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CLINICAL MANAGEMENT OF WOUNDS IN DOGS AND CATS WITH VARIOUS NOSOLOGICAL PROFILES USING HYDROGEL APPLICATIONS

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Summary. The article analyzes the peculiarities of the wound-healing process in dogs and cats with tissue defects that healed by secondary tension. The study was conducted on 34 animals at a private veterinary clinic and involved 60 days of prospective observation. The etiological structure of the wounds was determined, revealing that bite injuries dominated. The study demonstrated the dependence of the topographic localization of wounds on the type of animal, with predominant damage to the limbs and cervical region. The focus is on the peculiarities of the regeneration process during secondary healing, particularly hemostasis, inflammation, proliferation, and remodeling. It has been confirmed that the duration of the wound healing process is independent of animal species, breed, sex, or injury location. The decisive role of tissue deficit volume and complicating medical history in regeneration dynamics is emphasized. The feasibility of using hydrogel applications in the complex treatment of wounds of various etiologies that heal by secondary intention is supported. Experimental studies have proven that hydrogels promote wound cleansing, maintain optimal moisture levels, and stimulate granulation tissue. These results confirm the effectiveness of a comprehensive approach to treating purulent and complicated wounds in small domestic animals, and they can be applied in the field of practical veterinary medicine

Keywords: diagnosis, assessment, body injuries, sharp objects, healing, scars, animals

Introduction. In veterinary practice, wounds are one of the most common pathologies. Their timely and adequate treatment is essential to maintaining the quality of life of animals (Wilkinson and Hardman, 2020). The wound healing process is complex and dynamic, ensured by closely coordinated cellular, molecular, and tissue interactions. Any traumatic injury triggers an inflammatory response, leading to excessive production of inflammatory mediators and the activation or recruitment of immune cells. Under certain conditions, this can lead to a systemic inflammatory response in the body (Yu et al., 2015).

Depending on their origin, wounds are classified as traumatic, surgical, or burn wounds. Traumatic wounds result from mechanical damage, such as cuts, bites, or blows. They are often accompanied by significant soft tissue damage and microbial contamination (Gillespie et al., 2020). Surgical wounds result from surgical interventions and are usually controlled. However, an increased number of operations on patients with comorbidities increases the risk of surgical site infection and wound complications (Cochetti et al., 2020). Burn wounds are caused by high temperatures, chemicals, electric currents, or radiation, and they can vary in depth and extent of damage (Jeschke et al., 2020). Severe burn injuries lead to significant immune, inflammatory, and metabolic disruptions. These injuries can lead to multiple organ dysfunction and have a long-term negative impact on patients' physical and psychoemotional states (Jeschke et al., 2020).

Depending on the nature of the injury, wounds are classified as superficial, deep, or penetrating. Superficial wounds only affect the epidermis and usually heal without complications. Deep wounds penetrate the dermis and subcutaneous tissue, requiring longer treatment. Penetrating wounds are characterized by damage to internal organs or cavities, posing an immediate threat to the animal's life. Depending on the degree of contamination, wounds are classified as clean, contaminated, or infected. This classification is important for choosing a treatment strategy. Wounds are classified as acute or chronic based on healing time. Acute wounds usually heal within two to three weeks, while chronic wounds do not show a tendency to heal within three weeks and often recur. Chronic wounds pose a significant biological and socioeconomic burden. Their pathogenesis is associated with pathologically prolonged inflammation, hypoxia, ischemic-reperfusion injury, and bacterial colonization (Zhao et al., 2016).

Wound healing occurs in successive, partially overlapping phases. Disruption of any phase can lead to chronicity or excessive scarring (Fernández-Guarino et al., 2023). Excessive scar tissue formation leads to hypertrophic scars and keloids, which may be accompanied by pain, itching, and functional impairment (Martin and Nunan, 2015).

The classical model distinguishes four main phases of wound healing: hemostasis, inflammation, proliferation, and remodeling. The initial hemostatic phase is characterized by microvascular damage, fibrin clot formation, platelet aggregation, and fibroblast activation. Fibroblasts play a key role in subsequent tissue repair. The inflammatory phase involves the infiltration of neutrophils, monocytes, and lymphocytes into the wound, cleansing the wound defect and creating conditions for subsequent regeneration stages (Knoedler et al., 2023).

The proliferative phase is characterized by the formation of granulation tissue, active angiogenesis, migration, and proliferation of fibroblasts and epithelial cells. Granulation tissue consists of newly formed vessels and an extracellular matrix rich in collagen and other structural proteins. Cell adhesion to the extracellular matrix via integrin receptors is a prerequisite for cell cycle progression and effective tissue regeneration (Kamranvar, Rani and Johansson, 2022).

At the same time, epithelialization occurs, during which epithelial cells migrate from the edges of the wound and form a new epidermis, providing the skin with barrier, protective, and immune functions (De Szalay and Wertz, 2023). Myofibroblasts play an important role in wound contraction and scar tissue formation by synthesizing and organizing the extracellular matrix, restoring the mechanical integrity of tissues; however, their excessive or prolonged activity can lead to fibrosis (Schuster et al., 2022).

Exudation is an essential component of the wound healing process. Wound fluid, derived from blood plasma, contains oxygen, nutrients, cytokines, growth factors, and cellular elements necessary for cleansing and healing the wound (Saravi et al., 2023). However, excessive or prolonged exudation, especially if it is cloudy or purulent, indicates infection or necrotic tissue. In this case, more active surgical intervention and a correction to the treatment strategy are required (Beraja et al., 2025).

The type of wound treatment required depends on the stage of the wound healing process, the type of injury, and the patient's overall health. The main principles are: thorough cleansing of the wound; prevention or control of infection; maintenance of an optimal moist environment; and stimulation of tissue regeneration (Fani et al., 2024). Modern wound treatment approaches are based on using dressing materials that promote physiological healing. Moist-dry dressings, which were previously widely used, are no longer considered the standard of care due to their negative impact on tissue regeneration (Ousey et al., 2016).

Hydrogel dressings are becoming increasingly popular as they provide an optimal moist environment, reduce inflammatory reactions, and stimulate repair processes, particularly in chronic wounds (Huang et al., 2022). Calcium-based alginate dressings, polyurethane foam dressings, hydrocolloids, and hydrogels are among the most widely used modern wound dressings. Calcium alginate is particularly effective for deep, exuding wounds and has a pronounced hemostatic effect (Zhang, Cheng and Ao, 2021). Hydrocolloid dressings create a moist environment and are typically used to treat chronic wounds and second-degree burns (Zaika et al., 2024). Polyurethane foam dressings are used for wounds with moderate to heavy exudation, providing mechanical protection and gas exchange (Holmes et al., 2022). Hydrogels, due to their high water content and biocompatibility, are promising materials for treating wounds during the granulation and epithelialization phases. Additionally, they can incorporate antibacterial components (Liu et al., 2022).

Thus, the above-discussed works indicate the relevance of wound treatment in veterinary medicine, given the high prevalence of wounds and the risk of complications. The introduction of biocompatible wound dressings — particularly hydrogel applications — is seen as a promising direction in modern practice, as they allow targeted influence on the course of the wound healing process and increase the effectiveness of therapy.

The study **aims** to analyze the nosological forms of wounds in dogs and cats of various origins for which hydrogel applications are the optimal treatment option, and to confirm their clinical efficacy through experimentation.

Materials and methods. The study involved dogs and cats with wounds accompanied by significant tissue defects that prevented primary approximation of the edges. This resulted in the wounds healing by secondary intention, through granulation with the formation of a normotrophic scar. The animals were selected based on absolute clinical and anamnestic indications for surgical wound debridement, followed by conservative treatment and secondary healing.

In this study, the term 'secondary tension' refers to the process of wound healing involving suppuration, the formation of granulation tissue, and subsequent scar remodelling. This type of regeneration is characteristic of wounds with a large tissue deficit, where primary approximation of the edges is impossible, and of wounds complicated by an infectious process.

A total of 34 animals (dogs, n = 19; cats, n = 15) were involved in the study. The empirical base was the private veterinary clinic 'Dovira' (Kharkiv). Each animal underwent a clinical examination using a well-known algorithm, which concluded that there were no pathologies directly affecting the wound healing process. The total observation period lasted 60 days. Skin regeneration was monitored every 15 days. The assessment criteria for tissue regeneration were a reduction in classic signs of inflammation and total epithelialization.

The study included animals of all ages, sexes, and breeds. The clinical cases presented demonstrate the effectiveness of diagnostic and therapeutic measures, as well as the dynamics of the wound-healing process, when treating wounds of various profiles using hydrogel-based products (Fig. 1).

Figure 1. Hydrogel-based dressings from different manufacturers: a — Sorbalgon; b — DuoDerm; c — Kaltostat; d — Hydrocoll.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

Results and discussion. ***Etiological characteristics of wounds.*** Analysis of the etiology of wound injuries showed that in most cases, dogs and cats were diagnosed with bite wounds. The proportion of such injuries was 39% in dogs and 33.3% in cats (Table 1).

Lacerations ranked second in frequency (26.3% in dogs and 20% in cats). Cut wounds were found in 21% of dogs and 20% of cats. Cases of burn injuries are of interest from the perspective of their statistical distribution. Chemical burns caused by various compounds were recorded at the same proportion across species, namely 15.7% in dogs and 20% in cats.

Table 1 — Distribution of wounds according to their etiology (n = 34)

Type of damage	Dogs, n = 19		Cats, n = 15	
	n	%	n	%
Bite wound	7	39	5	33.3
Laceration	5	26.3	3	20
Cut wound	4	21	3	20
Chemical burn	3	15.7	3	20
Thermal burn	0	0	1	6.7

However, only one case of thermal burn was recorded in cats, accounting for 6.7% of all cases.

Bite wounds were characterized by irregular edges and variable depths, increasing the risk of bacterial contamination. Lacerations were caused by tissue rupture, had uneven edges, and were usually less infected than bite wounds. Cut wounds were caused by sharp objects, had smooth edges, and were accompanied by significant bleeding. The probable origins of recorded cases of chemical and thermal burns in dogs and cats can be explained by specific conditions of animal husbandry, behavioral responses, and anthropogenic factors.

Localization of wound damage. During the clinical examination, a relationship was established between the type of animal and the topographical localization of wounds, as reflected in the statistical distribution of nosological units (Table 2).

Table 2 — Distribution of clinical cases of wounds depending on their location (n = 34)

Topographic location	Dogs, n = 19		Cats, n = 15	
	n	%	n	%
Thoracic limb	6	31.5	6	40
Neck	6	31.5	2	13.3
Pelvic limb	4	21.3	3	20
Torso	3	15.7	4	26.7

In cats, the most commonly affected areas were the thoracic limbs (40%) and torso (26.7%), less commonly the pelvic limbs and neck (13.3% each). In dogs, the largest proportions of injuries were to the thoracic limbs and neck (31.5% each), followed by the pelvic limbs (21.3%) and the torso (15.7%).

The course of the wound healing process under secondary tension. Based on our clinical observations, the wound-healing process under secondary tension progressed through four consecutive stages: hemostasis, inflammation, proliferation, and remodeling (Table 3).

Table 3 — Duration of wound regeneration stages (n = 34)

Regeneration stage (unit)	Duration of the stage (M ± m)
Hemostasis (min)	7.5 ± 0.14
Inflammation (h)	4 ± 0.06
Proliferation (days)	12.5 ± 0.43
Remodeling (days)	57.5 ± 0.14

The hemostasis stage lasted an average of five to ten minutes, which is why most animals arrived at the clinic without signs of active bleeding. The inflammatory stage developed within three to five hours after the injury. Signs of proliferation were observed between days 5 and 20, whereas the remodeling stage lasted between days 55 and 60 after the injury.

The following are several clinical cases involving the use of hydrogel to treat animals with wounds of various origins admitted to a veterinary medical facility.

A cat nicknamed 'Chance' was diagnosed with a chronically infected wound on its neck, showing signs of necrosis and purulent inflammation. This was caused by a bite from another animal. Following surgical debridement of the wound, primary closure was not possible due to significant tissue loss, so secondary tension treatment was chosen, involving the application of hydrogels.

The cat's chronic infected wound gradually healed in stages, corresponding to the classic course of wound healing by secondary tension.

Following surgical debridement (Fig. 2a), necrotic tissue and purulent exudate were removed from the wound. On the 1st day of observation (Fig. 2b), a reduction in edema, a clean wound bed, and moderate exudation were noted. Using hydrogel applications helped to maintain a moist environment, which is optimal for further reparative processes.

On the 15th day of observation (Fig. 3a), there were clear signs of active granulation in the wound: the wound bed was evenly filled with pink-red granulation tissue; the amount of exudate had decreased significantly; and there were no signs of purulent inflammation. The animal's general condition remained satisfactory. By the 30th day (Fig. 3b), the wound defect had further reduced in size due to the contraction of the wound edges and intensive growth of granulation tissue. Signs of marginal epithelialization began to appear, indicating that the healing process had transitioned to the proliferation phase.

On the 45th day of observation (Fig. 4a), the wound was significantly smaller in size, and most of it was covered with young epithelium. The granulation tissue became denser and paler, which corresponded to the stage of scar tissue maturation. There were no inflammatory changes. On the 60th day (Fig. 4b), almost complete healing of the wound was noted. The surface was covered with formed epithelium, with only a slight scar remaining without signs of inflammation or infection. The functional and cosmetic results of the treatment were assessed as satisfactory.

The prolonged proliferative stage was clearly associated with the animal's immunodeficiency. Complete wound healing occurred, resulting in the formation of a normotrophic scar.

A cat nicknamed 'Asya' developed a chronic wound as a result of a thermal burn to its right hind limb. Following primary surgical treatment, hydrogel applications were used.

The cat's chronic thermal wound gradually healed by secondary tension, which was prolonged due to the depth of the injury and insufficient home care.

Before surgical debridement (Fig. 5a), the wound on the right pelvic limb was characterized by the presence of necrotic tissue, inflammatory changes, and uneven edges typical of thermal burns. On the 1st day after primary surgical treatment (Fig. 5b), cleansing of the wound bed from non-viable tissues, a decrease in the severity of inflammation, and moderate exudation were noted. The use of hydrogel applications helped maintain the moist environment necessary for the activation of reparative processes.

On the 15th day of observation (Fig. 6a), the first signs of granulation tissue formation appeared in the wound, but the process was uneven. In some areas, there was still high moisture content, and the wound was not cleansing properly, partly due to poor hygiene and the fur around the wound not being removed promptly.

By the 30th day of treatment (Fig. 6b), there was a gradual decrease in wound size and more pronounced granulation tissue formation. The wound edges began to contract, but healing remained slow, and maceration of the edges was periodically observed due to violations of the care regimen.

The healing process was slowed down due to insufficient wound care at home, in particular, the untimely removal of hair around the injury. On the 45th day of observation (Fig. 7a), the wound defect was significantly smaller, and the wound bed was mainly filled with mature granulation tissue. There were clear signs of marginal epithelialization, and inflammation was minimal. The general condition of the animal was assessed as satisfactory.

On the 60th day of treatment (Fig. 7b), complete wound healing was noted. The surface of the injury was covered with formed epithelium, and a moderately pronounced scar remained without signs of active inflammation or infection.

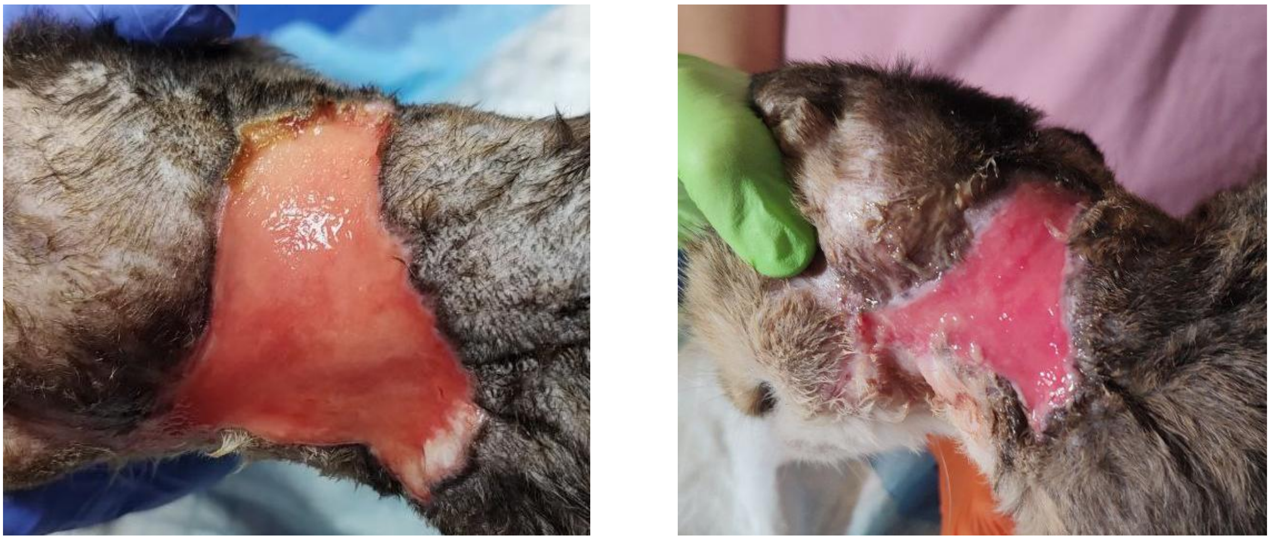
Thus, despite the slow course of reparative processes caused by insufficient wound care at home, the use of hydrogel applications in combination with surgical debridement ensured gradual wound cleansing, granulation tissue formation, epithelialization, and final healing on the 60th day of treatment.

A dog named 'Filimon' developed chemical necrosis of the tissues of the left chest limb as a result of doxorubicin extravasation during chemotherapy for lymphoma. Due to intense exudation at the initial stage, hydrocolloid dressings were used, and after the exudation decreased, hydrogel and alginate applications were used. Comprehensive therapy contributed to the gradual cleansing of the wound, reduction of the severity of inflammation patterns, and tissue regeneration.

Before surgical debridement (Fig. 8a), the wound was characterized by pronounced tissue necrosis, intense exudation, and significant inflammatory changes typical of chemical damage caused by cytostatic drugs. On the 1st day after surgical treatment (Fig. 8b), partial cleansing

a **b**

Figure 2. Chronic infected wound on a cat's neck: a — before surgical treatment; b — 1st day after treatment.



a **b**

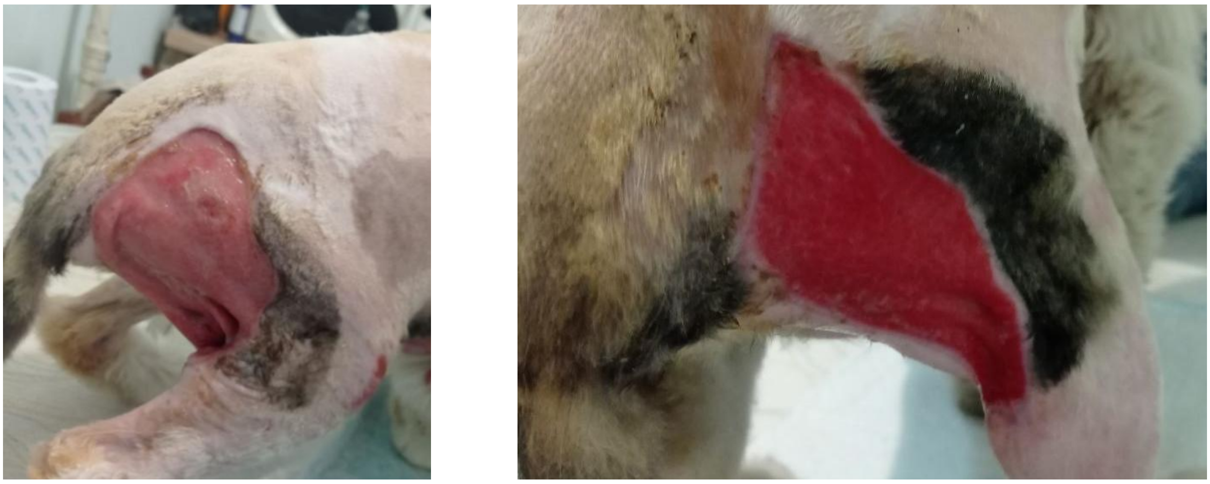
Figure 3. Chronic infected wound on a cat's neck: a — on the 15th day of observation; b — on the 30th day of observation.

a **b**

Figure 4. Chronic infected wound on a cat's neck: a — on the 45th day of observation; b — on the 60th day of observation.

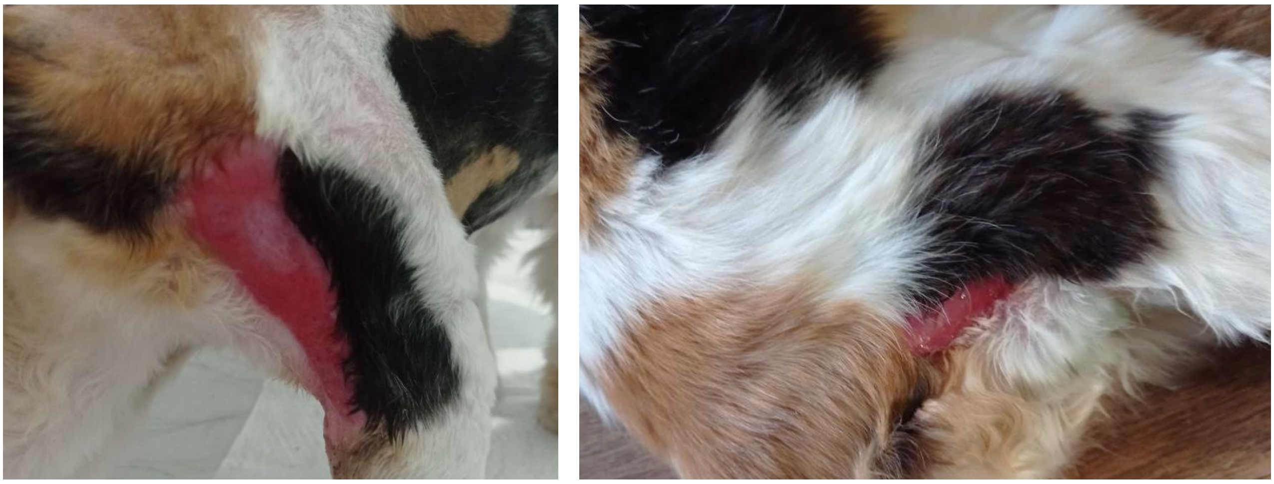
a **b**

Figure 5. Thermal wound on the right pelvic limb of a cat: a — before surgical treatment; b — on the 1st day after treatment.



a **b**

Figure 6. Thermal wound on the right pelvic limb of a cat: a — on the 15th day of observation; b — on the 30th day of observation.



a **b**

Figure 7. Thermal wound on the right pelvic limb of a cat: a — on the 45th day of observation; b — on the 60th day of observation.

a b

Figure 8. Wound on the left thoracic limb of a dog caused by a chemical burn: a — before surgical treatment; b — on the 1st day after treatment.

a b

Figure 9. Wound on the left thoracic limb of a dog caused by a chemical burn: a — on the 15th day of observation; b — on the 30th day of observation.

a b

Figure 10. Thermal wound on the right pelvic limb of a dog: a — on the 45th day of observation; b — on the 60th day of observation.

of the wound bed and a reduction in the amount of necrotic tissue were noted, but profuse exudation persisted. In this regard, hydrocolloid dressings were used at the initial stage of treatment, which effectively absorbed the exudate and created a protective environment for the wound.

On the 15th day of observation (Fig. 9a), the intensity of exudation decreased significantly, the wound bed became cleaner, and areas of young granulation tissue appeared. The severity of inflammatory patterns decreased, which allowed us to proceed to the use of hydrogel and alginate applications to stimulate reparative processes. On the 30th day of treatment (Fig. 9b), active formation of granulation tissue and a gradual reduction in the area of the wound defect were observed. The wound edges became smoother, and there were no signs of secondary infection, indicating stabilization of the local process.

On the 45th day of observation (Fig. 10a), most of the wound was filled with mature granulation tissue, and intense marginal epithelialization was occurring. Inflammatory manifestations were minimal, and exudation was practically absent. The general condition of the animal was assessed as satisfactory. On the 60th day of treatment (Fig. 10b), almost complete wound healing with scar tissue formation was noted. The surface of the lesion was covered with epithelium, limb function was preserved, and no patterns of active inflammation or necrosis were observed.

The results obtained indicate that the use of hydrogel applications during the clinical management of wound healing under secondary tension promotes wound surface cleansing, maintenance of a moist environment, stimulation of granulation, and scar tissue formation even in complex clinical cases complicated by secondary infection, immunodeficiency, or chemotherapy.

Our observations have shown that tissue healing does not differ depending on the location or type of wound, or the species of animal. However, it does differ significantly depending on the extent of tissue loss due to bodily injury and aggravating factors in the animal's medical history (Knoedler et al., 2023).

Thus, wound healing is a complex, multistage biological process that restores damaged tissue integrity. This occurs through a sequence of interrelated stages, each with its own cellular and molecular mechanisms. Hemostasis is the initial and critically important stage of the wound healing process, ensuring that bleeding stops and that blood remains fluid in the vascular bed. Hemostasis is considered to be the body's initial response to tissue damage and involves three interrelated processes: vasoconstriction (the reflex narrowing of the lumen of the damaged vessel), platelet aggregation resulting in the formation of a primary thrombus, and the activation of the blood coagulation cascade resulting in the formation of a stable fibrin plug. This has been confirmed by data from other researchers (Martin and Nunan, 2015).

Damage to blood and lymph vessels causes blood and lymph to leak out, filling the wound defect and helping to mechanically clean the wound surface of foreign particles and necrotic elements. Almost immediately after the injury, the blood vessels undergo reflex vasoconstriction, and damage to the endothelium leads to the activation of platelets with the subsequent formation of a thrombus directly in the defect area. The formed blood clot performs a protective function, isolating the wound from the external environment, dries out over time with the formation of a scab, and creates optimal conditions for the subsequent stages of tissue repair. The duration of vasoconstriction is usually 5–10 minutes, but the data available in the literature on this indicator are contradictory (Zhao et al., 2016).

The proliferation stage is characterized by active cell growth and migration, particularly of fibroblasts, endothelial cells, and epithelial cells. Fibroblasts gradually fill the wound defect and initiate the synthesis and deposition of the extracellular matrix, which is mainly composed of collagen fibers and glycosaminoglycans. At the same time, neovascularisation occurs to ensure the formation of granulation tissue, which is rich in newly formed capillaries, cellular elements, and the intercellular substances necessary for further regeneration (Kamranvar, Rani and Johansson, 2022).

Remodelling, or tissue reorganization, occurs after the main proliferative processes have been completed. During this period, epithelial cells proliferate in the basal and suprabasal (spiny) layers of the epidermis along the viable edges of the skin defect using pseudopods for migration. The epithelial cells gradually destroy the blood clot and stroma formed in the wound by secreting proteolytic enzymes, particularly collagenases and plasminogen activators. This replaces the temporary covering with fully viable tissue, as confirmed by several scientific studies (Schuster et al., 2022).

Thus, during remodeling, epithelial cells undergo significant phenotypic changes: they lose dense intercellular contacts with the dermis and apical-basal polarity, which ensures their mobility and ability to effectively repair damaged surfaces. Subsequently, collagen fibers are restructured, the number of cellular elements decreases, and the functional integrity of the tissue is gradually restored.

Conclusions. 1. The nosological structure of wounds in cats and dogs was determined based on the analysis of the patient journal. Bite wounds were most frequently diagnosed (39% of cases in dogs and 33.3% in cats), with a predominant topographical localization in cats in the thoracic limbs and torso (40% and 26.7% of cases, respectively), and in the thoracic limbs and neck and pelvic limbs, respectively, 31.5% and 21.3% of all registered cases.

2. The characteristics of the wound healing process have been identified. Clinical observations have confirmed the existence of characteristic stages of tissue regeneration: hemostasis, inflammation, proliferation,

and remodeling. Hemostasis stops bleeding, inflammation cleans the wound surface, proliferation forms new granulation tissue, and remodeling completes the healing process by restoring damaged epithelium.

3. It has been established that healing processes do not depend on breed, sex, location, or type of wound. However, important factors affecting healing are the extent of the damage and the presence of a complicating medical history.

4. The clinical effectiveness of hydrogel applications in the comprehensive treatment of purulent wounds has been confirmed. The findings highlight the necessity of an all-encompassing treatment approach that considers the animal's overall health. This can ensure more effective healing and reduce the risk of complications.

5. It has been experimentally proven that clinical management of non-surgical wounds is based on ensuring secondary tension by creating conditions for hemostasis, healing, and autolysis of necrosis. The main therapeutic measures include surgical wound debridement and the use of interactive hydrocolloid-based dressings.

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PSYCHO-EMOTIONAL STATE OF DOGS WITH DIFFERENT TEMPERAMENT CHARACTERISTICS UNDER THE INFLUENCE OF AN ACOUSTIC STIMULUS

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Summary. This study aimed to assess the psycho-emotional state of German Shepherd dogs with different temperament characteristics under acute stress and anxiety conditions. A modified five-point FAS-M (Fear, Anxiety, Stress) scale, adapted from the original eight-point system (Gatehouse et al., 2025), was applied. The experiment was conducted on 30 male dogs aged 3.9 ± 0.2 years, tested under open-field conditions for five consecutive days. On days 2–3, acute stress was induced by an acoustic stimulus (thunder, 90–100 dB), whereas on days 1, 4, and 5, the anxiety state was evaluated without stimulus exposure. Temperament traits were assessed using the C-BARQ questionnaire, allowing the classification of dogs by aggression, fear and anxiety, excitability, trainability, and obedience. It was established that acute stress caused a significant increase in psycho-emotional scores compared with baseline levels ($p < 0.001$), with the most pronounced reaction observed on the second experimental day. On non-stress days, gradual score reduction indicated adaptation to the testing environment. Highly aggressive and overexcitable dogs exhibited the strongest reactions to the stressor (up to 3.45 a. u.), whereas obedient and calm dogs showed the lowest reactivity and the fastest behavioral stabilization. Correlation analysis revealed a strong positive relationship between excitability and psycho-emotional state ($r = 0.80$; $p < 0.001$) and between aggression and excitability ($r = 0.68$; $p < 0.001$). Conversely, trainability and obedience were significantly negatively correlated with psycho-emotional state ($r = –0.65$; $p < 0.001$), confirming their protective role against stress development. These findings indicate that individual temperament traits determine the level of stress resistance in dogs and can be used to predict behavioral reactivity in veterinary practice, training, and animal welfare assessment

Keywords: stress, anxiety, aggression, excitability, obedience, FAS-M

Introduction. In contemporary veterinary behavioral science, the study of emotional and physiological responses of dogs to stressful stimuli is crucial for understanding mechanisms of adaptation, welfare, and the effectiveness of human–animal interaction. Psycho-emotional disturbances caused by chronic or acute stress can affect not only behavior but also neuroendocrine regulation, immune reactivity, and overall animal health (Beerda et al., 2000; Koolhaas et al., 2011). Clinical and experimental observations indicate that dogs respond sensitively to environmental changes, acoustic stimuli, social isolation, or unfamiliar surroundings, which manifest as increased anxiety, excitability, or aggression (Stanford, 1981; Döring et al., 2009). In veterinary practice, psychogenic stress is recognized as one of the main causes of behavioral disorders, examination difficulties, and reduced welfare (Hekman, Karas and Sharp, 2014; Hauser et al., 2020).

Stress responses in dogs exhibit both individual and typical components related to temperament, learning history, and socialization experience (Stephen and Ledger, 2005; Somppi et al., 2022). Behavioral indicators such as body posture, facial expression, vocalization, or avoidance, combined with physiological markers (cortisol level, heart rate), reflect the animal's emotional state (Flint et al., 2024; Mârza et al., 2024). Assessing these parameters forms the basis for developing scientifically grounded methods of stress control and prevention. Some studies have demonstrated the effectiveness of standardized scales such as FAS (Fear, Anxiety, Stress) for quantifying fear and anxiety during veterinary procedures (Mercier et al., 2023; Gatehouse et al., 2025). The use of such instruments enables correlation of behavioral responses with physiological changes, increasing the accuracy of emotional state assessment (Kim et al., 2022; King et al., 2022).

Recent works also emphasize the importance of interpreting 'frustration' as a distinct emotional response that can significantly affect the performance of working and search-and-rescue dogs (Dickinson and Feuerbacher, 2025). Similarly, chronic stress exposure can alter behavioral lateralization, indicating profound neurophysiological reorganization (Salgirli Demirbas et al., 2023). Moreover, McMahon, Youatt and Cavigelli (2022) highlight the significance of an animal's physiological profile as an indicator of individual behavioral differences: combining heart rate parameters, hormonal levels, and behavioral traits allows for more accurate interpretation of temperament's functional role. Collectively, these findings underscore that studying stress in dogs requires integrating neuroendocrine, cardiovascular, and behavioral indicators to achieve a comprehensive understanding of emotional regulation and adaptation mechanisms. Therefore, the complex assessment of stress-related behavioral and physiological traits in dogs, taking into account their temperament, is essential for developing scientifically grounded methods for evaluating adaptive responses. This has both theoretical and practical relevance — improving animal welfare, enhancing veterinary procedure efficiency, and strengthening the quality of human–dog interactions (Teo et al., 2022).

The study **aimed** to investigate the behavior of dogs, their level of psycho-emotional reactivity, trainability, and obedience under the influence of stress reactions in accordance with individual temperament traits, using an open field model with an acoustic stimulus.

Materials and methods. The study was conducted on 30 healthy male German Shepherd dogs aged 3.9 ± 0.2 years and weighing 34.0 ± 3.1 kg. The animals were kept at the Police Cynological Center in Kyiv and the 'vom Yambwelle Hof' kennel. All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University (protocol No. 3, April 22, 2025).

Temperament traits were assessed using the standardized C-BARQ (Canine Behavioral Assessment & Research Questionnaire) method recommended by the University of Pennsylvania (2023). Standard questionnaires completed by the owners were analyzed (Serpell, 2015). Among 14 behavioral criteria, four were selected as most indicative of individual reactivity differences: aggression, fear and anxiety, excitability, and trainability and obedience. Each trait was divided into three expression levels.

For aggression:

Calm dogs — did not display aggression toward people or other animals;

Moderately aggressive — reacted aggressively only in specific threatening or competitive contexts;

Aggressive — showed pronounced aggression (growling, biting, or attack attempts).

For fear and anxiety:

Brave dogs — showed no signs of fear or avoidance;

Moderately anxious — exhibited short-term alertness or mild worry;

Fearful dogs — displayed pronounced fear responses, avoided contact, trembled, or hid.

For excitability:

Calm — stable behavior, low reactivity;

Moderately excitable — transient periods of increased activity under stimulation;

Highly excitable — hyperreactive, excessive motor activity, poor behavioral control.

For trainability and obedience:

Obedient — easily trainable, promptly responded to commands;

Occasionally disobedient — responded selectively or required repetition;

Disobedient — had training difficulties, often ignored the trainer's cues.

These behavioral profiles were used to form experimental groups and to compare responses of dogs with different temperament traits during acute stress modeling. This approach enabled assessment of how individual temperament features influence behavioral and physiological reactivity intensity under standardized testing conditions.

Acute stress modulation was performed using the open-field test. A 16 m^2 test arena was equipped with a video camera for behavioral monitoring. Testing was performed over five consecutive days between 9:00 and 10:00 a. m. *Day 1:* the dog was left alone for 10 minutes for environmental adaptation. *Days 2–3:* the dog was left alone for 9 minutes — the first 3 minutes in silence, then 3 minutes with a thunder sound (90–100 dB), followed by 3 minutes of silence. *Days 4–5:* the dog was left alone for 5 minutes in silence to record anxiety-related behavior.

Behavioral responses were scored using a modified FAS-M (Fear, Anxiety, Stress) scale adapted from the original eight-point system (Gatehouse et al., 2025) to a five-point format. Two independent experts observed and rated each dog to minimize subjectivity. 0 points: no visible behavioral changes; calm and relaxed; 1 point: mild tension or concern without aggression or excessive movement; 2 points: fearfulness or agitation without aggression, frequent movements, or avoidance; 3 points: pronounced arousal with high mobility, vocalization, moderate aggression; 4 points: strong aggression — attack or active defense attempts. Additionally, typical signs of acute stress were recorded, including trembling, yawning, tongue protrusion, tail tucked between legs, avoidance of eye contact, and reactions toward the handler and experimenter (Gutiérrez et al., 2019; Kartashova et al., 2021). This combination of quantitative and qualitative assessments enabled a comprehensive characterization of behavioral responses to stress stimuli.

Statistical analysis was performed using MS Excel 2025 with the built-in 'Data Analysis' tool. Descriptive statistics, correlation, and one-way ANOVA were applied. Differences between means were tested using Student's *t*-test, with significance levels set at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$.

Results and discussion. Modern research in canine neurophysiology confirms that stress responses are closely linked not only to individual temperament traits but also to early-life experiences and the quality of human–dog interactions. Buttner, Awalt and Strasser (2023) demonstrated that dogs exposed to early-life stressors show altered physiological and behavioral responses during social stress-buffering paradigms in adulthood, including reduced ability to mitigate stress through social contact (Buttner, Awalt, and Strasser, 2023). These findings are consistent with Höglin et al. (2021), who reported that long-term stress in dogs correlates with the quality of their relationship with the owner and certain personality traits such as emotional stability and anxiety level (Höglin et al., 2021).

Table 1 presents baseline results of the emotional and psychological state evaluation of German Shepherd dogs using the modified FAS-M (Fear, Anxiety, Stress) scale during a five-day experimental period. Measurements were taken before and after the dogs' exposure to the testing arena ('open-field' conditions), where acute stress (acoustic stimulus) and anxiety states were modeled. It was found that on the first experimental day, the mean pre-exposure score was 1.2 a. u. (ranging from 0 to 2), while after exposure, it significantly increased to 2.0 a. u. ($p < 0.001$), indicating the development of a mild stress response associated with environmental novelty and isolation.

Table 1 — Emotional and psychological state of German Shepherd dogs under acute stress and anxiety conditions (n = 30; a. u.)

Study period		Statistical indicators				
		M *(min.–max.)*	SD	SEM	S2	CI (95%)
Day 1	Before arena	1.2 *(0–2)*	0.79	0.14	0.63	0.3
	After arena	2 *(0–3)****	0.81	0.15	0.65	0.3
Day 2	Before arena	1.2 *(0–2)*	0.71	0.13	0.51	0.27
	After arena	2.6 *(2–4)****	0.67	0.12	0.46	0.25
Day 3	Before arena	1.6 *(1–3)*	0.67	0.12	0.45	0.25
	After arena	2.7 *(2–4)****	0.76	0.14	0.57	0.28
Day 4	Before arena	1.8 *(1–3)*	0.65	0.12	0.42	0.24
	After arena	2.3 *(1–3)***	0.66	0.12	0.44	0.25
Day 5	Before arena	1.8 *(1–3)*	0.68	0.12	0.46	0.25
	After arena	2.1 *(1–3)**	0.63	0.11	0.4	0.23

Notes: significant differences between pre- and post-exposure values: * — $P \leq 0.05$; ** — $P \leq 0.01$; *** — $P \leq 0.001$.

On the second and third days, when the dogs were exposed to an auditory stimulus (recorded thunder sound), emotional arousal scores increased significantly after exposure compared to baseline. Specifically, on day 2, the mean value rose from 1.2 to 2.6 a. u. ($p < 0.001$), and on day 3 — from 1.6 to 2.7 a. u. ($p < 0.001$), indicating a pronounced response to acute stress. On the fourth and fifth days, when anxiety was assessed without external stimuli, there was still a significant increase after arena exposure. The mean score increased from 1.8 to 2.3 a. u. on day 4 ($p < 0.01$) and from 1.8 to 2.1 a. u. on day 5 ($p < 0.05$).

The obtained results demonstrate that the modified FAS-M scale is a sensitive tool for detecting changes in the dogs' psycho-emotional state under the influence of acute stress and situational anxiety in open-field conditions. A significant increase in post-exposure scores was already observed on the first day of testing, despite the absence of a sound stimulus, reflecting a response to the novel environment and social isolation. Similar findings regarding the stressogenic effects of unfamiliar surroundings, isolation, and the veterinary environment were reported by Stanford (1981), Döring et al. (2009), and Hekman, Karas and Sharp (2014), who showed marked increases in anxiety levels and behavioral disturbances even in the absence of additional stressors. These observations confirm that testing conditions themselves can act as a significant source of psychogenic stress.

Fig. 1 presents the differences (Δ) between emotional and psychological state scores (according to the FAS-M scale) recorded before and after the dogs' exposure to the testing arena under acute stress conditions (days 2–3, with sound stimulus) and anxiety conditions (days 1, 4, and 5, without external stimuli).

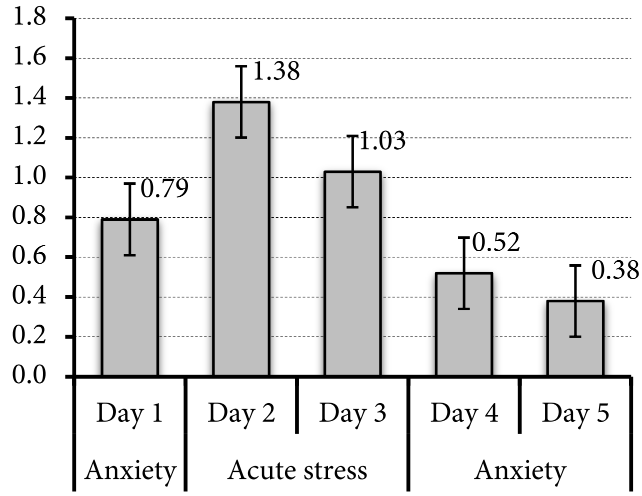

Figure 1. Difference (Δ) in emotional and psychological state scores of dogs under acute stress and anxiety conditions before and after exposure in the arena (n = 30; arbitrary units).

The largest difference between pre- and post-exposure scores was recorded on day 2 (acute stress) — 1.38 a. u. ($p < 0.001$), indicating a pronounced response of dogs to the sound stimulus. On day 3, also under acute stress, this difference slightly decreased to 1.03 a. u., suggesting partial desensitization or early adaptation to the repeated stimulus. On the days when only anxiety was assessed (without targeted stress exposure), the difference was less pronounced — 0.79 a. u. on day 1, 0.52 a. u. on day 4, and 0.38 a. u. on day 5, reflecting a gradual reduction in reactivity and partial habituation to the testing environment over the course of the experiment.

Thus, the highest psycho-emotional responses were registered on the second and third days of the experiment, during exposure to the auditory stimulus (thunder sound), which was accompanied by a sharp rise in FAS-M scores and the greatest pre-/post-test difference. This pattern aligns with the concept of acute stress as a rapid adaptive response characterized by activation of behavioral and neuroendocrine mechanisms (Koolhaas et al., 2011; Beerda et al., 2000). Comparable findings were reported by Flint et al. (2024) and Gobbo and Zupan Šemrov (2021), who observed pronounced changes in behavioral and cardio endocrine indicators in dogs exposed to intense sensory or social stimuli. The reduction in Δ between days 2 and 3 (from 1.38 to 1.03 a. u.) in this study may reflect initial adaptation or partial habituation to the repeated stressor, consistent with earlier observations of habituation to recurring stimuli while maintaining general physiological tension (Beerda et al., 2000; Hauser et al., 2020).

On the non-stimulus days (1, 4, 5), a lower intensity of stress response and a progressive decrease in pre-/post-exposure differences were observed, indicating habituation of the animals to the arena and testing procedures. These findings correlate with results by King et al. (2022), Mercier et al. (2023), and Gatehouse et al. (2025), who demonstrated that standardized testing conditions, repeated exposure, and predictable environments reduce fear and anxiety in dogs during clinical visits. Hence, the dynamics of mean FAS-M values confirm the validity of the applied acute stress model, illustrating both stress-induced and adaptive components of behavioral responses.

Furthermore, Gobbo and Zupan Šemrov (2021) showed that aggressive reactivity in dogs is accompanied by activation of neuroendocrine and cardiovascular mechanisms, including increased cortisol levels and heart rate, emphasizing the tight coupling between emotional and physiological regulation. Similarly, Grigg et al. (2022) established that dogs' emotional states mirror those of their handlers, as synchronization of behavioral and cardiovascular parameters reflects emotional contagion within the human–dog dyad.

Fig. 2 presents the dynamics of the psycho-emotional state of dogs with varying degrees of aggressiveness (calm — n = 9, moderately aggressive — n = 12, aggressive — n = 9) under acute stress and anxiety conditions throughout the five-day experiment, according to the modified FAS-M scale (a. u.). Calm dogs exhibited the lowest baseline psycho-emotional scores (0.78–1.67 a. u.). Their reactivity increased markedly on the second and third days, reaching peaks of 2.56 and 2.44 a. u., respectively, indicating a moderate yet distinct response to acute auditory stress. Under anxiety-only conditions (days 4 and 5), their emotional state remained relatively low (1.67–2.11 a. u.), demonstrating good adaptability.

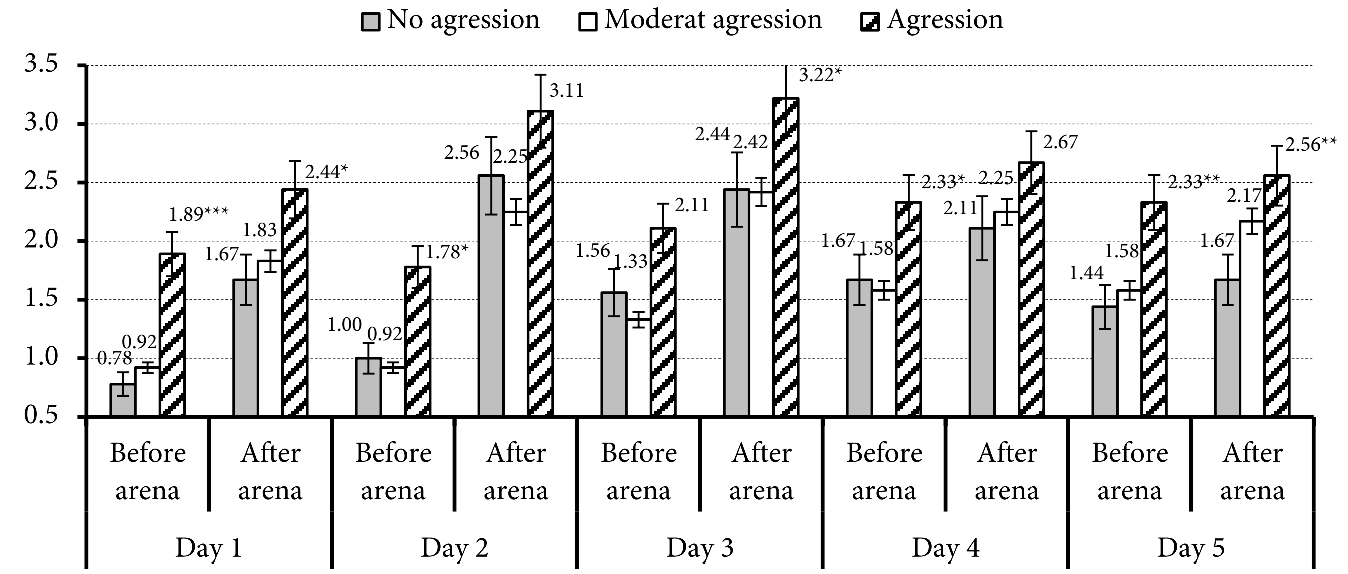

Figure 2. Dynamics of the psycho-emotional state of dogs with different levels of aggressiveness under acute stress and anxiety during the experiment (a. u.). Significant differences compared to calm dogs: * — P < 0.05; ** — P < 0.01; *** — P < 0.001.

Moderately aggressive dogs showed slightly higher baseline scores (0.92–1.58 a. u.) than calm ones. The most significant increases occurred under acute stress (days 2 and 3), when post-exposure scores reached 2.25 and 2.42 a. u., respectively. During anxiety evaluation (days 4 and 5), their psycho-emotional state remained somewhat elevated (2.17–2.25 a. u.) compared to calm dogs, indicating moderate susceptibility to stress reactions.

Dogs with pronounced aggressive behavior exhibited consistently high pre-exposure psycho-emotional levels (1.78–2.33 a. u.), which were significantly higher than those of the other groups. In response to acute stress (days 2 and 3), this group showed the highest scores, reaching peak values of 3.11 and 3.22 a. u., respectively. During the evaluation of anxiety without a stressor (days 4 and 5), the excitation levels of aggressive dogs remained elevated (2.67 and 2.56 a. u.), indicating the lowest adaptive capacity among the studied groups.

Dogs without evident signs of fear or anxiety had the lowest and most stable baseline psycho-emotional scores (1.0–1.55 a. u.), showing only moderate reactions to the acute auditory stimulus (days 2 and 3), when post-exposure scores increased to 2.45 and 2.55 a. u., respectively (Fig. 3). On non-stimulus days (days 4 and 5), their anxiety levels remained low, ranging from 1.82 to 2.0 a. u., reflecting good adaptability.

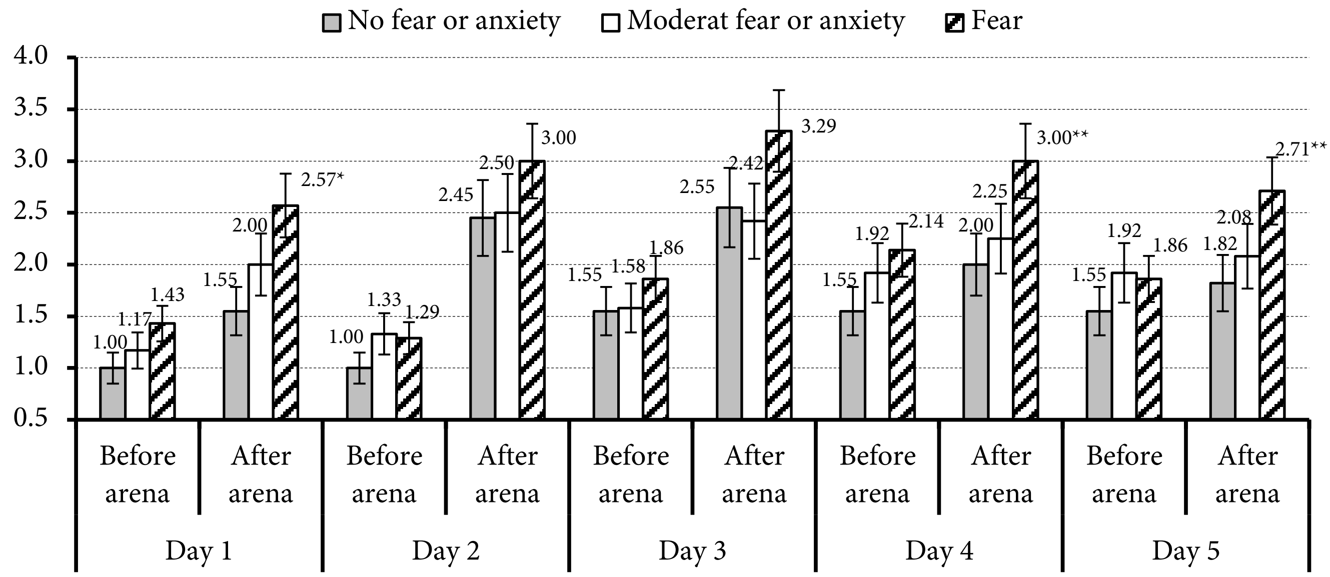

Figure 3. Dynamics of the psycho-emotional state of dogs with different levels of fear and anxiety throughout the experiment (a. u.). Significant differences compared to dogs without pronounced fear and anxiety: * — P < 0.05; ** — P < 0.01; *** — P < 0.001.

Animals with a moderate degree of fear and anxiety had slightly higher baseline scores (1.17–1.92 a. u.) and exhibited more pronounced increases in response to acute stress on days 2 and 3 (up to 2.5 and 2.42 a. u.). Under anxiety-only conditions (without the stressor), their post-exposure scores (2.08–2.25 a. u.) were somewhat higher than those of fearless dogs, indicating less effective adaptation.

Dogs with pronounced fear and anxiety showed the highest baseline psycho-emotional levels (1.29–2.14 a. u.) and the most intense reactions to acute stress, reaching up to 3.29 a. u. on day 3, which significantly exceeded the corresponding values in other groups. During the evaluation of ordinary anxiety (days 4 and 5), their emotional state remained elevated (2.71–3.0 a. u.), indicating a persistent predisposition to high anxiety levels and low adaptability.

Fig. 4 presents the dynamics of the psycho-emotional state of dogs with varying levels of excitability, assessed using the modified FAS-M scale under conditions of acute stress and anxiety throughout the five-day experiment. Dogs with calm behavior displayed the lowest baseline psycho-emotional levels (0.43–1.57 a. u.). Under acute stress (days 2 and 3), their scores increased significantly to 2.57 and 2.43 a. u., respectively, though remaining lower than in other groups. During anxiety evaluation days (4 and 5), the values stabilized at low levels (1.57–2.0 a. u.), reflecting good adaptive capacity to stressful situations.

Moderately excitable dogs had higher baseline psycho-emotional scores (0.83–1.5 a. u.) compared to calm animals. Under acute stress (days 2 and 3), their reactions moderately intensified (2.0 and 2.08 a. u.), demonstrating average sensitivity to stress factors. During anxiety assessment (days 4 and 5), their scores remained stable at about 2.08 a. u., indicating moderate reactivity and sufficient adaptability. Highly excitable dogs exhibited the highest initial psycho-emotional levels (1.82–2.36 a. u.) and the most pronounced response to acute stress (days 2 and 3), reaching peak values of 3.27 and 3.45 a. u., respectively. Even during ordinary anxiety conditions (days 4 and 5), their psycho-emotional levels remained elevated (2.55–2.82 a. u.), reflecting poor adaptability to stressful environments. These findings indicate that the level of excitability significantly affects dogs' psycho-emotional responses and adaptive capacity under acute stress and anxiety. Dogs with excessive excitability exhibit high reactivity and low adaptability, whereas calm animals show minimal reactivity and strong stress resistance.

Fig. 5 illustrates the dynamics of the psycho-emotional state of dogs with different levels of trainability and obedience. Well-trained dogs (obedient) had the lowest baseline psycho-emotional scores (0.45–1.36 a. u.) and showed a moderate response to acute stress (day 2 — 2.09 a. u., day 3 — 2.0 a. u.). Under normal anxiety conditions, their scores remained low and stable (1.73–1.82 a. u.), indicating high adaptability and low reactivity.

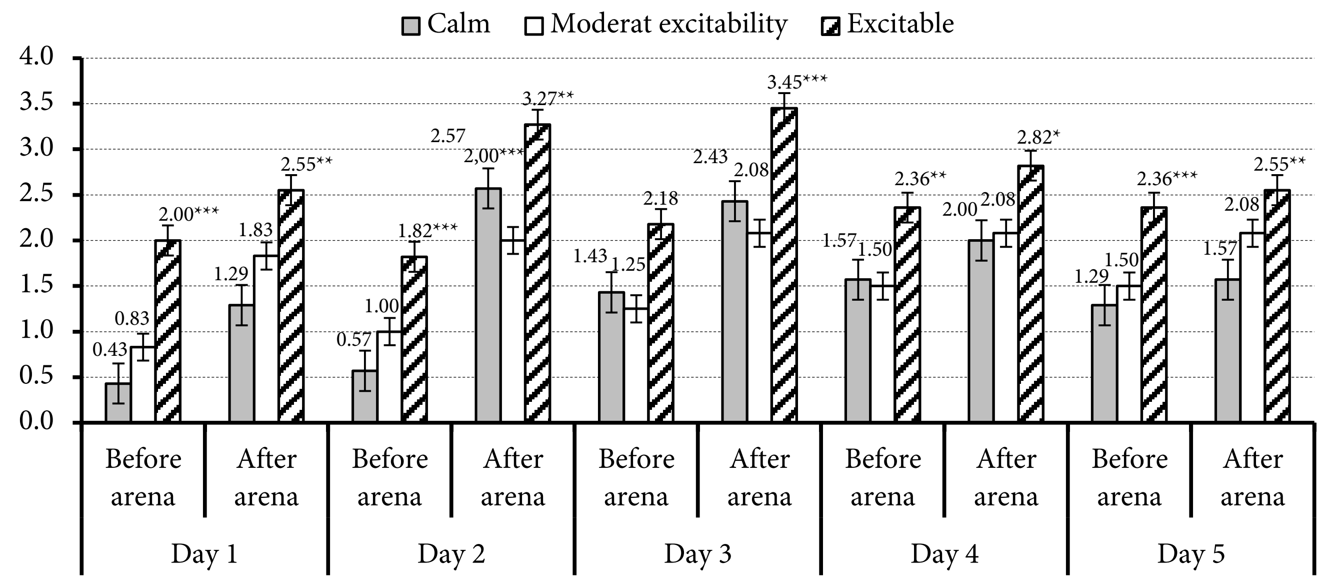

Figure 4. Dynamics of the psycho-emotional state of dogs with varying degrees of excitability during the experiment (a. u.). Significant differences compared to calm dogs: * — P < 0.05; ** — P < 0.01; *** — P < 0.001.

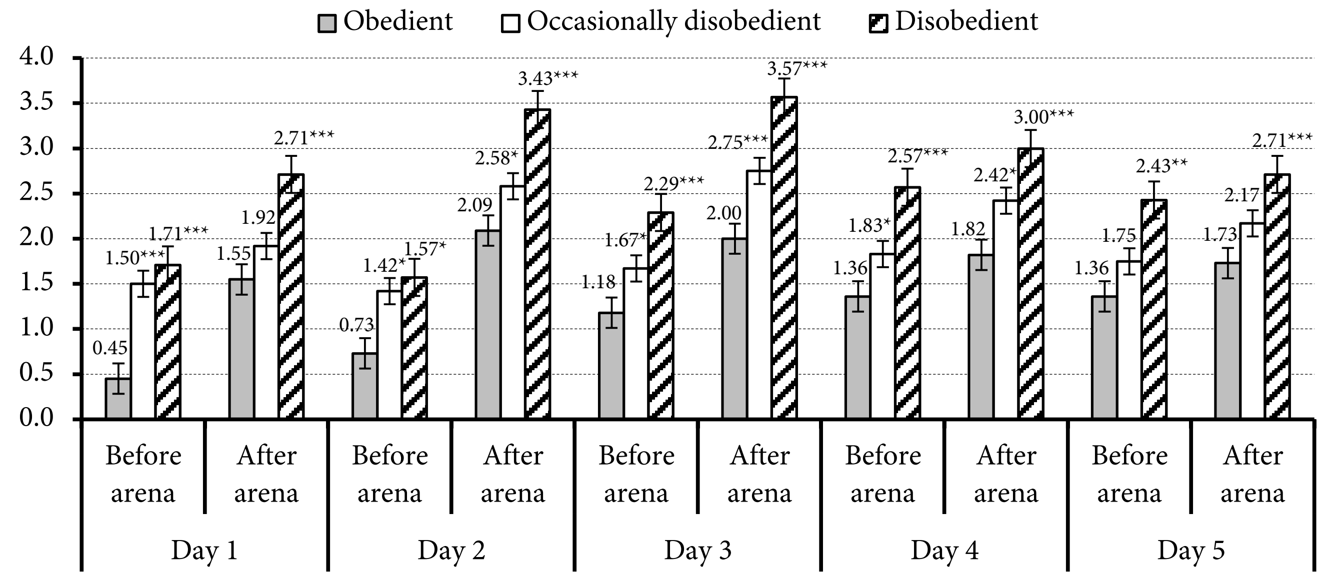

Figure 5. Dynamics of the psycho-emotional state of dogs with different levels of trainability and obedience during the experiment (a. u.). Significant differences compared to obedient dogs: * — P < 0.05; ** — P < 0.01; *** — P < 0.001.

Dogs that were occasionally disobedient had higher baseline scores (1.42–1.83 a. u.) than obedient dogs. Their stress response was more pronounced (day 2 — 2.58 a. u., day 3 — 2.75 a. u.), and anxiety scores in non-stress conditions remained elevated (2.17–2.42 a. u.), reflecting lower adaptability compared to obedient dogs. Disobedient dogs displayed the highest initial psycho-emotional levels (1.57–2.57 a. u.) and maximum reactivity to acute stress (day 2 — 3.43 a. u., day 3 — 3.57 a. u.). Even on anxiety-assessment days, their emotional state remained high (2.71–3.0 a. u.), indicating poor adaptability and heightened stress sensitivity.

The dynamics of the influence of various temperament traits (aggression, fear and anxiety, excitability, trainability, and obedience) on the emotional and mental state of dogs over the five-day experiment are shown in Fig. 6, based on their relative effect strength. The ‘aggression’ trait exhibited the strongest influence on the first day of the experiment before exposure to the arena (0.55 a. u.; P < 0.001), followed by a significant decline in the subsequent days, reaching its minimum on day 3 after exposure (0.16 a. u.). However, by the end of the experiment (day 5), its influence increased again (0.46 a. u. after exposure; P < 0.01), indicating a gradual re-emergence of aggressive tendencies under prolonged stress. The 'fear and anxiety' trait showed an initially low impact (0.09 a. u. on day 1 before exposure), but its significance gradually increased throughout the experiment, peaking on days 4 and 5 (0.42 a. u. before and after exposure; P < 0.01). Excitability had the highest impact on the psycho-emotional state at the start (0.86 a. u. before exposure on day 1; P < 0.001), followed by a gradual decline over the next days (0.27 a. u. after exposure on day 4). Despite the downward trend, excitability remained moderately significant at the end of the experiment (0.56 a. u. before exposure on day 5; P < 0.001). The 'trainability and obedience' trait maintained a consistently strong influence throughout the entire experiment, particularly pronounced on days 2 and 3 (0.75 and 0.86 a. u. after exposure; P < 0.001). This underscores the essential role of obedience and learning capacity in shaping dogs' responses to acute stress and anxiety-inducing conditions.

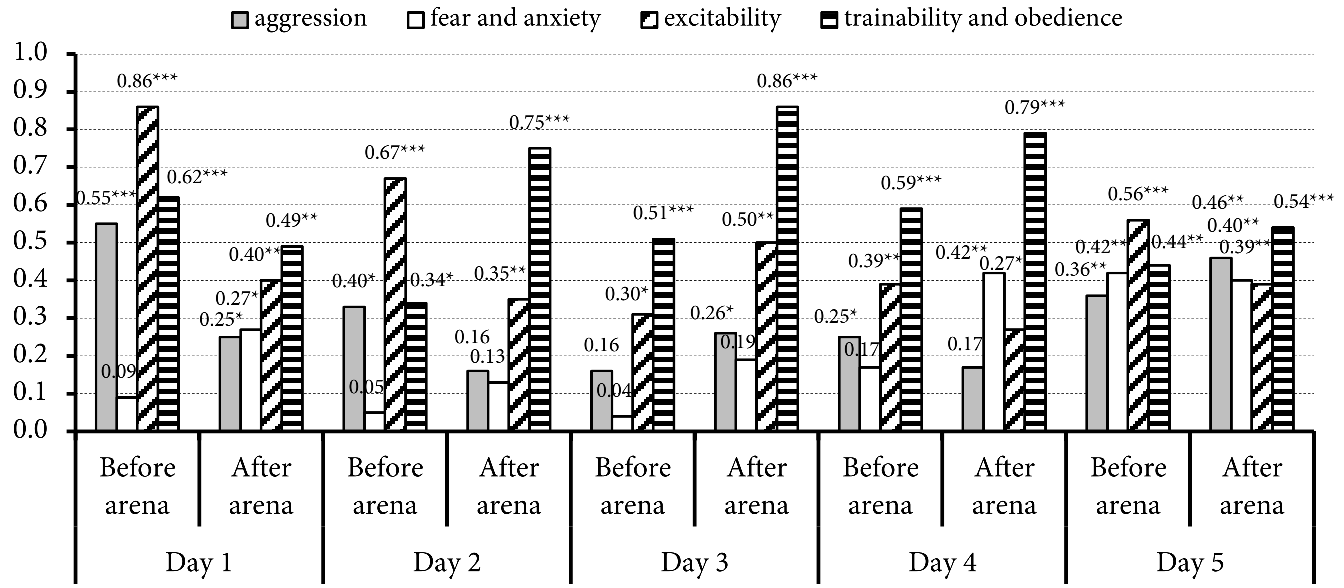

Figure 6. Dynamics of the influence of various temperament traits on the emotional and mental state of dogs during the experiment (η^2_χ; a. u.). Significant at: * — P < 0.05; ** — P < 0.01; *** — P < 0.001.

The temperament subgroup analysis revealed a clear differentiation of behavioral responses. Dogs with calm behavior, low aggressiveness, mild or absent anxiety, and high obedience had minimal baseline values and only moderate reactions to acute stress, followed by rapid state stabilization. In contrast, dogs with pronounced aggressiveness, high excitability, or persistent fear demonstrated elevated baseline scores, maximal reactivity to auditory stressors, and maintained high levels even on non-stress days. These findings align with McMahon, Youatt and Cavigelli (2022), who emphasized that an individual's physiological and behavioral profile (temperament) determines the functional significance of stress reactivity, as well as with Somppi et al. (2022) and Höglin et al. (2021), who found that dogs' personality traits and the quality of human–dog interactions are linked to long-term stress levels and emotional stability.

Table 2 presents the correlation analysis between the psycho-emotional state of dogs and key temperament characteristics. Aggression showed a strong positive correlation with excitability (r = 0.68; p < 0.001), indicating a close relationship between increased excitability and aggressive behavior. At the same time, aggression was negatively correlated with trainability and obedience (r = –0.39; p < 0.05), suggesting that aggressive dogs are less responsive to control and learning. Fear and anxiety showed a moderately negative correlation with trainability (r = –0.43; p < 0.05), implying that more anxious animals are less obedient and harder to train.

Table 2 — Correlations between the psycho-emotional state of dogs and various temperament characteristics (n = 30; r, a. u.)

Parameters		Degree of manifestation			
		Aggression	**Fear and anxiety**	**Excitability**	**Trainability and obedience**
Degree of manifestation	Fear and anxiety	0.23	–	–	–
	Excitability	0.68***	0.03	–	–
	Trainability and obedience	–0.39*	–0.43*	–0.37*	–
Psycho-emotional state index		0.55***	0.21	0.80***	–0.65***

Notes: significant differences: * — P < 0.05; ** — P < 0.001.

Excitability demonstrated a very strong positive relationship with the psycho-emotional state ($r = 0.80$; $p < 0.001$), confirming that high excitability is the primary factor amplifying emotional responses in stressful conditions. Similar associations between heightened emotional excitability, aggressive behavior, and increased neuroendocrine activation were reported by Beerda et al. (2000), Gobbo and Zupan Šemrov (2021), and Stephen and Ledger (2005). The observed positive correlations between overall psycho-emotional state and aggression ($r = 0.55$; $p < 0.001$) and a trend toward association with anxiety support the concept that aggression in some animals has a frustration–anxiety basis and may represent maladaptive coping strategies under stress.

Moreover, the psycho-emotional state exhibited a strong negative correlation with trainability and obedience ($r = -0.65$; $p < 0.001$). The negative associations of obedience with aggression and anxiety confirm that a high level of trainability and behavioral control plays a protective role in mitigating stress-related behavioral reactions. These findings are consistent with Mercier et al. (2023) and Grigg et al. (2022), who highlighted the importance of structured owner–dog interaction, predictable cues, and training in reducing emotional dysregulation. The results also correspond with the social stress-buffering hypothesis, emphasizing the role of stable social bonds in lowering stress reactivity (Teo et al., 2022; Buttner, Awalt and Strasser, 2023).

The observed increase in the role of fear and anxiety, along with the partial restoration of aggression significance at the end of the experiment, may indicate that under repeated environmental influences, some animals transition to a more stable yet less adaptive response pattern characteristic of chronic anxiety or frustration. This shift in the balance between active and passive coping strategies aligns with modern stress models, which emphasize the risk of transitioning from adaptive to maladaptive responses under prolonged or repeated exposure to stressors (Koolhaas et al., 2011; Salgirli Demirbas et al., 2023).

Thus, the obtained results indicate the dominant influence of excitability and aggressiveness on the psycho-emotional state of dogs, as well as the key role of trainability as a factor that reduces anxiety and stress response levels.

Conclusions. Overall, the study results demonstrate that: the open-field model with an auditory stimulus is an effective tool for standardized induction of acute stress in dogs; the level of psycho-emotional reactivity is largely determined by individual temperament traits, primarily excitability and aggressiveness; high trainability and obedience are associated with lower stress indicators and can be considered markers of better adaptive capacity. These findings are consistent with leading research in veterinary behavioral medicine and emphasize the importance of integrating standardized behavioral scales with temperament assessment in the development of stress prevention and correction protocols in dogs.

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FEATURES OF THE MICROSCOPIC STRUCTURE OF THE YEMENI CHAMELEON (*CHAMAELEO CALYPTRATUS*) INTESTINAL

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Summary. Due to its attractive appearance and behavioral characteristics, the Yemeni chameleon (*Chamaeleo calyptratus*) is one of the most common reptile species in home terrariums and zoos. Due to violations of the detention conditions and feeding, pathologies of the digestive canal are quite common. At the same time, detailed information on the structure of the intestine is fragmentary and contradictory, making it impossible to develop adequate methods for diagnosing and treating its pathological conditions. The microscopic features of the intestine of 1-year-old Yemeni chameleons were determined. Histological preparations were prepared from a cross-section of the middle section of the duodenum, jejunum, ileum, colon, rectum, and colonic diverticulum, which were stained with hematoxylin and eosin, as well as according to Mallory. Three membranes were found in the intestinal wall of the Yemeni chameleon: mucous, muscular, and serous. Four layers were found in the composition of the mucous membrane: epithelial, lamina propria, muscularis mucosae, and submucosa. The epithelial layer is represented by a single-layered, single-row prismatic epithelium, in which two main cell types were identified: border and goblet cells. Characteristic structures of the intestinal mucosa were longitudinal folds that expanded the intestinal cavity to process large, undigested food items (e. g., insects). In the jejunum and ileum, such folds had a thin wall formed by an epithelial layer and a thin lamina propria, several rows of myocytes, and contained a slit-like cavity — a lymphatic sinus lined with endothelial cells. Given the presence of significant lymphatic sinuses, a small thickness of the lamina propria and muscularis mucosae, the submucosal base of the mucosa, the muscular membrane, the jejunum, and the ileum are probably the sites of absorption of feed lipids and water. In our opinion, specific structures similar to villi in the intestines of mammals and birds are intercryptal protrusions (ICP), which are bundles of epithelial cells between the mouths of neighboring crypts. No crypts were found in the jejunum and ileum of chameleons. A feature of the Muscular membrane of the intestine was a gradual increase in the relative thickness of the outer longitudinal layer in the direction from the duodenum to the rectum

Keywords: reptiles, digestion, morphometric indicators

Introduction. The evolution of reptiles has led to significant differences in the morphological and functional organization of organ systems, with the greatest diversity observed in the digestive system. Reptiles are among the most ecologically and evolutionarily significant groups of living organisms, having accumulated enormous diversity in morphology, behavior, ecology, life cycles, and defensive strategies (Fry et al., 2006). Such features of reptiles pose a challenge for veterinary medicine specialists during diagnosis and treatment (Mitchell and Diaz-Figueroa, 2005). Given the digestive system's extreme efficiency, the specific nature of food and feeding behavior across animal classes leaves its mark on the features of its morphofunctional organization (Karasov and Douglas, 2013). The difference between reptiles and mammals lies in the absence of true teeth, which are adapted only for capturing prey and exclude mechanical grinding (mastication) of food, the absorption of large particles, slow digestion, and prolonged processing in the digestive tract (Fritz et al., 2010). They are characterized by long periods of starvation between large meals, which require significant morphophysiological plasticity in the digestive system (Zhong, Zheng and Wang, 2023; Yang, Wang and Wu, 2025). It should be noted that the presence of lizards in captivity alters the composition of the intestinal microbiota, which in turn influences the immune status not only of this organ but also of the entire organism (Kohl et al., 2017; Wang, Wu and Yang, 2024).

Reptiles are of significant veterinary and medical importance as paratenic and intermediate hosts for many parasites (Manoj, 2024). Reptilian digestive tracts are associated with diseases of both infectious and non-infectious etiology. They are recognized as reservoirs of bacteria, and the expansion of the global trade in pet reptiles has led to reptile-associated salmonellosis becoming a significant public health problem (Muslin et al., 2025). Researchers have reported numerous cases of cryptosporidiosis causing inflammation of both the large and small intestines in green iguanas (*Iguana iguana*) (Kik et al., 2011; Gałęcki and Sokół, 2018), leopard geckos (*Eublepharis macularius*) (Terrell, Uhl and Funk, 2003), and agama lizards (Hallinger et al., 2019). Various coccidian species are common in Madagascar chameleons and inhabit different parts of the intestine (Modrý et al., 2001). In such animals, acute inflammation of the mucous membrane of the stomach, duodenum, colon, and gallbladder has been noted. It is known that reptiles are carriers of various parasites of the digestive tract. Various types of intestinal helminths are quite common — the parapharyngodont nematodes (Oxyurida) in the Egyptian variable lizard (*Agama mutabilis*) (Morsy et al., 2019), the oxyuridae nematodes (Pharyngodonidae) in agama lizards (Hallinger et al., 2019), the nematodes *Pharyngodon mamillatus*, *Thelandros alatus*, and *Parapharyngodon micipsae*, as well as the intestinal cestode *Oochoristica tuberculata* in the spotted skink (*Chalcides ocellatus*) (Ibrahim, Fadiel and Nair, 2005). An important environmental problem is the mass poisoning of reptiles with various chemical means of protecting agricultural plants, which primarily affect the digestive organs (Cakici and Akat, 2012; Simbula et al., 2021).

One of the most common domestic chameleon species, which attracts humans with a change in skin color and behavioral features, is the Yemeni (veil) chameleon (*Chamaeleo calyptratus*). Due to their significant prevalence, violation of the conditions of detention and feeding, they are often patients of veterinary clinics (Melero et al., 2023). In addition, chameleons, as representatives of scaly lizards, are used as a valuable biological model of organisms in order to study the mechanisms of adaptation to specific conditions of existence and nutrition (Diaz et al., 2015). At the same time, out of 202 species of Chamaeleonidae, 38.6% are under threat of extinction. Zoological institutions play an important role in the conservation of these animals. Currently, almost a thousand individuals of 36 different chameleon species are kept in zoological institutions around the world (Aduriz et al., 2024). Although numerous species of reptiles are widely studied by researchers, information describing the detailed structure of individual organs in many reptiles, including chameleons, is insufficient and, sometimes, contradictory (Cizek et al., 2019).

Thus, the analysis of scientific sources indicates the prevalence of digestive system diseases, including intestinal diseases, among different species of lizards. Such a problem requires detailed research and an understanding of intestinal micromorphology. This is necessary for interpreting clinical signs of diseases and determining treatment methods.

The work **aimed** to establish the features of the microscopic structure of the intestine of a 1-year-old Yemeni chameleon (*Chamaeleo calyptratus*).

The objectives of the study were to describe the histological structure and determine the main morphometric indicators of the microscopic structures of the duodenum, jejunum, ileum, colon, rectum, and colonic diverticulum of a 1-year-old Yemeni chameleon.

Materials and methods. The material for histological studies was intestinal samples of a 1-year-old Yemeni chameleon (n = 5): duodenum, jejunum, ileum, colon, colonic diverticula, and rectum. Animals were obtained from veterinary clinics where they died for reasons not caused by digestive diseases. After evisceration, the intestines were fixed in 10% neutral formalin solution. According to the classical method, samples of different intestines were washed in running water, after dehydration and compaction in alcohol solutions of increasing concentration, they were clarified in alcohol and chloroform solutions, and embedded in paraffin. Thin histological sections were prepared from paraffin blocks on an MC-2 microtome, which were stained with hematoxylin and eosin, as well as according to Mallory. The study of histological preparations was carried out on a 'Jenamed 2' microscope (Carl Zeiss, Germany). Using an ocular grid on preparations made from cross-sections of the intestines, the size of microscopic structures was determined: the outer diameter, the thickness of the membranes and their layers, the height of the folds and intercryptal protrusions, the depth of the crypts, and the area of the fold cavities. The serous membranes of different intestines had approximately the same thickness (approximately 5 µm), so their thickness was not measured.

The established indicators of intestinal microstructure size were analyzed using a one-way analysis of variance (ANOVA) in Biostat LE 7.3. With the determination of the arithmetic mean (M) and its standard deviation (SD). The reliability of differences in intestinal parameters among chameleon intestines was assessed using Tukey's criterion; differences were considered reliable at $P < 0.05$.

In analyzing scientific literature, data on the morphofunctional features of the intestines of other representatives of the suborder Iguania in the order Squamata in the class Reptilia were used.

Results and discussion. Taking into account the known data on the division of the digestive tract of reptiles into separate organs and their parts Engelke et al. (2020) for the bearded agama (*Pogona vitticeps*), Hamdi et al. (2014) for the African chameleon (*Chamaeleon africanus*), macroscopically in the composition of the intestine of the Yemeni chameleon (*Chamaeleo calyptratus*) in the small section we distinguished three intestines: duodenum, jejunum and ileum, and in the composition of the large intestine — colon, colonic diverticulum and rectum. Macroscopically, the small intestine had the appearance of a thin tube of approximately the same diameter and had a black color on the outside due to melanin, which is consistent with the corresponding data of Hamdi et al. (2014) for the intestine of the African chameleon. The duodenum, without a clear border, began at the pylorus of the stomach and was accompanied by the pancreas, which had the form of a thin pink cord. The next longest section of the small intestine, which did not contain the pancreas in the mesentery, was defined as empty. The ileum was defined at the place of transition of the small intestine into the large intestine, which had a significantly larger diameter (Table 1).

Table 1 — Intestinal diameter of the Yemeni chameleon (n = 5, x ± SD)

Intestine	Diameter, µm
Duodenum	5.4 ± 0.4^{a}
Jejunum	12.0 ± 1.1^{bc}
Ileum	13.2 ± 0.9^{c}
Colon	15.2 ± 1.4^{a}
Colonic diverticulum	9.0 ± 0.9^{b}
Rectum	11.0 ± 1.1^{bc}

Note. Different letters indicate the values significantly differing from one another within a column on the results of comparison using the Tukey test ($P < 0.05$).

The colon was the largest in diameter and contained a small, rounded protrusion — diverticula. The rectum had a smaller diameter and connected the colon and cloaca in the form of a straight tube. As established by Engelke et al. (2020), Skripka et al. (2020), and Kushch et al. (2024), a characteristic macroscopic feature of the internal relief of the mucous membrane of the digestive canal of reptiles is the presence of a significant number of longitudinal and, to a lesser extent, transverse folds. For some parts of the intestinal tube, the parallel arrangement of such folds is characteristic; for others, it is zigzag. The presence of such folds is due to the absence of teeth; reptiles swallow their prey (insects), often large in size, whole. Namely, thanks to the longitudinal folds, the cavity of the digestive canal can significantly increase its volume and accommodate such contents (Fritz et. al., 2010). Microscopically, the longitudinal folds in the duodenal wall of the Yemeni chameleon were protrusions that were formed by the mucosa with all its layers (Fig. 1). This is consistent with the classical view of the structure of the mucosal fold (Standring, 2020).

From the point of view of the layered structure, according to the results of our studies, in the wall of the duodenum of the Yemeni chameleon, we have identified three membranes: mucous, muscular, and serous. Four layers are distinguished in the composition of the mucous membrane: epithelial, lamina propria, muscularis mucosae, and submucosa. The results of determining their morphometric indicators are given in Table 2. The epithelial layer was formed by a single-layer prismatic epithelium. Two populations of prismatic enterocytes were found in its composition: border and goblet cells. Border cells had a basally located oval-shaped nucleus, oxyphilic cytoplasm, and a characteristic brush-like border at the apical pole. The less numerous goblet cells had a characteristic high goblet shape. They contained a transparent secret, consistent with the corresponding data reported by Zaher et al. (2012) and Engelke et al. (2020). The lamina propria of the mucosa was formed by fibrous loose connective tissue, in which few fibroblast nuclei and a delicate network of collagen fibers were found. The muscularis mucosae consisted of two layers of smooth muscle tissue.

In a cross-section of the duodenal wall of chameleons, 2 to 7 folds were consistently found, formed by all layers of the mucosa: epithelial, lamina propria, muscularis mucosae, and submucosa.

As is known, the mucous membrane of the small intestine of mammals and the entire intestine of birds is characterized by the presence of such microscopic formations as villi and crypts, which significantly increase the surface area of digestion and absorption of nutrients (Kardong, 2019). Villi are protrusions of a predominantly finger-shaped shape, which include all layers of the mucous membrane: epithelial layer, lamina propria, and muscularis mucosae. Crypts, or Liberkühn glands, are finger-shaped immersions of the epithelial layer into the lamina propria of the mucous membrane (Atkinson, Leedham and Byrne, 2026). Such a single structure, consisting of villi and crypts, constitutes a morphofunctional complex that performs the intestine's primary functions. It has been established that crypts are primarily sites of cell formation, and villi are the sites of digestion and nutrient absorption from the chyme (Mescher, 2021).

Table 2 — Morphometric parameters of the thickness of the wall and membranes of the intestine of the Yemeni chameleon (n = 5, x ± SD)

Intestine	Intestinal wall, µm	Mucous membrane		Muscular membrane	
		µm	%	µm	%
Duodenum	1,019.1 ± 94.2[bc]	513.6 ± 47.2[a]	50.4	505.2 ± 39.3[d]	49.6
Jejunum	1,392.9 ± 11.3[c]	1316.0 ± 17.0[c]	94.5	76.9 ± 5.0[b]	5.5
Ileum	1,458.8 ± 10.0[c]	1350.8 ± 12.2[c]	92.6	108.0 ± 7.3[bc]	7.4
Colon	849.0 ± 9.9[bc]	360.2 ± 28.0[a]	42.4	488.8 ± 38.7[cd]	57.6
Colonic diverticulum	427.1 ± 33.2[a]	376.1 ± 30.2[ab]	88.1	51.0 ± 4.9[a]	11.9
Rectum	791.2 ± 43.3[b]	464.9 ± 40.6[b]	58.8	326.3 ± 28.0[c]	41.2

Note. Different letters indicate the values significantly differing from one another within a column on the results of comparison using the Tukey test ($P < 0.05$).

The duodenal mucosa of the Yemeni chameleon contained cylindrical-shaped invaginations of the epithelium into the lamina propria, which we identified as crypts. They were densely arranged and separated from each other by thin layers of loose fibrous connective tissue of the lamina propria and a thin layer of 1–2 rows of myocytes of the muscularis mucosae. In addition, some of these crypts were also separated from the submucosa by a thin muscularis mucosae, consisting of two layers of smooth muscle cells — an inner circular and an outer longitudinal. In general, this microscopic structure of the crypts fully corresponds to that of both mammals and birds (Kardong, 2019), as well as other species of reptiles (Skripka et al., 2020). The submucosal base was represented by a very thin layer of fibrous loose connective tissue, containing a small number of cells and fibers and a significant content of amorphous substance, which is why on histological preparations it appeared as a thin, almost transparent strip between the muscularis mucosae and the inner layer of the muscular membrane.

According to the classical view of the structure of villi (Kardong, 2019), based on the results of our studies, we did not detect such structures of the mucous membrane in the duodenum of the Yemeni chameleon. At the same time, the mucous membrane of this intestine was characterized by the presence of such microscopic structures as intercryptal protrusions (ICP), which resembled villi in shape on the cut, but are the result of a specific folded organization of the epithelial layer. Such

ICPs represented a rise of the epithelial layer between the crypts buried between them and resembled a sheaf of grain. The microscopic organization of the duodenal ICP had a pronounced volumetric radial structure. A group of enterocytes emerging from the crypts forms a structure similar to a sheaf of grain. In such a structure, the narrow base ('sheaf crest') is located directly above the crypts — the zone of cell formation, and the tops of enterocytes diverge, which significantly increases the area of their contact with the chyme. The depth of the crypts was almost three times greater than the height of the ICP (Table 3).

Table 3 — Morphometric parameters of the intestinal mucosa of the Yemeni chameleon (n = 5, x ± SD)

Intestine	Height of the ICP, µm	Depth of the crypts, µm	Area of the sinuses of the folds, µm
Duodenum	75.0 ± 5.3[a]	226.7 ± 14.9[b]	
Jejunum			77.3 ± 5.4[b]
Ileum			27.6 ± 2.2[a]
Colon	150.0 ± 16.1[b]	87.3 ± 6.0[a]	
Colonic diverticulum	79.6 ± 7.7[a]	220.6 ± 17.6[b]	
Rectum	125.0 ± 9.8[b]	254.3 ± 18.9[b]	

Note. Different letters indicate the values significantly differing from one another within a column on the results of comparison using the Tukey test (P < 0.05).

In Yemeni chameleons, the ICP in the duodenum had a predominantly tooth-like shape. In general, the alternation of crypts and ICP gave the mucosa a scalloped appearance. Such a relief was characteristic of both the surface of the mucosal folds and the areas between them. Our data on the presence and structural features of ICP in the intestine of the Yemeni chameleon are consistent with the data of Engelke et al. (2020) on the presence of intercryptal folds or crests in the intestine of the bearded dragon. However, other researchers — Hamdi et al. (2014) in the intestine of the African chameleon, Zaher et al. (2012) in the intestine of the Egyptian spiketail (*Uromastyx aegyptiaca*), Skripka et al. (2020) in the sand lizard (*Lacerta agilis*), Awaad, Rushdy and Adly (2022) in the five-banded mabui (*Trachylepis quinquetaeniata*) the protrusions of the mucous membrane were determined as villi and folds.

The mucous membrane of the duodenum of the Yemeni chameleon consisted of two layers: a thicker inner circular layer and a thin outer longitudinal layer (Table 4). They are separated from each other by thin layers of fibrous loose connective tissue. Sometimes, ganglia of the myenteric nerve plexus were found in them. In a much smaller number, neurons of the nerve nodes were also found in the submucosal base. The data we obtained on the topography of the nerve plexuses in the intestine of the Yemeni chameleon are consistent with the corresponding information of Martinez-Ciriano et al. (2000) regarding their structure in the intestine of the Spanish wall lizard (*Podarcis hispanica*). Pigment cells with numerous small brown-black granules were found in the epithelial cells of the very thin serous membrane of both the duodenum and other parts of the intestine. The presence of melanin inclusions in melanomacrophages of the liver of the Yemeni chameleon was reported by Al-Doaiss et al. (2023).

Table 4 — Morphometric parameters of the muscular coat of the intestine of the Yemeni chameleon (n = 5, x ± SD)

Intestine	Muscular membrane, µm	Inner layer		Outer layer	
		µm	%	µm	%
Duodenum	505.2 ± 39.3[d]	425.0 ± 31.0[d]	84.1	80.2 ± 7.2[b]	15.9
Jejunum	76.9 ± 5.0[ab]	52.1 ± 5.6[ab]	67.8	24.8 ± 2.5[ab]	32.2
Ileum	108.0 ± 7.3[b]	77.6 ± 8.3[b]	71.9	30.4 ± 3.4[ab]	28.1
Colon	488.8 ± 38.7[d]	311.5 ± 27.1[c]	63.7	177.3 ± 22.0[c]	36.3
Colonic diverticulum	51.0 ± 4.9[a]	26.7 ± 3.2[a]	52.4	24.3 ± 17.0[a]	47.6
Rectum	326.3 ± 28.0[c]	52.3 ± 4.6[a]	16.0	274.0 ± 29.8[d]	84.0

Note. Different letters indicate the values significantly differing from one another within a column on the results of comparison using the Tukey test (P < 0.05).

Microscopically, the mucous membrane of the jejunum had significant differences from the duodenum (Fig. 2). Its mucous membrane is represented by numerous high folds, consisting mainly of an epithelial layer, a very thin lamina propria, one or two rows of myocytes and endothelium, which delimit a centrally located cavity — sinus.

Endothelial cells directly lined the lumen of the sinus; their nuclei had a flattened shape, often looked like thin dark lines, and protruded into the lumen (Fig. 3). Smooth myocytes lay outside the endothelial cells, had more voluminous nuclei of a rod-shaped or spindle-shaped shape, and were thicker compared to those of endothelial cells.

The areas of the mucosa between the folds were constructed similarly and consisted of a layer of epithelium, a very thin lamina propria, one or two layers of myocytes, and a thin submucosa. Sometimes, two-layer folds were found on histological preparations, which is probably a consequence of the contraction of the intestinal wall and, accordingly, the 'wedging' of the fold into itself.

The presence of large central vessels in the folds of the small intestine of bearded dragons and their accompaniment by smooth muscle cells is indicated by Engelke et al. (2020). In our opinion, the cavities in the folds of the mucosa of the jejunum are lymphatic sinuses.

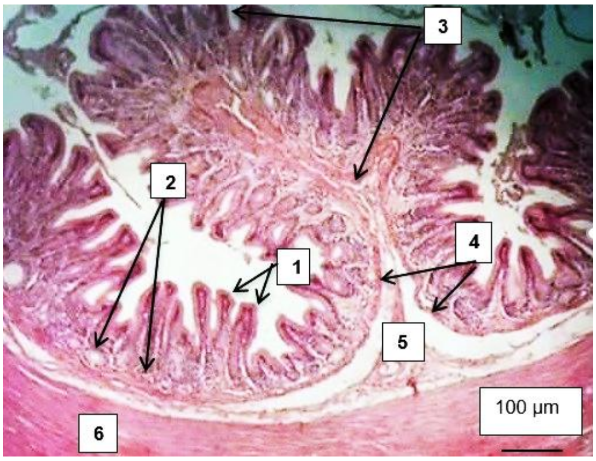

Figure 1. Duodenal wall of the Yemeni chameleon (histological preparation): 1 — intercryptal protrusions; 2 — crypts; 3 — mucosal fold; 4 — muscularis mucosae; 5 — submucosal base of the mucosa; 6 — inner layer of the muscularis mucosae; hematoxylin and eosin staining.

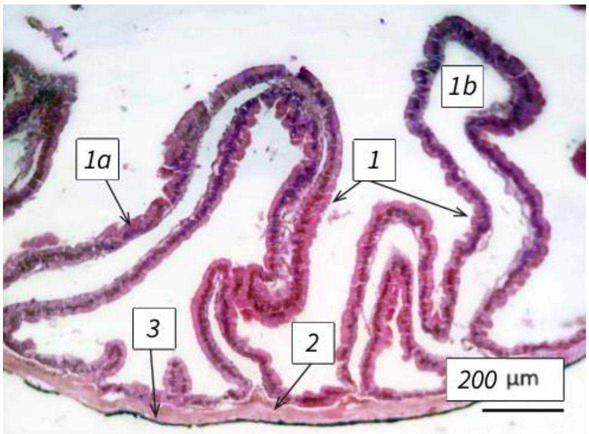

Figure 2. The wall of the jejunum of a Yemeni chameleon (histological preparation): 1 — mucosal fold: a — epithelial layer, b — lymphatic sinus; 2 — muscular membrane; 3 — serous membrane; hematoxylin and eosin staining.

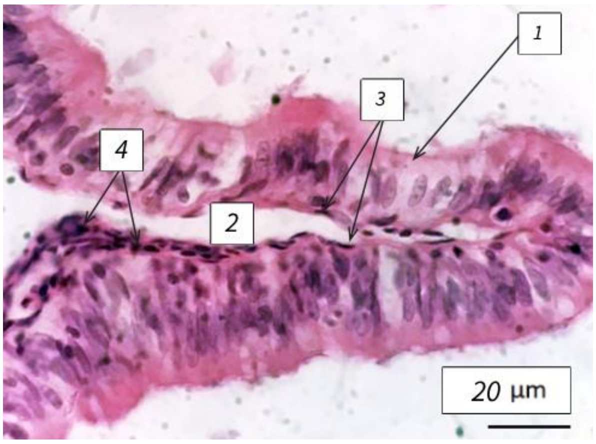

Figure 3. Longitudinal section of the ileal fold of the Yemeni chameleon (histological preparation): 1 — epithelial cells of the mucosa; 2 — lymphatic sinus cavity; 3 — nuclei of endothelial cells of the lymphatic sinus; 4 — nuclei of myocytes of the fold; hematoxylin and eosin staining.

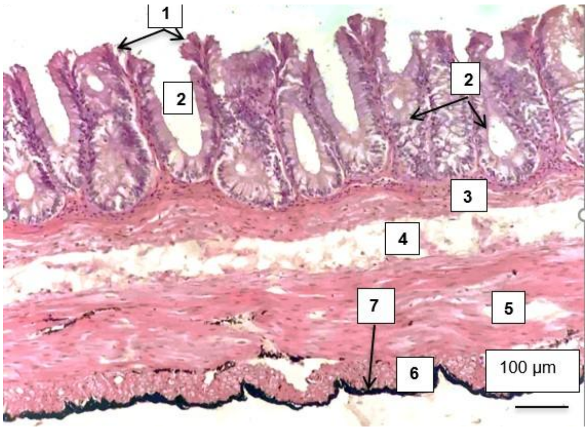

Figure 4. Colon wall of the Yemeni chameleon (histological preparation): 1 — intercryptal protrusions; 2 — crypts; 3 — muscularis mucosae; 4 — submucosa; 5 — inner muscular membrane; 6 — outer muscular membrane; 7 — serosa; hematoxylin and eosin staining.

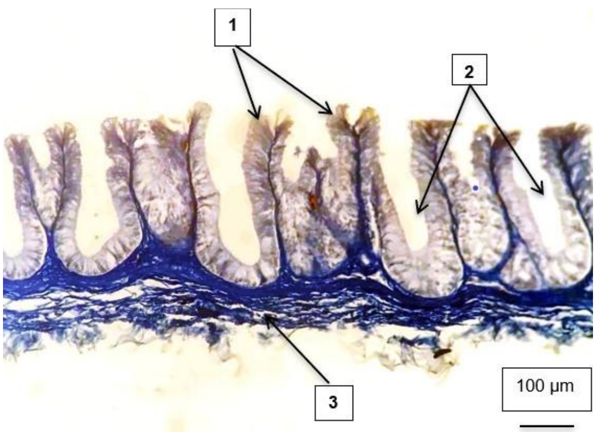

Figure 5. Wall of a colonic diverticulum of a Yemeni chameleon (histological preparation): 1 — intercryptal protrusions; 2 — crypts; 3 — submucosa; Mallory staining.

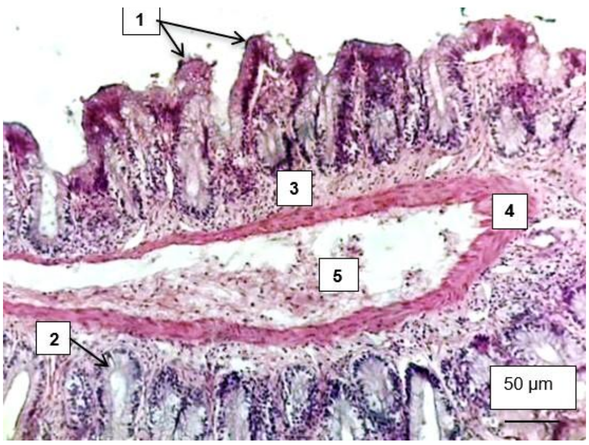

Figure 6. Rectal wall of a Yemeni chameleon (histological preparation): 1 — intercryptal protrusions; 2 — crypts; 3 — lamina propria; 4 — muscularis mucosae; 5 — submucosa; hematoxylin and eosin staining.

Evidence that such sinuses are lymphatic, and not blood-bearing, is the absence of blood cells in their lumen. During the study of longitudinal sections of this intestine, sinuses in these longitudinal folds were found to extend along their long axes, indicating a slit-like shape. Compared with the duodenum, the muscular coat of the jejunum wall was significantly thinner. The epithelium of the folds was indistinctly expressed ICP and had no crypts.

The structure of the ileum mucosa was similar to that of the jejunum and contained numerous longitudinal folds. At the same time, unlike the duodenum, its relief was distinguished by a smaller number and size of folds and, accordingly, a 64.3% smaller area of the lymphatic sinus cavity (Table 3). At the same time, the epithelial layer of the folds and interfold areas exhibited a more pronounced ICP and lacked crypts.

As is known, in the intestines of animals, the products of protein and carbohydrate breakdown are absorbed into the blood capillaries, while lipids are transported in the form of chylomicrons through the lymphatic channel (Tso and Balint, 1986; Karasov and Douglas, 2013). Given the microscopic structure of the chameleon jejunum, namely the presence of numerous folds of the mucous membrane with a significant volume of lymphatic sinuses, accompanied by myocytes that create a powerful pumping system (Zawieja, 2009; Razavi, Munn and Padera, 2025), it can be assumed that this area is the site of intensive lipid absorption. In addition to absorbing lipid breakdown products, the intestinal lymphatic system plays a significant role in water absorption, thereby regulating the interstitial volume of intestinal wall tissues (Kvietys and Granger, 2010).

Probably, such architectonics of the mucous membrane is a functional adaptation to the sporadic feeding of chameleons on insects (Yang, Wang and Wu, 2025), and the presence of myocytes in the wall of the sinuses indicates the active nature of lymphatic drainage (Zawieja, 2009). Given the smaller total volume of lymphatic cavities in the folds of the ileum compared to the jejunum, the absorption processes in this intestine occur less intensively.

According to the results of the longitudinal dissection of the transition of the small intestine into the large intestine, a transverse circular fold was found, at the base of which was the ileocolonic sphincter, which separated the ileum and colon. The presence of such a fold between the ileum and colon in the bearded agamas is indicated by Engelke et al. (2020), and in the sand lizard — Skripka et al. (2020). Microscopically, the wall of the colon was significantly different from that of the ileum and was somewhat similar to the duodenum (Fig. 4). Its wall had several folds, which included all layers of the mucous membrane, as well as the submucosa. Compared to the jejunum and ileum, the thickness of the muscularis mucosa was significantly greater, and it was separated from the muscular membrane by a rather thick submucosa base. The characteristic structures of the mucosa were crypts and the ICP located between them, the height of which, compared with other intestines, was the greatest. Unlike the duodenum, the tops of the ICP had not a tooth-shaped, but a rounded shape. It should be noted that compared with other intestines, in the colon, the thickness of the muscularis mucosa, as well as the muscularis mucosae, had the greatest values.

The wall of the colonic diverticulum was thinner than that of the colon itself and had no folds. Its mucosa contained densely arranged low crypts with rounded lumens and interspersed low ICPs of predominantly rounded shape (Fig. 5). Compared with other intestines, the thickness of the muscularis mucosa of the colonic diverticulum was the smallest.

Compared to the colon, the rectal wall had a smaller diameter and a thicker mucosa. The mucosa formed several folds, which in their middle part contained fibrous loose connective tissue of the submucosa base (Fig. 6).

The mucosa was composed of rod-shaped crypts with wide lumens and bundle-shaped ICPs located between them. Thin layers of fibrous loose connective tissue with strands of smooth muscle cells separated the crypts from each other. These cells formed the basis of the ICPs. Compared with the thin section, goblet cells were found in significantly greater numbers in the epithelial layer, consistent with the known pattern in the intestines of birds and mammals (Kardong, 2019). A relatively thick muscularis mucosa separated the bottom of the crypts from the submucosa, formed by loose connective tissue. Blood and lymphatic vessels of various diameters were found in its composition. Unlike other intestines, in the rectum, both the lamina propria and the submucosa, composed of fibrous loose connective tissue, contained a greater number of its cells, as well as lymphocytes.

A characteristic feature of the muscular membrane of the rectum was the greater relative thickness of the outer layer compared to the inner layer, which is consistent with the corresponding information of Engelke et al. (2020) regarding the bearded dragon. Moreover, unlike other intestines, in the rectum, muscle cells in the outer longitudinal layer formed bundles delimited by layers of fibrous loose connective tissue.

Conclusions. Based on microscopic and morphometric studies, the morphofunctional characteristics of the intestine of a 1-year-old Yemeni chameleon were provided. Its mucosa consisted of 4 layers: epithelial layer, lamina propria, muscularis mucosa, and submucosa, which had different degrees of development in different intestines. Two types of cells were found in the epithelial layer: border and goblet cells. The muscularis mucosae mainly consisted of two thin layers of smooth muscle cells: internal circular and external longitudinal. A feature of the mucosa of the duodenum, colon, and rectum was the presence of several longitudinal folds, in the formation of which all layers of the mucosa participated. A characteristic feature of the mucosa of the jejunum and ileum was the presence of numerous longitudinal folds with a centrally located cavity, which was determined as a lymphatic sinus.

Unlike mammals and birds, whose mucous membrane contains crypts and villi, the Yemeni chameleon's mucous membrane contains crypts and intercryptal protrusions. The muscular membrane consisted of two layers of smooth muscle tissue: the inner circular and the outer longitudinal. A characteristic feature of the muscular membrane was a gradual increase in the relative thickness of its outer layer and, accordingly, a decrease in the inner. Taking into account the revealed significant differences in the microscopic structure of individual intestines, they can be a morphological basis for understanding the peculiarities of pathological processes in different parts of the intestine. The revealed features of the histological structure of the intestinal wall of Yemeni chameleons reflect the trophic specialization of this species of insectivorous reptiles.

We consider the determination of the features of the development of the intestines of the Yemeni chameleon in the postnatal period of ontogenesis to be a **prospect for further research**.

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FUNCTIONAL STATE OF THE INTESTINE IN DOGS WITH MALABSORPTION SYNDROME FED A BUTYRATE-ENRICHED THERAPEUTIC DIET

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Summary. Malabsorption syndrome is a common clinical condition in dogs characterized by chronic disturbances in digestion and absorption, intestinal dysfunction, and impaired intestinal mucosal barrier function. This condition leads to a gradual decline in the animal's nutritional status. Under these circumstances, dietary management strategies that maintain intestinal function and stabilize its morphofunctional characteristics are crucial. The study aimed to assess the functional state of the intestines of dogs with malabsorption syndrome fed a therapeutic diet containing butyrate, using clinical, laboratory, and instrumental indicators. The study was conducted on dogs with clinically confirmed malabsorption syndrome of various etiologies. The animals' condition was assessed based on an analysis of clinical manifestations, hematological and biochemical blood test results, intestinal ultrasound, and coprological examination. The obtained parameters were analyzed over time during dietary feeding with a butyrate-containing therapeutic food. In the context of feeding butyrate-containing therapeutic feed, dogs showed favorable clinical outcomes, characterized by reduced frequency and severity of diarrhea, flatulence, and abdominal discomfort, improved feed tolerance, and stabilization of appetite. Laboratory indicators were characterized by a trend toward normalization of the hematological profile, improved protein metabolism, increased cobalamin levels, and decreased markers of low-grade inflammation. Ultrasound examination revealed a reduction in reactive changes in the intestinal wall and stabilization of intestinal motility. Coprological studies indicated improved feed digestibility, reduced mucus and undigested components, and decreased dysbiosis. The data obtained indicate that the functional state of the intestine in dogs with malabsorption syndrome against the background of the use of butyrate containing therapeutic diets is characterized by positive clinical, laboratory, and instrumental changes, confirming the advisability of using such diets in dietary management to support intestinal barrier function and normalize intestinal homeostasis.

Keywords: dietary therapy, butyrate salts, short-chain fatty acids, barrier function

Introduction. Chronic gastrointestinal disorders in animals are one of the main causes of prolonged digestive dysfunction and impaired nutrient absorption. These disorders accompany inflammatory enteropathies, exocrine pancreatic insufficiency, and chronic liver diseases. They also accompany post-infectious and parasitic intestinal lesions, often leading to malabsorption syndrome. Chronic enteropathies in dogs are multifactorial, developing due to the interaction between the immune system of the intestinal mucosa, the gut microbiota, environmental factors, and genetic predisposition. Evidence of this can be seen in breed-specific forms of immune-mediated gastrointestinal disorders. Early infectious lesions and dysbiotic shifts play an important role in the chronicity of the pathological process (Jergens and Heilmann, 2022; Cagnasso et al., 2024).

Enteropathies in dogs are typically characterized by chronic or recurrent diarrhea, loose stools, steatorrhea, gradual weight loss, and the development of deficiencies in protein, fat, and fat-soluble vitamins, as well as an impaired overall nutritional status. Against the backdrop of prolonged inflammatory and metabolic processes, the small intestine mucosa undergoes villous atrophy, crypt hyperplasia, decreased density of intercellular contacts, impaired barrier function, and dysbiosis, playing a leading pathogenic role in these conditions. These changes reduce the absorptive surface area and decrease digestive enzyme activity, resulting in varying degrees of maldigestion and malabsorption. This leads to chronic clinical manifestations and the need for long-term treatment.

Traditional approaches to dietary management of dogs with chronic gastrointestinal disorders involve the use of highly digestible therapeutic diets with controlled levels of protein, fat, fiber, and prebiotic components. Such diets help stabilize intestinal motility, form stools, and maintain the animals' nutritional status (Rudinsky, Rowe and Parker, 2018; Koyama et al., 2024). At the same time, in many cases, their effect is primarily symptomatic and does not ensure full restoration of the structural organization of the mucous membrane, tight intercellular junctions, and the intestinal barrier function, which is critically important for dogs with chronic enteropathies or malabsorption syndrome (AlShawaqfeh et al., 2017; Fritsch et al., 2022).

In this context, there is growing interest in functional food components capable of directly influencing epithelial regeneration, maintaining the intestinal barrier, and normalizing the microbiota. Among such bioactive compounds, short-chain fatty acids (SCFAs) attract particular attention, especially butyrate, a key metabolite of the colon microbiota. Butyrate is the primary energy substrate for enterocytes, stimulates their proliferation and differentiation, strengthens intercellular junctions, modulates the immune response, and exerts a pronounced anti-inflammatory effect (Pilla and Suchodolski, 2021).

Experimental and clinical studies indicate that butyrate increases mucus synthesis, stimulates the expression of tight junction proteins, maintains the integrity of the epithelial barrier, and exerts an immunomodulatory effect. Normal bacterial colonization and enteral nutrition are critical factors in maintaining the intestinal barrier function (Martinez-Guryn, Leone and Chang, 2019; Gaschen, 2006), whereas a deficiency of luminal nutrients or SCFAs impairs it (Rudinsky, Rowe and Parker, 2018).

In particular, butyrate mediates the microbiota's regulatory influence on the mucosal immune system by activating AMPK — an enzyme that coordinates energy metabolism and the regeneration of epithelial cells.

Disruption of the gut microbial balance, associated with reduced butyrate production, leads to increased intestinal permeability, activation of local inflammation, and a decrease in the mucosal reparative potential. Adequate production of SCFAs, primarily butyrate, enhances the function of tight junction proteins, limits the translocation of toxins and pathogens, and reduces the mucosal inflammatory response, underscoring their key role in maintaining epithelial integrity (Ma et al., 2021; Tamura, 2025).

In veterinary nutrition, butyrate is considered a promising functional ingredient for supporting gastrointestinal development and modulating the gut microbiota. Due to technological limitations in the use of free butyrate, its stabilized forms — sodium and calcium salts, as well as butyrate glycerides — are used in practice. It has been established that these derivatives optimize intestinal wall morphology, inhibit the growth of enteropathogens, reduce local inflammation, and promote the formation of a stable microbiota (Bedford and Gong, 2018; Elnesr et al., 2020). In addition, butyrate exhibits epigenetic activity through histone acetylation mechanisms, influencing tissue regeneration, immune response, and antioxidant protection (Lin et al., 2020; Correia et al., 2024; Burlakova and Dimitrov, 2025; Chen et al., 2025). According to recent studies, the use of butyrate-containing diets in dogs is associated with improved nutrient digestibility, modulation of the gut microbiota, normalization of hematological and metabolic parameters, and a reduction in oxidative stress, which contributes to the restoration of intestinal homeostasis without undesirable side effects (Schnorr et al., 2024). Additionally, SCFAs, particularly butyrate, support intestinal eubiosis by lowering local pH and limiting the growth of pathogenic microorganisms. Despite the existence of individual experimental and clinical observations (Schnorr et al., 2024), the evidence base regarding the use of butyrate-containing diets in dogs with malabsorption syndrome remains limited. This necessitates further systematic research and underscores the relevance and practical significance of this study in the context of improving dietary therapy for chronic gastrointestinal disorders in dogs.

This study **aims** to evaluate the functional status of the gastrointestinal tract in dogs with malabsorption syndrome receiving a butyrate-containing therapeutic diet. The evaluation will include an analysis of clinical status, hematological and biochemical blood parameters, and structural, functional, and ultrasound characteristics of the intestine.

Materials and methods. The study included 18 dogs, aged two to seven years, with clinically confirmed malabsorption syndrome. The sample included various breeds (Corgi, Cavalier King Charles Spaniel, Shih Tzu, Pug, Spitz, Beagle, Akita Inu, Great Dane, Laika, Setter, and mixed breeds), reflecting the clinical heterogeneity of the population. The animals exhibited typical manifestations of malabsorption: persistent or recurrent diarrhea, flatulence, abdominal distension, fluctuations in body weight, unstable or decreased appetite, and signs of incomplete food absorption. In some cases, the animals exhibited weakness and decreased activity.

Two groups of nine dogs each were formed based on clinical homogeneity and the feasibility of a standardized dietary treatment regimen. The experimental group received a diet containing butyrate salts, and the control group received a standard gastroenterological diet without butyrate. The diets were maximally harmonized in terms of energy and nutritional characteristics, differing primarily in the presence of butyrate salts. This allowed for an assessment of butyrate's specific dietary effect (Bedford and Gong, 2018). The experimental diet was Virbac HPM G1 Digestive Support (containing butyrate salts), and the control diet was Brit Veterinary Diet Gastrointestinal Low-Fat Grain-Free (without SCFAs). The pathogenetic effects of dietary butyrate on mucosal support, barrier function, and anti-inflammatory action have been described in experimental models (Wu et al., 2018; Sadurní et al., 2022; Chen et al., 2025). Animals with acute infections, severe systemic pathology, or conditions requiring urgent therapy were excluded from the study.

The initial assessment was conducted based on the medical history, housing conditions, previous diet, and frequency of exacerbations (Jergens and Heilmann, 2022). The clinical examination included a physical examination, measurement of body weight, and assessment of body condition (Levchenko et al., 2017). In the hematological analysis, the number of red blood cells (RBC), white blood cells (WBC), and hemoglobin (Hb) was determined, and a leukogram was generated; the neutrophil-to-lymphocyte ratio (NLR) was interpreted according to the following criteria: ≤ 3 — absence of a systemic inflammatory response, 3–5 — low-intensity/chronic inflammation, > 5 — more active inflammation; > 7–8 — systemic inflammation or protein-losing forms of enteropathy (Becher et al., 2021). Serum biochemical analysis included total protein, albumin, urea, creatinine, AST, ALT, and alkaline phosphatase; Additionally, cobalamin (B_{12}) was measured as a marker of absorption and C-reactive protein (CRP) as an indicator of systemic inflammation and the effectiveness of treatment (Levchenko et al., 2002).

Ultrasound examinations were performed using a Mindray M7 Vet portable ultrasound system (linear and convex transducers) to assess the thickness and layered structure of the intestinal wall (Van Hatten, 2023), peristalsis, mesenteric lymph nodes, and the presence of free fluid, as well as the condition of the liver, pancreas, bile ducts, and gallbladder (Penninck and d'Anjou, 2015; Linta et al., 2021). Small bowel thickness was interpreted taking into account weight-dependent reference ranges and a 'gray zone' of 4–6 mm, while also assessing the preservation of layering and echogenicity (Delaney, O'Brien and Waller, 2003).

Coprological analysis was performed using standard microscopic and chemical methods: digestibility (undigested muscle/plant fibers), the presence of mucus and fat, as well as microbial components (stained with methylene blue or Gram stain) were assessed (Englar, 2023; Vecchiato et al., 2025). The semi-quantitative severity of changes was determined on a 0–3 scale (Cavett et al., 2021). Fecal consistency was assessed using Purina's standardized fecal scoring chart for dogs (Purina, 2021).

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

Statistical analysis was performed using variational statistical methods, applying the Student's *t*-test for independent samples and the Mann–Whitney U test. A p-value of less than 0.05 was considered statistically significant.

Results. Based on the medical history, dogs in both groups exhibited a prolonged, recurrent course of gastrointestinal disorders characterized by poor tolerance of standard diets and the absence of a sustained response to previous dietary adjustments, consistent with the chronic course of malabsorption syndrome. Clinically, most animals exhibited recurrent diarrhea, flatulence, abdominal discomfort, unstable appetite, and fluctuations in body weight. Before the start of dietary therapy, hematological parameters in both groups were within the reference ranges or approached their lower limits. The mean values of hemoglobin, red blood cells, and white blood cells showed no intergroup differences (Hb: $p > 0.28$; RBC: $p > 0.44$; WBC: $p > 0.52$), confirming the clinical and laboratory equivalence of the sample (Table 1). The neutrophil-to-lymphocyte ratio (NLR) was consistent with a profile of low-grade chronic inflammation (Table 2) and did not differ significantly between groups ($p > 0.05$).

The serum biochemical profile before the start of dietary therapy (Table 3) in dogs from both groups showed no signs of severe organ dysfunction. Total protein concentration was within the low-normal range and amounted to 58.4 ± 0.72 g/L in the experimental group and 57.9 ± 0.69 g/L in the control group ($p > 0.05$), while the albumin level was 29.1 ± 0.83 g/L and 28.8 ± 0.79 g/L, respectively ($p > 0.05$).

Nitrogen metabolism parameters remained within the reference range: urea concentration was 5.6 ± 0.18 mmol/L in the experimental group and 5.8 ± 0.21 mmol/L in the control group, creatinine levels were 96.3 ± 2.7 μmol/L and 98.1 ± 2.9 μmol/L, respectively ($p > 0.05$ for all parameters), indicating preserved renal function.

In some dogs, cobalamin concentrations decreased to the lower limit of the reference range. Mean values were 248.6 ± 7.4 pg/mL in the experimental group and 252.1 ± 8.1 pg/mL in the control group ($p > 0.05$). This corresponded to an impaired absorption function of the small intestine in chronic enteropathies.

Some animals had moderately elevated C-reactive protein (CRP) concentrations, amounting to 12.4 ± 0.88 mg/L in the experimental group and 11.9 ± 0.91 mg/L in the control group. There were no statistically significant intergroup differences ($p > 0.05$), indicating the presence of a low-intensity chronic inflammatory response.

Overall, no intergroup differences were found for any biochemical parameters before dietary therapy ($p > 0.05$ by *t*-test and Mann–Whitney U), confirming baseline clinical and metabolic equivalence.

According to the results of the pre-treatment ultrasound examination (Table 4), the small intestine wall thickness was 3.27 ± 0.05 mm in the experimental group and 3.16 ± 0.04 mm in the control group ($p > 0.05$), and the mucosal thickness was 2.02 ± 0.03 mm and 1.93 ± 0.03 mm, respectively ($p > 0.05$). The dimensions of the mesenteric lymph nodes also did not differ between the groups (5.97 ± 0.12 mm vs. 5.77 ± 0.10 mm; $p > 0.05$), confirming the homogeneity of the initial morphological state of the intestine.

Before the start of dietary therapy, most dogs exhibited soft or paste-like stool consistency, the presence of mucus, undigested fibers, neutral fat, and signs of dysbiosis, which corresponded to the typical manifestations of maldigestion and malabsorption in chronic enteropathies (Table 5). Following dietary therapy, dogs in the experimental group had a 1.04-fold higher red blood cell count (6.23 ± 0.06 T/L) compared to the control group (5.97 ± 0.05 T/L; $p < 0.05$) and a 1.08-fold higher hemoglobin concentration (132.4 ± 1.14 g/L vs. 123.1 ± 0.94 g/L; $p < 0.05$). At the same time, the total white blood cell count in the experimental group was 1.07 times lower (10.96 ± 0.07 g/L) compared to the control group (11.78 ± 0.09 g/L; $p < 0.01$), indicating a reduction in the intensity of the low-grade inflammatory response (Table 6).

The neutrophil count in dogs in the experimental group was 1.10 times lower (58.00 ± 0.41%) compared to the control group (64.00 ± 0.41%), while the lymphocyte count, conversely, was 1.27 times higher (30.00 ± 0.41% vs. 23.67 ± 0.33%; $p < 0.01$). These shifts in the leukocyte formula (Table 7) led to a 1.40-fold decrease in the neutrophil-to-lymphocyte ratio (NLR) — to 1.93 ± 0.20 in the experimental group compared with 2.70 ± 0.25 in the control group ($p = 0.03–0.04$), indicating a reduction in the intensity of the systemic inflammatory and stress-associated response.

Table 1 — Hematological indicators in dogs before the start of the study

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Red blood cells (RBC), T/L	5.5–8.5	5.93 ± 0.05	5.88 ± 0.05	0.44	0.50
Hemoglobin (Hb), g/L	120–180	124.2 ± 1.14	123.8 ± 0.90	0.28	0.31
White blood cells (WBC), g/L	6.0–17.0	12.11 ± 0.10	12.01 ± 0.09	0.52	0.53

Table 2 — White blood cell count and neutrophil-to-lymphocyte ratio (N/L) in dogs with enteropathy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	P (Mann–Whitney U)
Basophils, %	0–1	0.44 ± 0.18	0.56 ± 0.18	0.661	0.684
Eosinophils, %	2–10	7.11 ± 0.68	7.67 ± 0.53	0.526	0.592
Neutrophils, %	60–77	68.11 ± 0.86	68.22 ± 0.80	0.925	1.000
Lymphocytes, %	12–30	15.33 ± 1.26	14.33 ± 1.19	0.572	0.594
Monocytes, %	3–10	9.00 ± 0.41	9.22 ± 0.36	0.690	0.702
N/L	1.5–3.5	4.75 ± 0.47	5.06 ± 0.46	0.638	0.595

Table 3 — Biochemical blood indicators in dogs with enteropathy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Total protein, g/L	55–75	57.1 ± 0.75	56.8 ± 0.73	0.62	0.84
Albumin, g/L	28–40	28.6 ± 0.82	28.8 ± 0.89	0.78	0.91
Urea, mmol/L	3.5–7.5	4.44 ± 0.10	4.54 ± 0.09	0.33	0.48
Creatinine, μmol/L	40–120	74.9 ± 2.25	75.6 ± 2.07	0.71	0.79
AST, U/L	15–45	28.4 ± 0.63	28.3 ± 0.60	0.89	0.95
ALT, U/L	10–65	31.3 ± 0.74	31.3 ± 0.67	0.98	0.98
Alkaline phosphatase, U/L	20–150	95.9 ± 2.72	95.3 ± 2.70	0.86	0.87
Vitamin B_{12}, pg/mL	250–900	256.7 ± 9.8	255.0 ± 9.2	0.81	0.89
CRP, mg/L	<10	13.6 ± 0.91	12.1 ± 0.75	0.19	0.28

Table 4 — Ultrasound indicators of the intestine in dogs before the start of dietary therapy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Small intestine wall thickness, mm	2.0–3.0	3.27 ± 0.05	3.16 ± 0.04	0.1266	0.1384
Mucosal thickness, mm	1.2–1.8	2.02 ± 0.03	1.93 ± 0.03	0.0739	0.0968
Mesenteric lymph node, mm	≤ 5.0–6.0	5.97 ± 0.12	5.77 ± 0.10	0.2166	0.2145

Table 5 — Coprological indicators in dogs with malabsorption syndrome

Indicator, points	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Stool consistency (0–3)	0–0.5	2.22 ± 0.08	2.18 ± 0.09	0.61	0.65
Mucus in stool (0–3)	0	1.89 ± 0.07	1.84 ± 0.08	0.48	0.52
Undigested dietary fiber (0–3)	0–0.5	1.67 ± 0.09	1.58 ± 0.08	0.44	0.50
Neutral fat/lipid inclusions (0–3)	0	1.33 ± 0.07	1.27 ± 0.07	0.39	0.45
Starch granules (0–3)	0	1.22 ± 0.06	1.18 ± 0.06	0.41	0.47
Microbial colonization/bacteria (0–3)	0–0.5	1.78 ± 0.08	1.73 ± 0.07	0.54	0.59
Yeast cells (0–3)	0	1.11 ± 0.05	1.07 ± 0.05	0.46	0.51

Table 6 — Hematological indicators in dogs after dietary therapy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Red blood cells (RBC), T/L	5.5–8.5	6.23 ± 0.06	5.97 ± 0.05	< 0.05	< 0.05
Hemoglobin (Hb), g/L	120–180	132.4 ± 1.14	123.1 ± 0.94	< 0.05	< 0.05
White blood cells (WBC), g/L	6.0–17.0	10.96 ± 0.07	11.78 ± 0.09	< 0.01	< 0.01

Biochemical changes following dietary therapy (Table 8) were more pronounced in the dogs of the experimental group. Total protein levels were 1.08 times higher (62.1 ± 0.63 g/L) compared to the control group (57.3 ± 0.67 g/L; $p < 0.01$), and similarly, the albumin concentration was 1.08 times higher (32.0 ± 0.71 g/L vs.

29.6 ± 1.00 g/L; p < 0.01). The concentration of cobalamin in dogs in the experimental group was 1.23 times higher than the control values (313.9 ± 6.05 pg/mL vs. 255.1 ± 6.76 pg/mL; p < 0.001), whereas the C-reactive protein level was 1.41 times lower (8.2 ± 0.46 mg/L vs. 11.6 ± 0.82 mg/L; p < 0.001), indicating a reduction in the systemic inflammatory response.

According to ultrasound examination data after 30 days of dietary therapy (Table 9), the experimental group showed a 1.05-fold reduction in small intestine wall thickness (2.92 ± 0.04 mm vs. 3.06 ± 0.05 mm; p < 0.01) and a 1.09-fold reduction in mucosal thickness (1.72 ± 0.03 mm vs. 1.87 ± 0.03 mm; p < 0.01). The size of the mesenteric lymph nodes was 1.06 times smaller in dogs fed a diet containing butyrate (5.47 ± 0.09 mm vs. 5.79 ± 0.10 mm; p < 0.05).

Coprological parameters (Table 10) confirmed a more complete restoration of digestive function in the experimental group: formed stools were recorded 1.77 times more frequently (78% vs. 44%), the average consistency score was 1.24 times lower (1.44 ± 0.06 vs. 1.78 ± 0.08; p < 0.01), and the mucus score was 1.41 times lower (1.11 ± 0.05 vs. 1.56 ± 0.07; p < 0.01). Signs of intestinal dysbiosis decreased 2.55 times more frequently in dogs in the experimental group (56% vs. 22%), which was consistent with improvements in clinical status and laboratory and instrumental parameters.

Thus, the results obtained suggest that a dietary regimen supplemented with butyrate salts can be considered an effective component in the treatment of functional disorders of the digestive tract in dogs with malabsorption syndrome.

Table 7 — White blood cell count and neutrophil-to-lymphocyte ratio (N/L) in dogs following dietary therapy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	P (Mann–Whitney U)
Basophils, %	0–1	1.00 ± 0.00	1.00 ± 0.00	—	—
Eosinophils, %	2–10	5.89 ± 0.26	5.44 ± 0.18	> 0.05	> 0.05
Neutrophils, %	60–77	58.00 ± 0.41	64.00 ± 0.41	< 0.01	< 0.01
Lymphocytes, %	12–30	30.00 ± 0.41	23.67 ± 0.33	< 0.01	< 0.01
Monocytes, %	3–10	5.11 ± 0.26	5.89 ± 0.11	> 0.05	> 0.05
N/L	1.5–3.5	1.93 ± 0.20	2.70 ± 0.25	0.03	0.04

Table 8 — Biochemical blood indicators in dogs with malabsorption syndrome on the 30th day of dietary therapy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Total protein, g/L	55–75	62.1 ± 0.63	57.3 ± 0.67	< 0.01	< 0.01
Albumin, g/L	29–43	32.0 ± 0.71	29.6 ± 1.00	< 0.01	< 0.01
Urea, mmol/L	3.5–9.0	4.4 ± 0.10	4.5 ± 0.06	> 0.05	> 0.05
Creatinine, μmol/L	20–150	73.1 ± 2.23	76.3 ± 1.58	> 0.05	> 0.05
AST, U/L	10–50	24.2 ± 0.52	28.0 ± 0.41	< 0.05	< 0.05
ALT, U/L	10–65	27.1 ± 0.54	31.4 ± 0.56	< 0.05	< 0.05
Alkaline phosphatase, U/L	12–143	89.8 ± 2.25	94.4 ± 2.44	> 0.05	> 0.05
Vitamin B_{12}, pg/mL	200–1000	313.9 ± 6.05	255.1 ± 6.76	< 0.001	< 0.001
CRP, mg/L	< 10	8.2 ± 0.46	11.6 ± 0.82	< 0.001	< 0.001

Table 9 — Ultrasound indicators of the small intestine in dogs on the 30th day of dietary therapy

Indicator	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Small intestine wall thickness, mm	2.0–3.0	2.92 ± 0.04	3.06 ± 0.05	0.0048	0.0062
Mucosal thickness, mm	1.2–1.8	1.72 ± 0.03	1.87 ± 0.03	0.0031	0.0054
Mesenteric lymph node, mm	≤ 5.0–6.0	5.47 ± 0.09	5.79 ± 0.10	0.0121	0.0157

Table 10 — Coprological indicators in dogs on the 30th day of dietary therapy

Indicator, points	Norm	Experiment (n = 9)	Control (n = 9)	p (*t*-test)	p (Mann–Whitney U)
Stool consistency (0–3)	0–0.5	1.44 ± 0.06	1.78 ± 0.08	0.0019	0.0034
Mucus in stool (0–3)	0	1.11 ± 0.05	1.56 ± 0.07	0.0048	0.0069
Undigested dietary fiber (0–3)	0–0.5	0.89 ± 0.06	1.33 ± 0.06	0.0031	0.0056
Neutral fat/lipid inclusions (0–3)	0	0.72 ± 0.05	1.18 ± 0.06	0.0057	0.0081
Starch granules (0–3)	0	0.67 ± 0.05	1.11 ± 0.06	0.0063	0.0098
Microbial colonization/bacteria (0–3)	0–0.5	1.11 ± 0.06	1.56 ± 0.07	0.0084	0.0121
Yeast cells (0–3)	0	0.56 ± 0.05	0.94 ± 0.06	0.0098	0.0146

Discussion. The clinical manifestations of intestinal disorders in dogs from both groups were chronic and recurrent in nature and were consistent with the current understanding of malabsorption syndrome as a clinical manifestation of chronic enteropathies. According to Holmberg et al. (2022) and Jergens and Heilmann (2022), such conditions are characterized by prolonged or intermittent diarrhea, flatulence, abdominal discomfort, unstable appetite, and a variable response to standard diets, which was consistent with clinical observations in the animals included in the study. In accordance with current approaches to the management of dogs with chronic enteropathies, the assessment of dietary response is considered a key step in the diagnostic algorithm and determines the subsequent course of therapeutic and corrective measures (Gaschen, 2006; Rudinsky, Rowe and Parker, 2018). In this context, the absence of statistically significant intergroup differences in clinical, hematological, biochemical, ultrasonographic, and coprological parameters before the start of the study confirmed the baseline clinical and metabolic homogeneity of the groups formed. As Rudinsky, Rowe and Parker (2018) points out, this homogeneity is necessary for correctly interpreting the results of dietary interventions in veterinary gastroenterology. Elevated neutrophil-to-lymphocyte ratios recorded before dietary therapy began reflected a low-intensity chronic inflammatory response. According to Becher et al. (2021), NLR is a sensitive marker of systemic inflammation and stress-associated immune activation in dogs. The significant reduction in NLR observed in dogs in the experimental group after 30 days of dietary therapy, combined with a decrease in total white blood cell count and normalization of the white blood cell differential, indicated a weakening of the systemic inflammatory response.

The observed hematological changes may be explained by the immunomodulatory properties of short-chain fatty acids. According to Pérez-Reytor et al. (2021), butyrate reduces the expression of pro-inflammatory mediators and maintains immune homeostasis in the intestinal mucosa. Similar mechanisms of immune response regulation are described in the work of Liu et al. (2023). Additionally, Bedford and Gong (2018) states that butyrate is a vital energy source for enterocytes and plays a crucial role in preserving the structural and functional integrity of the intestinal barrier, which is essential in chronic enteropathies. Positive trends in protein metabolism parameters in dogs receiving a diet supplemented with butyrate salts reflect improvements in digestion and absorption. As Rudinsky, Rowe and Parker (2018) noted, even a moderate increase in total protein and albumin concentrations in dogs with chronic enteropathies is clinically significant and associated with a better response to dietary correction. Similar effects of butyrate-containing diets in dogs are described in clinical studies by Abdelhady et al. (2022) and Schnorr et al. (2024).

The changes in cobalamin concentrations were particularly informative in this study. According to Kather et al. (2020), vitamin B_{12} is a sensitive marker of impaired small intestinal absorption and is often reduced in cases of chronic enteropathy and dysbiosis.

At the same time, according to Toresson et al. (2016, 2023), there is a close functional relationship between the state of the gut microbiome and cobalamin levels, underscoring its diagnostic and prognostic value. The observed increase in cobalamin concentration in dogs of the experimental group can be considered indirect confirmation of the restoration of absorptive function and stabilization of the microbiotic balance.

The decrease in C-reactive protein concentration in dogs of the experimental group further confirmed the attenuation of the systemic inflammatory response. According to Oliveira et al. (2024), C-reactive protein (CRP) can be used as an auxiliary biomarker of inflammatory activity and the effectiveness of dietary and therapeutic interventions in dogs with chronic enteropathies.

Ultrasound findings, including reduced thickness of the small intestinal wall and mucosa and decreased size of mesenteric lymph nodes, were consistent with regression of inflammatory and reactive changes. According to Penninck and d'Anjou (2015) and the British Medical Ultrasound Society (SoR and BMUS, 2021), intestinal wall thickening is a common, albeit nonspecific, ultrasound sign of enteritis in dogs. However, Collins-Webb, Chong and Cooley (2023) state that a decrease in these parameters over time indicates improvement in the morphofunctional state of the intestine. Coprological parameters following dietary therapy confirmed a more complete restoration of digestive function in the dogs of the experimental group. According to Cavett et al. (2021), a reduction in the amount of mucus, neutral fat, and starch granules in feces reflects the normalization of enzymatic and absorptive processes, whereas Pilla and Suchodolski (2021) associate these changes with the stabilization of the gut microbiome. Normalization of fecal consistency indicators, assessed using the Faecal Scoring Chart for Dogs (Purina, 2021), indicated an improvement in intestinal function and dietary tolerance.

Thus, the combination of clinical, hematological, biochemical, ultrasonographic, and coprological changes indicated more pronounced functional recovery of the digestive tract in dogs that received a dietary ration with added butyrate salts. Based on literature regarding the effects of butyrate on the intestinal epithelium, immune response, and microbiotic balance, incorporating butyrate into therapeutic diets is a sound approach for treating malabsorption syndrome in dogs (Bedford and Gong, 2018; Matheus et al., 2023).

Conclusions. 1. In dogs with chronic enteropathies complicated by malabsorption syndrome, the use of a diet enriched with butyrate salts resulted in a better clinical response due to improved intestinal barrier function and restoration of absorption processes: clinical improvement was observed in 77.8% of dogs in the experimental group versus 33.3% in the control group, accompanied by a reduction in the frequency of diarrhea, flatulence, and abdominal discomfort, as well as better feed tolerance.

2. On a diet containing butyrate salts, a significant improvement in biochemical indicators was observed: total protein levels were 62.1 ± 0.63 g/L compared to 57.3 ± 0.67 g/L in the control group ($p < 0.01$), albumin concentration was 32.0 ± 0.71 g/L versus 29.6 ± 1.00 g/L ($p < 0.01$) in the control group.

3. Absorption function parameters were better in the experimental group: the cobalamin level was 313.9 ± 6.05 pg/mL compared to 255.1 ± 6.76 pg/mL in the control group ($p < 0.001$), while C-reactive protein concentration was lower — 8.2 ± 0.46 mg/L ($p < 0.001$).

4. According to hematological studies, a reduction in the systemic inflammatory response was observed: the neutrophil/lymphocyte ratio in the experimental group was 2.98 ± 0.31 versus 3.89 ± 0.38 in the control group ($p < 0.05$).

5. Based on the results of ultrasound and coprological studies, a significant decrease in intestinal wall thickness ($p < 0.01$), normalization of intestinal motility, and a reduction in the severity of dysbiosis and digestive disorders were noted.

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Part 2. Biosafety

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TOXICOKINETICS OF ZINC IN RATS AFTER A SINGLE ORAL ADMINISTRATION OF ZINC CARBONATE NANOPARTICLES

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Summary. Creating modern feed additives based on biologically active nanomaterials is an important area of research for improving mineral metabolism in animals and poultry. A significant amount of research focuses on developing zinc nanoparticles with a wide range of pharmacological effects. However, most zinc nanocomposites are toxic, even in low doses, and can accumulate in the body, leading to long-term adverse effects. To address this issue, zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone were synthesized and found to be non-toxic. The study aimed to advance preclinical research on the toxicokinetics of these nanoparticles by conducting an acute toxicity experiment. To this end, 96 male Wistar rats were administered a single oral dose of a colloidal zinc carbonate nanoparticle solution at the following doses: 50 mg/kg b. w. (group I), 500 mg/kg b. w. (group II), and 5,000 mg/kg b. w. (group III), based on the absolute mass of the drug. The toxicokinetic profile of the studied nanoparticles showed typical dynamics of changes in zinc content in the organs and tissues of rats — an increase in the level of this microelement in the blood (only in experimental group III on the 1st day after administration of nanoparticles by 17.5%), liver (on the 1st day in experimental group II by 18.5% and in experimental group III by 52.1%; subsequently, changes were observed only after administration of the maximum dose of the drug — on the 3rd day by 30.7%, on the 7th and 14th days, there was a tendency to increase) and kidneys (by 25.0% in experimental group II and by 36.2% in experimental group III on the 1st day after administration of nanoparticles, on the 3rd day in experimental group II it was higher by 14.9% in experimental group III by 15.9%, on the 7th day of the experiment, the zinc content remained higher than the control values by 13.4% in experimental group II and by 17.7% in experimental group III). Regardless of the dose of administered nanoparticles, zinc did not accumulate in the heart, muscles, or skin with hair. By day 14, the zinc levels in all of the rats' examined organs and tissues were similar to those of the control group. No significant changes in zinc content were observed in experimental group I throughout the experiment. Therefore, zinc carbonate nanoparticles are safe regarding toxicokinetic parameters and do not cause long-term accumulation

Keywords: toxicity, distribution, organs, tissues

Introduction. Bioactive nanoparticles are a leading research topic in veterinary pharmacy, particularly in the field of microelementology. They are being studied as potential agents for improving mineral metabolism with high bioavailability and low toxicity (Jafary, Motamedi and Karimi, 2023; Li Y. et al., 2024b; Yang, Xiong and Long, 2025). However, there are still issues regarding the determination of optimal dosages and particle parameters for different species of animals and birds. Additionally, long-term safety studies are needed to eliminate the risk of nanoparticle accumulation in tissues (Malik, Muhammad and Waheed, 2023; Naumenko et al., 2023; Ashraf et al., 2025).

In recent years, the use of nanostructured zinc, an essential element for metabolic processes, has become widespread. Zinc's bioavailability in its macroform is low, and its toxicity is high (Li Y. et al., 2024a; Yang et al., 2025). One promising approach is the targeted delivery of zinc to specific organs and tissues. This method can increase efficiency, reduce possible side effects, and allow the use of zinc-based nanoparticles as nanocontainers for other pharmacological agents (Do Carmo Neto et al., 2024; Yue et al., 2024).

The toxicokinetics of zinc nanoparticles (ZnNPs) in rats differ from those of traditional zinc salts, demonstrating distinct biological patterns based on physicochemical parameters (Zhang et al., 2018; Bautista-Pérez et al., 2024). Depending on the route of administration, ZnNPs are rapidly absorbed and pass through epithelial barriers in the intestine or respiratory tract to enter the systemic bloodstream (Fujihara and Nishimoto, 2024). They are mainly distributed in the liver, spleen, lungs, and kidneys, where ultrastructural changes in cells are observed, including mitochondrial damage and DNA fragmentation (Chen et al., 2016; Abo-El-Sooud et al., 2023). The metabolism of ZnNPs is associated with interaction with metallothioneins and activation of antioxidant systems — they can induce oxidative stress, enhance the formation of reactive oxygen species, and disrupt the balance of enzymatic processes, affecting various types of metabolism in the body (Cho et al., 2013; Kausar et al., 2023). Elimination occurs through bile and urine; however, ZnNPs tend to remain in tissues longer than soluble salts due to their lower solubility and intracellular accumulation. This increases the risk of subchronic intoxication with prolonged use (Baek et al., 2012; Pei et al., 2022). In contrast, some ZnNPs forms, particularly zinc oxide nanoparticles (ZnONPs), release Zn^{2+} ions too quickly, causing toxic effects. ZnNPs can accumulate in their original form without dissociation when interacting with animals or birds, contributing to increased zinc content in organs and tissues as a trace element (Liu et al., 2016). In contrast, low toxicity is exhibited by zinc carbonate nanoparticles (ZnCNPs) synthesized by the authors of the article — a modern feed additive that is an effective method for correcting mineral metabolism with antioxidant action (Koshevoy et al., 2025a), which requires the determination of toxicokinetic parameters and strict control of dosages to complete a series of preclinical studies.

When assessing toxicokinetics, it is necessary to establish differences in the routes by which ZnNPs enter the body and to determine their *in vivo* distribution. ZnNPs initially enter the systemic circulation rapidly and circulate in both bound and free forms. They are then distributed and accumulated in organs and tissues, primarily in the liver, the main organ of deposition and detoxification. There, ZnNPs interact with metallothioneins, forming intracellular complexes that alter mitochondrial structure and energy metabolism (Paek et al., 2013; Ali et al., 2023). The second most important target organ for ZnNPs is the spleen, where they are retained by macrophages and other reticuloendothelial system cells and are accompanied by immune activation and, in some cases, damage to cellular organelles (Wang et al., 2016). The lungs also accumulate significant amounts of ZnNPs, particularly when inhaled or administered intratracheally, where they elicit local inflammatory responses and can persist for extended periods in alveolar macrophages (Rahman et al., 2022). In the kidneys, ZnNPs are primarily distributed to the cortical tissue, where they affect tubular transport and may cause nephrotoxicity with prolonged exposure (Hashim et al., 2025). To a lesser extent, they are found in the heart and brain, and in endocrine and sex glands, but with chronic administration, their translocation across histohematological barriers is possible (Yun et al., 2015; Deore et al., 2021; Rehman et al., 2024).

Numerous studies in recent years have characterized in detail the toxicological profile of ZnNPs in laboratory animals, including four sequential ADME parameters: A — absorption, D — distribution, M — metabolism, E — excretion, mainly focusing on the route of administration — intratracheal, intraperitoneal, intragastric, etc. (Fujihara et al., 2015; Li et al., 2017; Liang et al., 2022).

The primary objective of our study was to characterize the ADME profile of ZnNPs following oral administration. First, ZnNPs are absorbed via rapid penetration through intestinal epithelial barriers. Due to their small size and their ability to interact with cell membranes, ZnNPs exhibit more rapid and greater systemic uptake than macrostructural salts (Park et al., 2017). Second, ZnNPs are distributed in hepatocytes and Kupffer cells in the liver, in macrophages and other reticuloendothelial system cells in the spleen, in alveolar macrophages in the lungs, and in the cortical substance of the kidneys (Bayat et al., 2023).

Thirdly, in most cases, the typical mechanism of zinc metabolism in nanoform is binding to blood proteins, metallothioneins, and other substrates; ultimately, ZnNPs are eliminated primarily through bile and urine (Lee et al., 2016; Hadrup, Vogel and Jacobsen, 2025). Thus, the ADME profile of ZnNPs in rats demonstrates high absorption, accumulation in the liver and kidneys, metabolic activation of antioxidant systems, and slow excretion. These characteristics make ZnNPs an effective source of microelements, but also pose toxic risks associated with their use, necessitating long-term safety studies (Keerthana and Kumar, 2020; Czyżowska and Barbasz, 2022).

Given the large amount of research on the toxicity of ZnONPs both *in vitro* and *in vivo* in laboratory and productive animals and poultry, the authors of this article decided to synthesize zinc-based NPs with reduced toxicity parameters, both through the use of a safe synthesis method that complies with the provisions of 'green chemistry' and the use of a biocompatible stabilizer (El-Saadony et al., 2024; Fatima et al., 2024).

Thus, zinc carbonate nanoparticles (ZnCNPs), stabilized with polyvinylpyrrolidone (PVP) and previously shown not to exhibit acute toxicity in white mice at a maximum dose of 40,000 mg/kg b. w., were synthesized (Koshevoy et al., 2023). The use of PVP as a stabilizer can significantly alter the toxicokinetic profile of ZnNPs. PVP is widely used in pharmaceuticals and biomedicine as a polymer capable of forming a protective shell around nanoparticles and preventing their aggregation (Sarcinelli et al., 2021; Shahrousvand et al., 2023; Abdelhakeem et al., 2025).

In theory, using PVP as a stabilizer for NPs may affect their ADME profile, especially during the absorption phase. PVP stabilization increases the dispersibility and stability of NPs in biological environments. This allows for a more uniform passage through epithelial barriers and reduces the risk of local aggregation in the intestine. The result is more controlled and less traumatic for tissue absorption (Iqbal et al., 2021; Cao et al., 2024).

During the distribution phase, PVP-modified NPs are less likely to precipitate rapidly in the liver and spleen because the protective polymer shell reduces their interaction with plasma proteins and cell membranes. This reduces the likelihood of large intracellular aggregates forming. This may lead to a more even distribution across organs and reduce the severity of local toxic effects (Choi and Choy, 2014; Li W. et al., 2024b).

During metabolism, PVP acts as a barrier, slowing direct contact between HPs and cell organelles. This reduces the intensity of induced oxidative stress and the risk of mitochondrial and DNA damage (Ding et al., 2012; Ferdous et al., 2018).

During elimination, PVP may promote the slower removal of HPs because stabilized complexes are more stable and less soluble. This prolongs their circulation time in the blood, delays their uptake in tissues, and reduces the likelihood of acute organ damage (Kermanizadeh et al., 2018; Li W. et al., 2024a).

Thus, the toxicokinetics become prolonged, and using PVP as a stabilizer contributes to shifting the toxicokinetic profile of NPs from rapid and aggressive to milder and more prolonged (Fennell et al., 2017; Ćurlin et al., 2021).

The study **aimed** to determine the toxicokinetics of zinc in the organs and tissues of rats exposed to zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone in an acute toxicological experiment.

Materials and methods. This study employed zinc carbonate nanoparticles (ZnCNPs), stabilized with polyvinylpyrrolidone and synthesized at the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine. The colloidal ZnCNPs solution used had a concentration of 2.5 g/dm^3, a pH value of 7.5, and contained spherical nanoparticles. The experiment was conducted on 96 sexually mature male Wistar rats, which were divided into four groups: a control group (C) and three experimental groups (E I, E II, and E III), with 24 rats in each group. Experimental group I received a single dose of 50 mg/kg b. w. of colloidal ZnCNPs solution, experimental group II received a single dose of 500 mg/kg b. w., and experimental group III received a single dose of 5,000 mg/kg b. w., all administered orally. The control group received a similar volume of distilled water. Throughout the study, the clinical condition and ethological parameters of all animals were monitored.

To determine the toxicokinetics of zinc on days 1, 3, 7, and 14 after the start of the experiment, six animals from each group were anesthetized and decapitated. A pathomorphological study was performed and samples of organs and tissues were collected, including blood, liver, kidney, heart, muscle, and hair with skin.

The parenchymal organs were separated from the connective tissue and the muscle from the tendon. The samples were then treated with concentrated nitric acid. The zinc content was subsequently determined using inductively coupled plasma mass spectrometry.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the State Biotechnology University.

The results were analyzed statistically. A Student's *t*-test was used. A difference was considered significant at $P < 0.05$. (Van Emden, 2019).

Results and discussion. Evaluation of rat internal organs and tissues revealed differences in zinc content after a single dose of zinc carbonate nanoparticles (ZnCNPs). While the obtained data indicate typical changes in the toxicokinetics of zinc after the oral administration of its compounds — namely, an increase in zinc content in the blood, liver, and kidneys, which are the main organs involved in its metabolism and excretion — the effect of ZnCNPs exhibited certain peculiarities. First, we studied the zinc content in the rats' blood because it is the primary route for the absorption and distribution of trace elements in the body.

On the first day after the NPs solution was administered, the zinc content in the blood of the rats in experimental group III increased by 17.5% (1.14 ± 0.04 mg/kg, $p < 0.01$). A tendency toward an increase in this indicator was observed in experimental group II. In experimental group I, the zinc content was at the level of the control values (Fig. 1). From the third to the fourteenth day of the main period of the experiment, there were no significant changes in zinc content in the experimental animals. These results are consistent with a previous study on the hematotoxicity of ZnCNPs conducted by the authors of this article. The study's results indicate no toxic effects on the blood system (Koshevoy et al., 2025b).

Secondly, it was important to determine changes in zinc content in the liver and kidneys because they are responsible for excreting this metal in bile and urine. Additionally, the liver is the site of zinc deposition, metabolism, and related enzymatic systems. Fig. 2 shows that introducing ZnCNPs into the livers of rats caused changes in zinc content.

Administration of the minimum dose (50 mg/kg b. w.) to rats in experimental group I did not cause changes in zinc content during the experiment. In animals in experimental group II, zinc content in the liver increased by 18.5% (29.79 ± 1.12 mg/kg, $p < 0.01$) only on the first day of the study. On the third day, this indicator tended to increase. On the seventh and fourteenth days, zinc content in the liver was at the level of the control group. Significant changes were observed in experimental group III. After the administration of ZnCNPs at a dose of 5,000 mg/kg b. w. on the first day of the study, the zinc content in the rats' livers exceeded that of the control group by 52.1% (38.23 ± 1.16 mg/kg, $p < 0.001$). On the third day, the zinc content remained 30.7% higher (34.14 ± 1.03 mg/kg, $p < 0.001$). Subsequently, on days 7 and 14 of the experiment, the zinc content in the animals tended to increase within the same group. This increase demonstrates the bioavailability and ability to accumulate ZnCNPs during acute intake. Previous studies by the authors of this article also indicate the absence of ZnCNP toxicity, particularly concerning liver protein synthesis and hepatospecific enzyme activity (Koshevoy et al., 2024a).

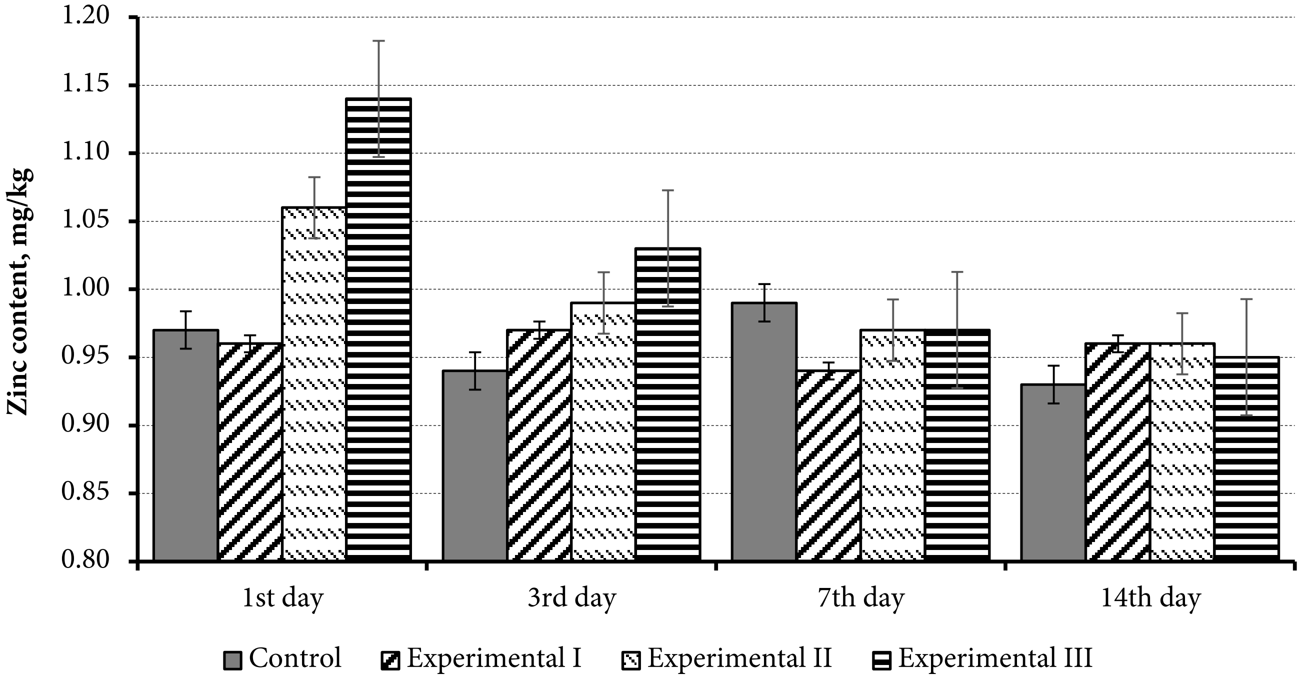

Figure 1. Zinc content in the blood of rats after a single oral administration of zinc carbonate nanoparticles.

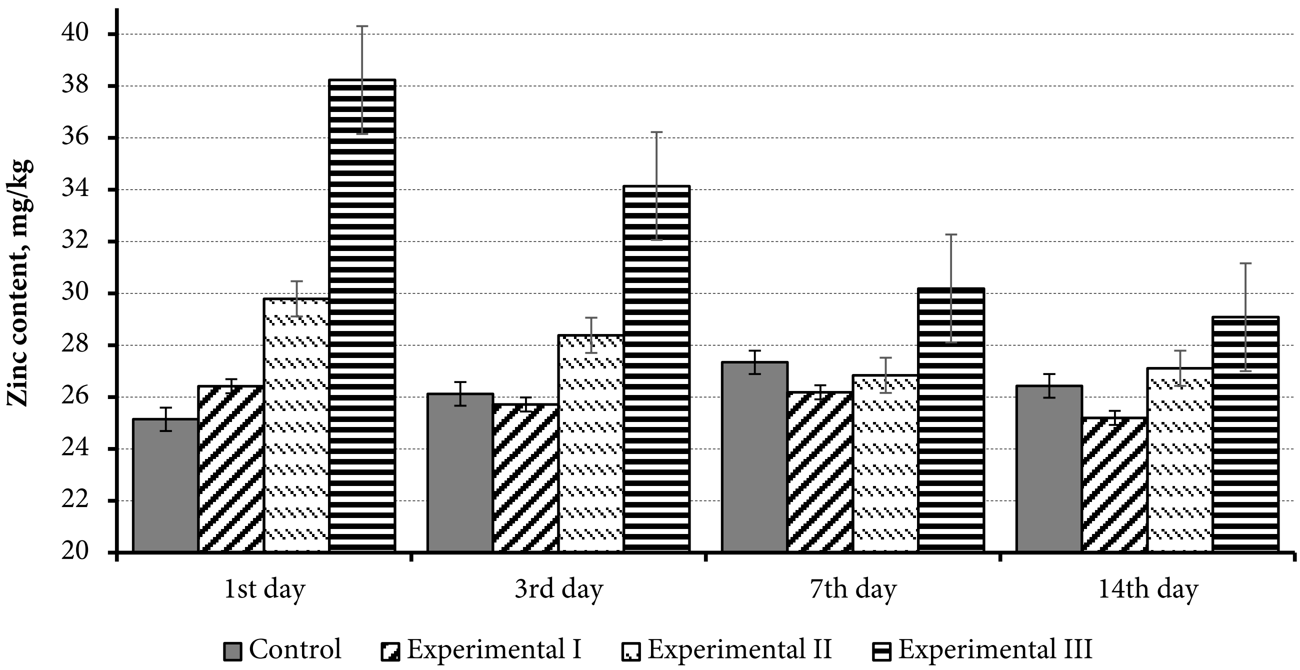

Figure 2. Zinc content in the liver of rats after a single oral administration of zinc carbonate nanoparticles.

Fig. 3 shows the effect of ZnCNPs administration on zinc content in rat kidneys. After the maximum dose of the test compound was administered orally, the zinc content increased by 36.2% (19.44 ± 0.84 mg/kg, $p < 0.001$) in rats in experimental group III. The changes in experimental group II, which received a lower dose, were less pronounced; the zinc content exceeded the control data by 25.0% (17.84 ± 0.56 mg/kg, $p < 0.01$). Meanwhile, no significant changes in zinc content were observed in rats of experimental group I on the first day of the experiment.

Subsequently, on the third day, the zinc content demonstrated similar changes. In experimental group III, the zinc content was 15.9% higher (17.23 ± 0.71 mg/kg, $p < 0.05$). In experimental group II, the zinc content was 14.9% higher (17.07 ± 0.49 mg/kg, $p < 0.05$). In experimental group I, the zinc content only tended to increase compared to the control rats. This study noted prolonged excretion of zinc from the rats' bodies after ZnCNPs administration, as the zinc content in the kidneys was higher than the control values on the seventh day of the experiment: by 17.7% in group III (16.23 ± 0.68 mg/kg, $p < 0.05$), by 13.4% in group II (15.64 ± 0.61 mg/kg, $p < 0.05$), and at the control level in group I. By the end of the experiment on day 14, there were no significant changes in microelement content among any of the experimental groups. The accumulation of zinc in kidney tissue indicated that the kidneys were excreting it over a longer period of time. Additionally, biochemical studies revealed that ZnCNPs exhibited no signs of nephrotoxicity (Koshevoy et al., 2024b). The effect of ZnCNPs on zinc content in the hearts, muscles, skin, and hair of rats was also determined (Table 1).

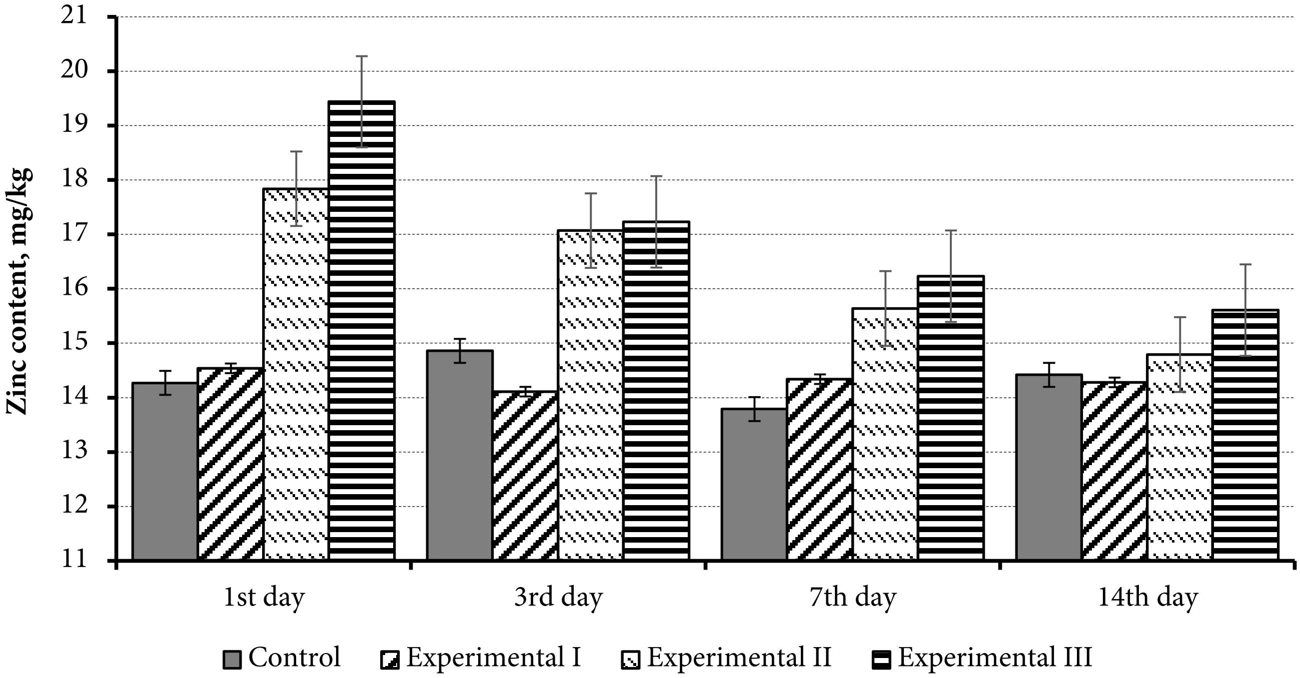

Figure 3. Zinc content in rat kidneys after a single oral administration of zinc carbonate nanoparticles.

Table 1 — Dynamics of zinc content in the heart, muscles, skin, and hair in rats following a single oral administration of zinc carbonate

Research day / group		Zinc content, mg/kg (M ± m, n = 6)		
		Heart	Muscles	Skin and hair
1	C	16.23 ± 0.51	11.78 ± 0.41	110.47 ± 3.36
	E I	16.31 ± 0.57	11.71 ± 0.39	107.62 ± 3.24
	E II	16.91 ± 0.53	11.84 ± 0.37	114.59 ± 3.51
	E III	17.04 ± 0.54	12.49 ± 0.47	120.34 ± 4.14
3	C	16.41 ± 0.49	11.44 ± 0.37	109.18 ± 3.71
	E I	16.37 ± 0.52	11.74 ± 0.43	109.88 ± 3.34
	E II	16.59 ± 0.51	12.08 ± 0.46	113.17 ± 3.41
	E III	16.87 ± 0.57	13.23 ± 0.54*	114.23 ± 4.34
7	C	16.31 ± 0.48	11.51 ± 0.44	111.36 ± 4.08
	E I	16.34 ± 0.56	11.68 ± 0.52	112.58 ± 3.47
	E II	16.47 ± 0.53	11.92 ± 0.57	111.89 ± 3.38
	E III	16.69 ± 0.62	12.06 ± 0.51	112.74 ± 3.91
14	C	16.21 ± 0.54	11.32 ± 0.39	110.12 ± 3.47
	E I	16.29 ± 0.52	11.64 ± 0.51	111.74 ± 3.42
	E II	16.36 ± 0.61	11.81 ± 0.49	109.94 ± 3.32
	E III	16.33 ± 0.78	11.89 ± 0.43	111.41 ± 3.86

Note. * — $p < 0.05$ statistically significant changes compared to the control group data.

As shown in Table 1, the administration of ZnCNPs solution did not increase zinc content in the heart. Only in experimental groups II and III on the first and third days of observation was there a tendency for this indicator to increase. However, there were no significant changes compared to the control group during the experiment in any of the experimental groups. Similar dynamics were observed in the study of wool samples with skin; throughout the 14-day experiment, zinc content did not differ significantly. Administering ZnCNPs did not lead to zinc accumulation in the muscles of rats. Only the maximum dose of 5,000 mg/kg b. w. increased the content of this trace element by 15.6% three days after administration. Thus, a single oral administration of ZnCNPs has no significant effect on zinc content in the hearts, muscles, skin, or hair of male rats.

Conclusions. The toxicokinetic profile of newly synthesized zinc carbonate nanoparticles stabilized with polyvinylpyrrolidone exhibited characteristic changes in zinc content in the organs and tissues of male rats. Administering dosages to three groups of animals at tenfold increases of 50–500–5,000 mg/kg b. w., compared to the control group, caused an increase in zinc levels in the blood, liver, and kidneys of rats in experimental groups II and III. There was no accumulation of zinc in the heart, muscles, or skin with fur in any of the experimental groups of rats. On day 14, zinc levels in all examined organs and tissues did not differ from those of the control group. No significant changes in zinc content were observed in group I during the experiment.

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ECOTOXICOLOGICAL ASPECTS OF MICROPLASTIC CONTAMINATION OF BEEKEEPING PRODUCTS

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Summary. The article summarizes recent scientific findings on the presence of microplastics in bee products and highlights the key trends reflecting the impact of human activities on the environment. It examines the factors that cause the accumulation of synthetic polymer particles in honey, pollen, bee bread, propolis, and wax, and analyzes the biological response of bees to the impact of microplastics from the perspectives of physiology, toxicology, and ecology. Particular attention is given to the methodological aspects of identifying microplastic particles in bee products, as well as the challenges of standardizing this process. The presented data aim to integrate existing research results in the context of assessing risks to human health and ecosystem stability and developing veterinary and sanitary control methods. This material is intended for specialists in veterinary medicine, food safety, apitherapy, and ecology
Keywords: honey bee, synthetic polymers, human health, veterinary and sanitary control

Introduction. The accumulation of microplastic particles in the natural environment has become one of the key environmental challenges of the 21st century. The scale and speed of the spread of polymer particles of various origins are forming new patterns of pollution that affect all components of the biosphere — the atmosphere, soil, surface and groundwater, and plant and animal organisms. In recent years, the attention of scientists (Gómez-Méndez et al., 2024; Al Naggar et al., 2024; Pasquini et al., 2023; Kadarsah et al., 2025) has increasingly focused on assessing such contamination in food products, especially in bee products, which are traditionally considered natural, organic, and biologically valuable. Bee products, especially honey, have historically been highly trusted by consumers and are widely used in nutrition, medicine, pharmacology, veterinary medicine, and cosmetology. This, along with the unique ecological properties of bees as super bioindicators, makes the study of microplastics in honey and other bee products particularly relevant.

The honey bee (*Apis mellifera* L.) is a highly sensitive bioindicator for monitoring the ecological state of the environment. In the process of collecting nectar and pollen, bees interact with a full range of environmental factors. Since worker bees have a flight radius of up to 5 km, they gather information about the state of the environment over large areas. This allows their products to serve as indicators of environmental pollution levels.

In this regard, honey, pollen, bee bread, and wax are increasingly being considered not only as food products but also as matrices that accumulate microplastics. Studies show that contamination with synthetic particles is not a random phenomenon, but a systemic characteristic of modern apiaries (Basaran et al., 2024; Rani-Borges et al., 2024; Gómez-Méndez et al., 2024). These data raise new scientific questions about the mechanisms of interaction between microplastics and bees, the physicochemical properties of particles, and the processes of their migration and accumulation in bee products.

The problem's relevance is exacerbated by the fact that microplastics can carry a wide range of toxic substances, including phthalates, bisphenol A, pesticides, heavy metals, and organic toxicants. Combined with the active metabolism and high physiological vulnerability of bees, these factors can cause behavioral disorders, reduced immune defense, impaired larval development, and general weakening of colonies. For humans, the main risk is the possible entry of synthetic particles into the digestive system during honey consumption, but the indirect toxic effect through adsorbed impurities may be more significant. Microplastics are considered a promising marker of food chain contamination in the One Health system, so a systematic study of them in bee products is important for human food safety.

Due to the absence of standardized methods for detecting microplastics in bee products, the presence of various identification techniques, and the limited systematic research conducted both regionally and globally, there is a pressing need for a comprehensive review of existing scientific data. This review should also establish directions for future research in this area.

The work **aims** to systematize current information on the characteristics of microplastic contamination of bee products, to identify the mechanisms of their entry into the 'environment-bee-product' system, analyzing data on the possible toxicological effects on bees and humans, and evaluating existing analytical control methods and prospects for further research in the field of veterinary and food safety.

Materials and methods. Theoretical research methods were used, namely analysis and synthesis of scientific sources, systematization of data, and generalization of results to form directions for further empirical research. To form the review material, 38 scientific sources from the last 15 years were analyzed, including publications in peer-reviewed journals of international databases, FAO reports, and data from experimental studies in ecology, toxicology, and beekeeping.

The reliability of the obtained data was ensured through structural, comparative, and contextual analyses. Information on the mechanisms by which microplastics enter the bodies of bees and bee products was integrated. A critical assessment of the advantages and limitations of modern analytical methods was also carried out.

Results and discussion. Bertosh (2025) considers the problem of microplastics in drinking water and food products as a potential threat to human health. The author emphasizes the ability of synthetic particles to adsorb toxic chemical compounds (phthalates, bisphenol A, pesticides, heavy metals) and their possible interaction with the gut microbiota. Particular attention is paid to nanoplastics, which, due to their ability to penetrate biological barriers, may have increased toxicological potential. Other authors also emphasize the global nature of the microplastics problem and its impact on the environment. For example, Kirsanova (2024) highlights the persistence of polymer particles and their ability to remain in the biosphere for long periods of time, posing a threat to ecological balance and human health. For their part, Kotovenko et al. (2024) analyze the current state of microplastic pollution, identify the main sources of its release into the environment, and consider methods of drinking water purification, emphasizing the need to improve control technologies.

The presence of microplastics in bee products is a topic of active research around the world. The number of scientific publications on this subject is constantly growing (Cortés-Corrales et al., 2024; Rodrigues et al., 2025). The available literature discusses not only the detection of microplastic particles, but also the mechanisms of their migration, the biological reactivity of bees to the effects of microplastics, and the potential toxicological consequences for consumers of bee products (Rani-Borges et al., 2024; Al Naggar et al., 2024).

The first systematic evidence of honey contamination by synthetic fibers came from studies conducted by G. Liebezeit and E. Liebezeit (2015). The researchers found polymer particles in honey from various European countries. The authors noted that the number of microparticles found ranged from several dozen to several hundred per kilogram of product, and most of the particles were fibers of textile origin. These results became the starting point for subsequent studies, in which the presence of microplastics in honey was not only confirmed but also studied, taking into account environmental, technological, and regional factors.

A study by Basaran et al. (2024) analyzed honey from different regions of Turkey, where microplastic particle concentrations ranged from 0 to 1,280 particles/kg. The authors describe significant regional contrasts, which they attribute to varying levels of urbanization, traffic intensity, local industrial facilities, and the nature of agricultural activities. Urbanized areas are characterized by higher concentrations of polyester fibers, which is likely due to textile waste and significant atmospheric pollution.

Similar trends have been described in studies in Latin America. Rani-Borges et al. (2024), analyzing *Melipona quadrifasciata* honey, recorded the presence of microplastics in 100% of samples. Here, too, synthetic fibers predominated, and their size and color allowed the authors to suggest various sources of contamination, ranging from synthetic fabrics to elements of car tires and fragments of packaging materials. These data are consistent with the results of studies from South Korea, where Choi et al. (2024) found 10–1,020 particles/kg in honey from different regions of the country.

A common pattern noted by authors from different countries is the stable dominance of fibers over other forms of microplastics. This indicates that the main source of contamination is atmospheric air containing particles of synthetic textiles that enter bee products during the collection of nectar and pollen. At the same time, the presence of polyethylene and polypropylene fragments indicates an additional contribution of plastic, which may originate from river basins, technological elements of beehives, or surface waters used by bees.

The study by Buteler et al. (2025) investigated the presence of synthetic and natural microfibers in honey and bees in Argentina. The authors showed that fibers constitute the vast majority of particles found, which is consistent with previous international studies. They emphasized that the main source of contamination is atmospheric air saturated with textile fibers, which enter bee products during the collection of nectar and pollen. In addition, the paper draws attention to the potential environmental consequences of such pollution for bee health and honey quality, as well as the need to develop methods for monitoring and reducing the impact of microplastics in agroecosystems.

According to Gómez-Méndez et al. (2024), plant pollen is significantly more contaminated with microplastics than honey. In their study, all samples of bee pollen contained various types of microplastics, ranging from microfibers to whole fragments. This is due to the special structure of the exine of pollen grains, which has microscopic grooves, spikes, and ridges that can trap foreign particles. In addition, pollen from wind-pollinated plants is carried over long distances by the wind and can also stick to the exoskeleton of bees and be added to pollen by bees, which increases the likelihood of further contamination of the hive environment and the products obtained.

Most studies emphasize the link between pollen contamination and the state of the atmospheric environment. Pollen from urban areas has a higher number of microplastic fibers, while samples from rural areas more often contain polyethylene fragments. This is probably due to agricultural films and the use of synthetic packaging. These data are important for interpreting bee products as bioindicators of environmental health because they allow us to conclude local sources of pollution.

Bee bread is a natural fermented product made from bee pollen, honey, and enzymes from the digestive glands of worker bees. This process results in lactic acid fermentation, alcoholic fermentation, and the synthesis of biologically active substances. While the origin of bee bread determines its high nutritional value, it also contributes to the accumulation of microplastics. Studies analyzing bee bread alongside bee pollen and honey reveal higher microplastic concentrations in bee bread than in bee pollen. Scientists suggest that this may be due to fermentation processes increasing the ability of bee bread to retain hydrophobic particles.

Bee bread is a long-term food reserve for bees, making it important for assessing the continuous influx of microplastics into bee colonies' food chains. The accumulation of microplastics in bee bread can harm the development of larvae, since it serves as their main source of protein and minerals.

Wax occupies a special position among bee products because it can accumulate not only microplastics but also a wide range of chemical pollutants, including pesticides, heavy metals, polycyclic aromatic hydrocarbons, and veterinary drug residues (Mullin et al., 2010). Its lipid nature is the reason for its high affinity with hydrophobic molecules and particles. A number of studies suggest that old honeycombs can accumulate and contain significantly more microplastics than new ones, indicating cumulative pollution processes.

Data from simultaneous analysis of wax and honey are particularly revealing. Microplastic levels in wax often exceeded those in honey by 3–10 times. This highlights the importance of wax as the 'long-term memory' of the apiary, reflecting the historical state of pollution (Cortés-Corrales et al., 2024; Buteler et al., 2025). Laboratory studies by Al Naggar et al. (2023) have shown that chronic exposure to polystyrene fragments leads to a decrease in feed consumption and the weight of worker bees. Despite no change in mortality rates, the researchers note that the decrease in body weight may be associated with metabolic imbalance or compensatory responses of the body to the presence of foreign particles in the intestine.

A number of studies have reported a decrease in the speed of bees returning to the hive, which may indicate a disturbance in their spatial orientation. The authors suggest that microplastics may affect the nervous system of insects by modifying ion channels or interfering with receptor systems, which is consistent with data on its effect on the cognitive functions and behavior of bees (Pasquini et al., 2023; Buteler et al., 2025; Al Naggar et al., 2024).

The presence of microplastics in human tissues is associated with a number of potential risks. Studies show that they can cause local inflammatory reactions, oxidative stress, and disruption of cellular processes. Animal experiments confirm a possible impact on the cardiovascular system, in particular, accelerating the development of atherosclerosis. Microplastics have already been found in human arteries, highlighting their possible contribution to vascular pathology (Pittalwala, 2025).

Its impact on reproductive health is of particular concern. The detection of microplastics in the placenta and reproductive organs indicates a risk to fetal development and reproductive system function. In addition, the surface of microplastics can serve as a substrate for pathogenic and antibiotic-resistant bacteria, posing an additional threat to human health (Savchuk, 2025; Abbas, Ahmed and Ahmad, 2025).

An analysis of current scientific research shows that the problem of microplastic contamination of bee products is multifactorial and lies at the intersection of food safety, ecotoxicology, bee physiology, and global environmental change. Most authors emphasize that microplastics should be considered a new class of pollutants capable of migrating through various environments and entering food chains (Garrido Gamarro and Costanzo, 2024). The variety of approaches to its detection in honey, pollen, and bee bread often complicates the comparison of results, but the available data allow us to form an integrated picture of the scale and mechanisms of contamination.

Most studies indicate that microplastics are present in bee products, regardless of the geographical location of the study. This has been observed in urbanized areas of Europe and in subtropical regions of Latin America (Basaran et al., 2024; Rani-Borges et al., 2024). Such consistency in results indicates the wide migratory capacity of polymer particles and their persistence in the environment. Particularly indicative is the dominance of synthetic microfibers, which, due to their low mass and high aerodynamic stability, are easily transported by air currents and settle on plant surfaces, flowers, and water sources. This explains the detection of microfibers in honey even from remote apiaries located in relatively clean regions (Zhang et al., 2023).

The mechanisms by which microplastics enter honey, bee bread, and pollen are complex and involve some complementary pathways. The main one is considered to be atmospheric transport: bees come into contact with particles in the air during flight, and these particles are adsorbed onto the covering structures of their bodies. Particles also enter the hive through plant surfaces, where they accumulate as a result of atmospheric dust deposition (Gómez-Méndez et al., 2024). Bees use surface water for thermoregulation and food preparation, and even low concentrations of microplastics in water bodies cause them to re-enter the bee colony.

Another vector may be the abrasive destruction of plastic components of equipment or containers, although this mechanism is usually considered secondary. The formation of bee bread creates additional conditions for the retention of fine particles, as pollen is compacted and mixed with honey and bee enzymes to form a matrix capable of accumulating microplastics.

In general, the entry of particles into hive products is a multifactorial process that is sensitive to seasonality, landscape structure, and the intensity of anthropogenic pressure.

Physiological effects of microplastics on bees. The presence of microplastics in hive products indicates contact with bees, which has been confirmed by some experimental studies. According to Al Naggar et al. (2023), prolonged exposure to polystyrene particles in feed leads to a decrease in body weight and changes in feed consumption intensity. This is probably due to mechanical irritation of the intestinal walls or impaired intestinal motility.

Larvae may be vulnerable to the effects of microplastics, as bee bread is the main source of protein and biologically active substances in their diet. However, there are currently virtually no systematic studies in this area. Bee products are increasingly being recognized as valuable indicators of environmental health. As bees collect food within a radius of 3–5 km, analyzing honey, pollen, or bee bread provides an integrated assessment of atmospheric, soil, and technogenic pollution levels in the area (Garrido Gamarro and Costanzo, 2024). The presence of synthetic fibers in honey is correlated with traffic intensity, population density, and the type of industrial facilities in the region. Some researchers propose using bee products to monitor polymer aerosols, which is particularly relevant in urban and industrial areas.

Although honey's contribution to the overall dietary intake of microplastics is insignificant compared to seafood or mineral salt, the issue of chronic exposure remains important. Potential risks include the interaction of particles with the intestinal microbiota, the possible penetration of particles smaller than 20–50 µm into colon crypts, and the transfer of chemical contaminants on their surface (Zhang et al., 2023). Of particular concern is nano plastics, which, due to their nanoscale size, are capable of overcoming cellular barriers, but remain difficult to detect using current methods.

Methods for studying microplastics. The study of microplastics in food products usually involves several consecutive steps. First, samples are prepared, which involves chemical or enzymatic destruction of the organic matrix and subsequent filtration to isolate particles (Huang, Hu, and Wang, 2022).

The next step is morphological analysis using optical (Maes et al., 2017; Kalaronis et al., 2022) or electron microscopy, which allows the origin, shape, and size of microplastics to be determined (Misumi et al., 2018; Lou et al., 2023). To confirm the polymeric nature, spectroscopic methods are used, in particular FTIR (Primpke et al., 2017; Araujo et al., 2018; Cunsolo et al., 2021) and Raman spectroscopy (Gago et al., 2016; Huang, Hu, and Wang, 2022), as well as pyrolysis gas chromatography with mass spectrometry (Py GC/MS) (Seeley and Lynch, 2023; Zhang et al., 2023), which allows the types of polymers to be identified.

The final step involves quantitative assessment, integrating imaging results and chemical identification to provide a comprehensive overview of the level and nature of microplastic contamination in food products.

Recent studies demonstrate the significant potential of machine learning for identifying microplastics using SEM, fluorescence, FTIR, and Raman spectroscopy (Kedzierski et al., 2019; Shi et al., 2022; Meyers et al., 2022; Hufnagl et al., 2022). In particular, the PlasticNet model (Zhu, Parker, and Wong, 2023), trained on spectra of 11 types of virgin polymers, achieved over 95% classification accuracy, confirming the promise of deep learning in this direction.

At the same time, its effectiveness for environmental samples remains unexplored. Another approach (Weber, Zinnen, and Kerpen, 2023), combining algorithms and expert assessment, used over 64,000 Raman spectra from the environment and wastewater, achieving high accuracy (recall ≥ 99.4%, precision ≥ 97.1%) and reducing annotation time from several hours to less than one hour per sample.

Hu et al. (2024) proposed the use of artificial intelligence for the rapid identification of microplastics and the prediction of their environmental behavior. The authors developed a model capable of automatically recognizing microplastics in complex environmental matrices and simulating their migration and transformation. This approach not only significantly reduces sample analysis time but also opens up prospects for the creation of real-time microplastic monitoring systems, which could become an important tool for environmental policy and risk management.

At the same time, the lack of internationally agreed standards makes it difficult to compare the results of different studies. Significant differences exist in sample preparation methods, filter pore sizes, organic matrix cleaning procedures, and polymer composition verification methods (FTIR, Raman, and Py-GC/MS). A lack of control over laboratory contamination can also lead to false positive results. Therefore, developing a unified protocol for determining microplastics in bee products is one of the key tasks of modern food analytics.

Prospects for further research. Further development in this area should cover several key aspects. First, it is necessary to create an international standard for the detection of microplastics in food products, including honey. Second, the development of highly sensitive methods for analyzing nanoplastics, which have a significantly higher toxicological potential, is promising. Third, there is an urgent need to study the impact of microplastics on the intestinal microbiota of bees, which plays a decisive role in feed digestion, immune response, and behavioral reactions. Another important area of research is the migration of microplastics within bee colonies through food chains and the assessment of risks to vulnerable populations, such as children, people with chronic gastrointestinal diseases, or those with altered immune reactivity.

Conclusions. Microplastics are a stable, multi-component contaminant in bee products, reflecting levels of anthropogenic pressure and urbanization. Honey, bee pollen, bee bread, and wax can potentially contain synthetic particles regardless of geography or landscape, confirming the scale and persistence of polymeric micro-pollutants in the biosphere.

The main type of microplastic is textile fibers, which spread through the air and enter hive products due to their mobility and stability. Meanwhile, the presence of polyethylene and polypropylene indicates local sources of pollution, such as agriculture, transport, and households. The presence of microplastics in bee products can be used as an indicator of environmental health and as a tool for environmental monitoring.

Microplastics cause subclinical changes in bee physiology, including a decrease in body weight, changes in food consumption, and disorientation. Even minimal toxic effects can have consequences for the ecosystem, particularly with prolonged exposure, and the accumulation of synthetic particles in bee bread poses a risk to brood development.

While the risk to humans of consuming honey containing microplastics is low, it is significant in the context of chronic exposure. Of particular concern are the adsorption of chemical contaminants and interaction with the microbiota. Meanwhile, nanoplastics, which can penetrate biological barriers, are the least studied and potentially the most toxic.

A key challenge remains the standardization of methods for determining microplastics in bee products. Current differences in sample preparation, filtration parameters, identification methods, and contamination control make it difficult to compare results between studies. Improving these procedures is a necessary prerequisite for the development of internationally agreed standards and monitoring systems. Microplastic research is important for risk assessment, the development of new analytical technologies, and ensuring food safety.

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