spatial distribution within the cerebellar cortex.

Cytometric measurements combined with mathematical analysis revealed species-specific differences in the volume of Purkinje cells and their nuclei. In addition, the nuclear-to-cytoplasmic ratio varied among the examined domestic bird species, reflecting interspecific differences in neuronal morphology. These findings indicate the presence of species-specific structural characteristics of the cerebellar cortex. The application of morphometric analysis enabled an objective quantitative assessment of these differences and confirmed its high informative value for the investigation of neuronal populations (Rajković et al., 2016).

Evaluation of the histomorphometric characteristics of the cerebellar cortex represents an integral component of comprehensive morphological investigations; however, interpretation of these parameters requires consideration of the overall architecture of the organ. The macroscopic organization of the cerebellum determines the spatial arrangement of its structural components, the degree of cortical development, and the formation of distinct functional regions. Therefore, alongside the investigation of cerebellar cytoarchitecture, analysis of the surface morphology and its organizational patterns in different domestic bird species is essential.

In all examined domestic bird species, the cerebellum exhibited a well-developed folial organization. Its surface was formed by a system of transverse fissures that divided the cerebellar cortex into individual folia (*folia cerebelli*), which were further organized into lobules and larger morphological subdivisions. This organizational pattern substantially increases the surface area of the cerebellar cortex without a corresponding increase in the overall volume of the organ, thereby allowing the compact arrangement of a large number of neurons and the establishment of highly complex neuronal networks.

Comparative analysis demonstrated that the general organizational pattern of the cerebellar surface was conserved across all examined species, although several species-specific differences were identified. In domestic chickens, the cerebellar surface was divided by numerous transverse fissures into folia that were sequentially organized into lobules and cerebellar lobes. In turkeys, the fissures were more distinctly developed, resulting in clearer delineation of individual lobules. In domestic ducks, the cerebellum consisted of ten lobules grouped into three principal regions: the anterior, middle, and posterior divisions. Similarly, in domestic geese, the cerebellar surface exhibited a well-developed system of transverse fissures forming numerous folia and lobules, thereby creating a highly convoluted cortical surface.

The observed characteristics of folial organization indicate that domestic birds retain a common structural plan of the cerebellum while exhibiting moderate species-specific variation in the degree of surface development. An increased number of cerebellar folia and greater complexity of cortical folding are regarded as morphological adaptations that expand the surface area of the cerebellar cortex and enhance the integrative capacity of neuronal networks, thereby supporting more efficient motor coordination and increasingly complex behavioral responses among different bird species (Zilles et al., 1989; Kalisińska, 2005).

Histological examination demonstrated that the cerebellum of all investigated domestic bird species shared a common structural organization. Transverse histological sections clearly distinguished the gray and white matter. The gray matter formed a continuous cerebellar cortex covering the surface of the cerebellar folia, whereas the white matter occupied the central region, forming the conductive core of the organ. Despite interspecific differences in cerebellar size and the degree of folial development, the histoarchitecture of the cerebellar cortex exhibited a common organizational pattern in all examined species, reflecting the highly conserved structural organization of this brain region.

The cerebellar cortex of all investigated birds consisted of three well-defined layers: the superficial molecular layer, the Purkinje cell layer, and the deep granular layer. Each layer was characterized by a distinct cellular composition, specific patterns of neuronal organization, and specialized functional roles within the integrated neural circuitry responsible for information processing. This highly ordered laminar organization enables the integration of afferent signals, their processing within the cerebellar cortex, and the subsequent transmission of information to the effector structures of the central nervous system.

The molecular layer was the most superficial and comparatively sparsely populated with neurons. It was composed predominantly of stellate and basket cells, which formed an extensive network of interneuronal connections and functioned primarily as inhibitory interneurons. The axons of basket cells formed characteristic pericellular baskets surrounding the somata of Purkinje cells, whereas stellate cells established numerous synaptic contacts with their dendrites. Consequently, the molecular layer plays a pivotal role in regulating neuronal excitability, synchronizing the activity of cerebellar neuronal networks, and ensuring the high precision of coordinated movements.

The central component of the cerebellar cortical cytoarchitecture was the Purkinje cell layer, which consisted of a single row of large flask-shaped Purkinje neurons. These neurons represent the sole efferent output of the cerebellar cortex, transmitting information to the deep cerebellar nuclei and other regions of the central nervous system (Kim et al., 2012). Their extensively developed dendritic arborization enables the reception of a large number of afferent inputs, whereas the morphology, size, and spatial arrangement of Purkinje cells are directly associated with the efficiency of neuronal signal integration and the regulation of complex motor functions. Consequently, Purkinje cells constitute one of the principal targets of contemporary histomorphometric investigations, and their quantitative characteristics may reflect species-specific features of cerebellar functional organization in different animal species (Hoxha et al., 2018).

The granular layer is located adjacent to the white matter and exhibits the highest neuronal density among all layers of the cerebellar cortex. It is composed predominantly of small, round granule cells, which constitute the most abundant neuronal population of the cerebellum. These neurons are responsible for the initial processing of afferent information entering the cerebellar cortex and establish numerous synaptic contacts with the dendrites of Purkinje cells through their parallel fibers. The high density of granule cells provides the morphological basis for efficient integration of large volumes of sensory information, which is essential for maintaining balance, coordinating movements, and supporting complex behavioral responses in domestic birds.

Although the overall structural organization of the cerebellar cortex is highly conserved, its functional specialization is determined not only by its laminar architecture but also by the quantitative characteristics of its neuronal populations. Neuronal morphometric parameters, spatial organization, and cell density represent sensitive indicators of species-specific structural organization of the cerebellum. Therefore, the subsequent stage of the study was focused on a detailed histomorphometric characterization of neurons within each cortical layer of the cerebellum in the investigated domestic bird species.

Histomorphometric analysis of neurons in the Purkinje cell layer revealed pronounced interspecies differences in the morphometric characteristics of Purkinje cells. Although the overall pattern of their spatial organization was conserved among all examined species, significant differences were observed in perikaryal volume, nuclear size, and the nuclear-to-cytoplasmic ratio, all of which represent important quantitative indicators of the structural and functional organization of these neurons.

Analysis of the perikaryal volume of Purkinje cells demonstrated marked interspecies variability in this parameter (Fig. 1).

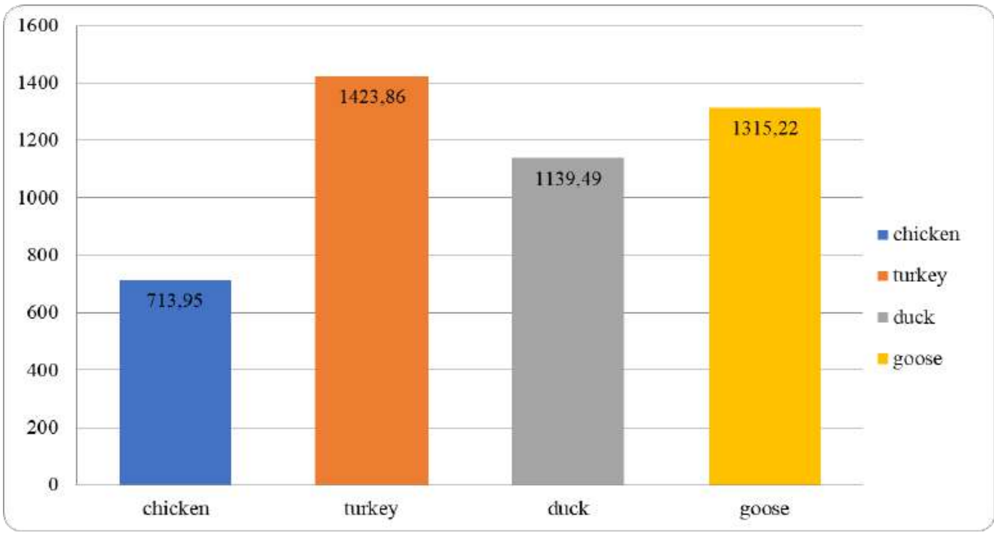

Fig. 1. Morphometric characteristics of the perikaryal volume of Purkinje cells in the cerebellar cortex of domestic birds (μm³)

The largest mean perikaryal volume was observed in turkeys (1423.86 μm³), followed by domestic geese (1315.22 μm³) and domestic ducks (1139.49 μm³), whereas the smallest value was recorded in domestic chickens (713.95 μm³). The mean perikaryal volume of Purkinje cells in domestic geese was 1.15-fold greater than that observed in domestic ducks and was significantly higher than that of domestic chickens by a factor of 1.84 ($P < 0.01$). The highest values were recorded in turkeys, in which the perikaryal volume was significantly greater ($P < 0.01$) than in domestic ducks and domestic chickens by factors of 1.25 and 1.99, respectively. These findings demonstrate pronounced interspecies differences in the morphometric characteristics of Purkinje cells, which are likely associated with species-specific functional demands placed on the cerebellum.

In contrast to the marked variation in perikaryal volume, the mean nuclear volume of Purkinje cells exhibited considerably lower interspecies variability (Fig. 2).

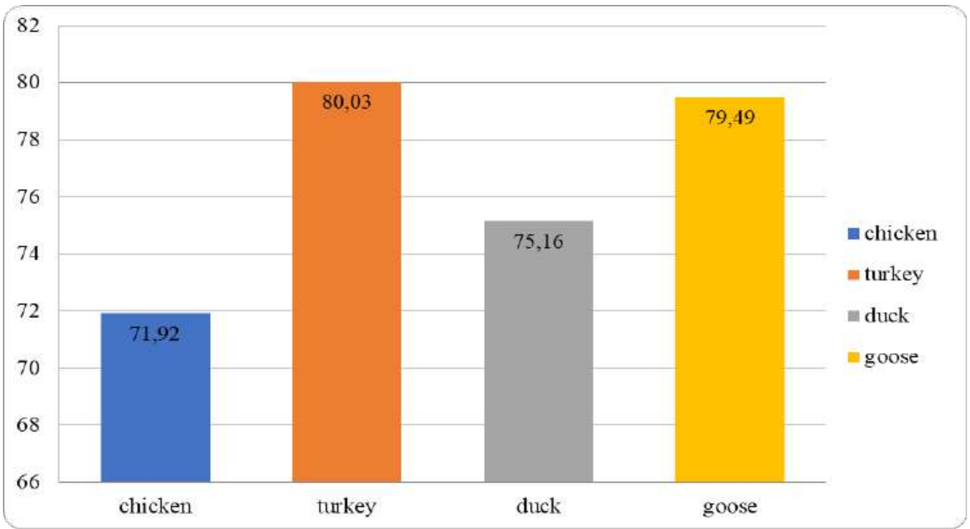

Fig. 2. Morphometric characteristics of the nuclear volume of Purkinje cells in the cerebellar cortex of domestic birds (μm³)

The largest mean nuclear volume was recorded in turkeys (80.03 µm³), followed by domestic geese (79.49 µm³) and domestic ducks (75.16 µm³), whereas the smallest value was observed in domestic chickens (71.92 µm³). However, statistical analysis revealed no significant interspecies differences ($P > 0.05$), indicating the relative stability of the nuclear component of Purkinje cells. These morphometric findings suggest that interspecies variability of Purkinje cells is determined primarily by differences in perikaryal size, whereas nuclear dimensions remain comparatively stable.

Particular attention should be paid to the nuclear-to-cytoplasmic ratio, which is considered one of the most informative morphometric indicators of the functional state of a cell (Nazarchuk, 2008; Cherniavskyi, 2019). The present study demonstrated that the lowest nuclear-to-cytoplasmic ratio was observed in Purkinje cells of domestic chickens (0.079 ± 0.013), whereas higher values were recorded in turkeys (0.083 ± 0.024), domestic ducks (0.087 ± 0.013), and domestic geese (0.089 ± 0.013) (Fig. 3).

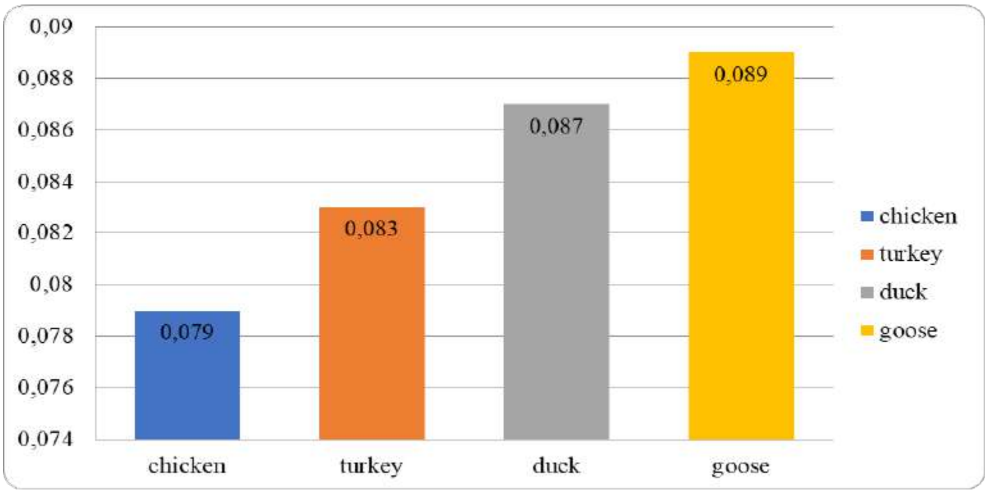

Fig. 3. Nuclear-to-cytoplasmic ratio of Purkinje cells in the cerebellar cortex of domestic birds

The nuclear-to-cytoplasmic ratio in domestic chickens was significantly lower ($P < 0.001$) than that observed in the other investigated species and was approximately 1.75-fold lower than the maximum value recorded. These differences were primarily attributable to variation in the extent of cytoplasmic enlargement despite only minor changes in nuclear volume, suggesting interspecies differences in protein synthetic activity, the intensity of intracellular metabolic processes, and the morphofunctional specialization of Purkinje cells in domestic birds.

4. Conclusions

The present comparative histomorphometric study demonstrated that, despite the conserved trilaminar histological organization of the cerebellar cortex in domestic birds, neurons of the Purkinje cell layer exhibit pronounced species-specific morphometric characteristics. These differences are primarily reflected in the variability of Purkinje cell perikaryal volume, nuclear volume, and the nuclear-to-cytoplasmic ratio, indicating distinct structural features of nervous tissue organization in domestic chickens, turkeys, domestic ducks, and domestic geese.

Among the investigated morphometric parameters, the perikaryal volume of Purkinje cells exhibited the greatest interspecies variability, whereas nuclear volume remained relatively stable across the examined species. Differences in the nuclear-to-cytoplasmic ratio suggest species-specific variations in the morphofunctional state of these neurons, the development of the protein-synthesizing apparatus, and the intensity of intracellular metabolic activity.

The obtained histomorphometric data expand current knowledge of the cytoarchitectonic organization of the cerebellar cortex in domestic birds, establish reference morphometric criteria for its normal structural organization, and may serve as a morphological basis for future comparative anatomical, histological, experimental, clinicopathological, and forensic veterinary investigations of the avian central nervous system.

Conflict of interest

The authors declare no conflict of interest.

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