MORPHOMETRIC CHARACTERISTICS OF SKIN IN THREE YEAR OLD HORSES OF VARIOUS COAT COLORS

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Abstract: *This scientific study investigates the morphological characteristics of the skin in three-year-old g'o'nons (young horses), specifically focusing on its thickness across different body regions and coat colors. During the study, a total of 140 skin biopsies were collected from 28 anatomical points on the bodies of 5 stallions of varying ages. The thickness of the collected samples was determined both macroscopically using a digital caliper under a magnifying glass and microscopically after preparing histological sections. The results revealed significant variations in skin thickness across different body regions; the thickest skin was recorded at the hoof dermal junction and the dorsal part of the body, while the thinnest skin was observed around the eyes and in the ventral region. Furthermore, black-coated g'o'nons were found to have slightly thicker skin compared to their bay and gray-coated counterparts. The minimal difference between macroscopic and microscopic measurements confirmed the reliability of the methodological approach. This information contributes to a deeper understanding of the functional adaptability, protective properties, and significance of skin in young horses for veterinary practice.*

Keywords: *Horses, gonon (young horse), skin, morphology, histology, thickness, age-related changes, stallions, coat color.*

Introduction. Skin is the outer covering of the mammalian organism, performing numerous vital functions, including mechanical protection, thermoregulation, sensory perception, immune response, and excretory functions. It serves as the primary barrier protecting the organism from harmful environmental influences. For horses, especially young three-year-olds (known as gonon), a healthy skin condition is extremely important for their growth, development, and resistance to various diseases. This age period is characterized by intensive growth and formation of the equine organism, during which the structural and functional characteristics of the skin undergo significant changes. Understanding the anatomical and histological features of the skin in young horses is of fundamental importance for preventing skin diseases, ensuring effective treatment, and maintaining overall health.

Existing scientific literature contains information regarding the general anatomical and histological structure of horse skin. Numerous studies have explored the skin characteristics of adult horses across various breeds. However, there is a relative scarcity of research dedicated to the in-depth study of the morphological specificities of the skin across different body regions and its age-related dynamics specifically in three-year-old horses. Histological parameters such as skin thickness, structure, hair follicles, and the distribution of sebaceous and sweat glands can vary depending on age, breed, sex, and even coat color. These changes directly impact the skin's durability, elasticity, thermoregulatory capacity, and potential to combat infections. The scientific novelty and relevance of this study lie in the comprehensive and complex analysis of these morphological features in young three-year-old horses, using animals of various coat colors as examples. The development of horse breeding in Uzbekistan and the growing interest in equestrian sports increase the necessity of maintaining the health of

young horses. Therefore, determining the morphofunctional characteristics of the skin in three-year-old horses holds practical significance for local veterinary medicine and the horse breeding industry.

Research Objective: To investigate the morphological characteristics of the skin, specifically its thickness, across various anatomical regions in three-year-old horses (*g'o'non*), and to identify variations related to age and coat color.

Research Tasks: 1. To collect skin samples from 28 anatomical points of three-year-old horses with different coat colors (gray, bay, and black).

2. To measure the total macroscopic thickness of the skin samples using digital calipers.

3. To prepare histological slides from the collected samples and conduct morphometric measurements of the skin layers (epidermis and dermis) under a microscope.

4. To compare the results of macroscopic and microscopic measurements and determine the correlation between them.

5. To analyze the differences in skin thickness across each anatomical region and among horses of different coat colors.

6. To evaluate the clinical and physiological significance of the obtained results and develop practical recommendations for the care and management of young horses.

Materials and Methods: This study was conducted during the period of 2023-2024 at the "Chori Chopag‘on" farm in the Boysun district of the Surkhondaryo region. The research subjects consisted of 5 clinically healthy, three-year-old stallions (gonon) of various coat colors (gray, bay, and black). Special attention was paid to selecting at least one horse of each coat color, with variations in age within the group where possible. All procedures involving animals were performed in strict accordance with veterinary ethical standards and under veterinary authorization.

During the study, skin biopsies were collected from 28 anatomical points on each stallion. The selected points covered areas of the skin with different functional and mechanical loads, including:

• Head region: The medial angle of the eye (mid-frontal part) and the center of the masseter muscle (*m. masseter*).

• Neck region: The lateral aspect of the C1-C2 cervical vertebrae and the lateral aspect of the C5-C6 cervical vertebrae.

• Dorsal region: The lateral aspect of the withers, the lateral aspect of the L5-L6 lumbar vertebrae, the surface of the croup, and the ischial region.

• Lateral region: The caudal border of the scapula, the area opposite the 15th-16th ribs, and 10 cm behind the last rib.

• Ventral region: The center of the pectoral muscles, the cranial and caudal parts of the sternum, the cranial and caudal umbilical regions, and the medial thigh.

• Forelimbs: The lateral and medial aspects of the antebrachium (forearm), the carpus, the metacarpus, and the hoof corium (periople).

• Hindlimbs: The semitendinosus and semimembranosus muscle region, the lateral and medial aspects of the crus (lower leg), the tarsus, the metatarsus, and the hoof corium (periople).

Circular skin samples with a diameter of 6 mm were obtained using a 6 mm biopsy punch, local anesthetics (lidocaine, procaine), and antiseptic solutions (iodine, chlorhexidine). Sterile forceps and suturing materials were used for the procedure. For histological analysis, the collected tissue samples were fixed in 10% neutral buffered formalin. The biopsy sites were treated under local anesthesia and subsequently processed under aseptic conditions using sterile gauze and adhesive tape. A total of 140 biopsy samples were collected.

The initial total thickness of the skin biopsies was measured "under a magnifying glass" using a digital caliper with a precision of ±0.01 mm. These

macroscopic measurements provided preliminary data regarding the overall skin thickness.

Histological Preparation and Morphometric Analysis: Histological slides were subsequently prepared from the collected samples. Each specimen was fixed in a 10% neutral buffered formalin solution for 24–48 hours. Following fixation, the samples were dehydrated through a graded series of ethanol (70%, 80%, 96%, and 100%), cleared in xylene, and embedded in paraffin wax at a melting point of 56–58°C. Sections with a thickness of 5–7 µm were cut from the paraffin blocks using an HM 355S Automatic Microtome. The sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) for general morphological evaluation. Additionally, Masson’s Trichrome staining was employed for collagen fiber identification, and Orcein staining was used to detect elastic fibers in selected samples.

The histological preparations were analyzed using an "Altami 1238" binocular microscope at magnifications of 40x, 100x, and 400x. High-resolution microphotographs were captured via an "Altami Vision" digital camera. Morphometric measurements were conducted on the captured images using ImageJ and "Altami Studio" software. Specifically, the thicknesses of the epidermis and the dermis (distinguishing between the papillary and reticular layers) were measured. For each slide, measurements were taken from 10 random fields to calculate the mean values.

Statistical Analysis: Statistical processing of the data was performed using Microsoft Excel 2016 and Statistica 10.0 software. Differences between groups were analyzed using Student’s t-test or one-way ANOVA. Results are presented as mean values (M) ± standard error (±m). Statistical significance was set at $p < 0.05$.

Methodological Validation: A critical finding was that the discrepancy between the initial macroscopic measurements (obtained via digital calipers)

and the microscopic measurements (from histological slides) was negligible. This suggests that the skin tissue did not undergo significant shrinkage or distortion during the fixation and processing stages, thereby confirming the high degree of reliability, precision, and objectivity of the research methodology.

Results: The results of this study revealed significant variations in the morphological characteristics of the skin in three-year-old stallions (*g'o'nonlar*), particularly regarding thickness across different anatomical regions and coat colors. The data provide critical insights into the functional adaptability of the skin in young horses and its response to environmental factors.

1. Distribution of Skin Thickness by Anatomical Region: The study identified substantial differences in skin thickness across various parts of the equine body. The thickest skin was observed in the hoof corium (forelimb: 3.85–3.94 mm; hindlimb: 3.90–3.97 mm) and the dorsal region, specifically the lateral aspect of the L5–L6 lumbar vertebrae (3.23–3.43 mm). These areas are subjected to constant mechanical stress, friction, and impact; therefore, their skin is thicker and more robust to provide effective protection. The croup surface (3.10–3.34 mm) and the metacarpal/metatarsal regions (forelimb: 2.85–2.92 mm; hindlimb: 3.10–3.35 mm) also exhibited relatively high thickness, reflecting their role in locomotion and weight-bearing.

Conversely, the thinnest skin was recorded in specific parts of the head, such as the mid-frontal area near the medial angle of the eye (1.23–1.37 mm), and in ventral regions, particularly the medial thigh (1.34–1.48 mm). These areas typically experience less mechanical stress and require higher sensitivity and elasticity. Additionally, the neck (averaging 1.80–1.90 mm) and other ventral parts of the trunk (1.75–1.89 mm) were found to be thinner compared to the dorsal segments.

2. Variations Based on Coat Color: A consistent trend was identified regarding skin thickness in relation to coat color. Black stallions consistently exhibited greater skin thickness across nearly all examined anatomical points compared to gray and bay stallions. For instance, in the head region, skin thickness in black horses averaged 1.45 mm, while it was 1.34 mm in gray horses and 1.36 mm in bay horses. A similar trend was observed in the dorsal region (black: 3.06 mm, gray: 2.89 mm, bay: 2.93 mm) and the hoof corium. Bay horses showed slightly higher skin thickness than gray ones, though this difference was less pronounced than the gap between black horses and others. Although these variations may require further statistical validation, they suggest that phenotypic traits, specifically coat color, may influence the structural characteristics of the skin.

3. Correlation Between Macroscopic and Microscopic Measurements: The study demonstrated a high correlation between macroscopic measurements obtained via digital calipers and microscopic measurements from histological slides. The negligible difference indicates that the skin samples underwent minimal shrinkage or deformation during the fixation and processing stages. This high degree of correlation confirms the reliability of the methodology and the accuracy of the results. (Detailed microscopic data for the epidermis and dermis layers should be inserted here if available).

Conclusion

In conclusion, the skin thickness of three-year-old stallions varies significantly depending on the body region, indicating a localized functional adaptation. The thickest skin is positioned where mechanical protection is paramount, while the thinnest skin is found in areas where sensitivity and mobility are essential. Furthermore, coat color appears to be a factor affecting skin thickness, with black stallions possessing slightly thicker skin than their gray or bay counterparts. These findings establish a foundation for

understanding the morphological maturation of skin in young horses and its dependence on various physiological factors.

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