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ANALYSIS OF KEY INDICATORS OF CHRONIC STRESS IN CATS AND DOGS

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Summary. Stress is an integral part of the life of every organism. This issue has become especially important now, during wartime, when stress affects both humans and animals. Military events have led to unprecedented changes in the lives of both humans and animals, affecting their daily routines, social interactions, and stress levels. The study was conducted on 12 dogs and 14 cats. The effects of stressors on cortisol, glucose, total leukocytes, and eosinophils levels were shown. The study's results show that during chronic stress, the studied animals showed an increase in cortisol and glucose levels, indicating increased stress in these animals in response to changes in their daily lives. Total leukocyte counts in dogs and cats were also higher than reference levels, indicating an immediate activation of the immune system in response to stressors. The differential response of eosinophils in animals underscores the complexity of the immune system's response to stress. Dogs, as social animals, may experience more pronounced immune modulation in response to stressors, potentially making them more sensitive to fluctuations in immune cell numbers. The study revealed important behavioral changes in dogs and cats. Behavioral manifestations are the most visible indicators of an animal's emotional well-being. Changes in behavior, including anxiety, hiding, vocalization, and altered social interactions, may reflect the emotional and psychological effects of stress. Our findings underscore the importance of considering individualized strategies for managing animal welfare in emergencies

Keywords: cortisol, glucose, leukocytes, war zone

Introduction. Animal welfare is becoming increasingly important as society becomes more aware of the ethical and humane aspects of animal treatment. An animal is in a state of well-being when it is healthy, comfortable, well-nourished, safe, able to behave naturally, and free from unpleasant conditions such as pain, fear, and suffering (as demonstrated by scientific evidence) (Nedosiekov et al., 2021). Animal welfare requires disease prevention and veterinary care, proper housing, maintenance, nutrition, and humane treatment. Ensuring comfortable conditions and respecting the physiological and psychological needs of animals affects their health (Amat, Camps and Manteca, 2016). Today's realities: the war in Ukraine, determines the need to bring Ukrainian veterinary medicine to a new level, focusing on the decent treatment of animals and their welfare.

Stress is an integral part of the life of every organism. This topic is especially relevant now, during war, when both humans and animals suffer from stress. Animals in war zones may suffer even more than humans because they do not understand what is happening and often live in a state of chronic stress. Stress as a complex and often destructive factor has long been studied in the context of animal health and welfare. In dogs and cats, stress can manifest itself in physiological and behavioral changes that often mirror human responses to environmental factors or situations. These responses, including changes in cortisol levels, immune system dynamics, and behavioral displays, provide insight into the welfare and adaptability of these animals during stressful situations. That is, stress can be considered an adaptive syndrome (Stella and Croney, 2016). The factors that cause stress can be physical, emotional, or social. Physical, when the animal feels uncomfortable due to environmental factors. For example, changes in temperature, pain, hunger and thirst, noise, or vibration. Emotional factors may include loneliness or separation from the owner. Social factors include competition or aggression from other animals (Grigg and Kogan, 2019).

Behavior is often the most visible indicator of an animal's emotional well-being. Changes in behavior, including anxiety, hiding, vocalization, and changes in social interactions, can reflect the emotional and psychological effects of stress (Beerda et al., 1999).

When an animal is under stress, all body systems begin to work more intensively. For a stress response to occur, it is important that the activity of the endocrine glands, especially the hypothalamus-anterior pituitary-adrenal cortex system, be intensified. Depending on the duration and intensity of the stressor, stress can be subtle, leading to adaptation, or develop into pathological stress that can cause illness or even death in animals (Part et al., 2014). Like any other response of the body, stress has several stages.

The first stage of stress called the anxiety or mobilization stage is a general activation of the body to combat negative external influences. This stage is characterized by the activation of the sympathetic nervous system and the release of stress hormones, including adrenaline and norepinephrine. Adrenaline stimulates the breakdown of glycogen in the liver and muscles, leading to an increase in blood glucose levels. This gives the muscles extra energy to respond quickly to stress. Activation of the sympathetic nervous system also stimulates the breakdown of fats (lipolysis) in adipose tissue, resulting in the release of free fatty acids into the bloodstream. These can be used as a source of energy for muscles and other tissues (Pizzino et al., 2017). In general, the overall metabolic rate of the body increases during this phase. This increases the availability of energy for the animal, allowing it to act more efficiently in the face of a threat.

The immune system also plays a critical role in the body's response to stress. During stressors, the immune system may become activated, leading to changes in the number of different immune cells, including white blood cells. An increase in the total number of white blood cells can indicate the immune system's response to stress. Eosinophils are involved in the body's immune response and can be affected by stress (Nagaraja et al., 2016).

The second stage called the resistance or adaptation stage develops with prolonged exposure to a stressor and is characterized by a significant increase in the size and activity of the adrenal glands, as well as an increase in the body's general and specific resistance. At this stage, there is an increased release of corticotropin from the pituitary gland, which stimulates the adrenal glands to increase production of glucocorticoids such as cortisol. Cortisol is known to help regulate stress and the body's response to danger, but this is only one, albeit important, function (Kooriyama and Ogata, 2021).

Cortisol, a steroid hormone produced by the adrenal glands, is a well-established marker of stress in both humans and animals. Stressful situations trigger the release of cortisol, which often leads to an increase in blood levels. The physiological response of dogs and cats to stress is assessed by monitoring cortisol levels (Nenadovic et al., 2017).

Cortisol plays a key role in maintaining blood glucose levels and adapting the body to prolonged stress. Under the influence of cortisol, gluconeogenesis is stimulated. This provides the body with energy under conditions of prolonged stress when glycogen stores can be depleted. To support the process of gluconeogenesis, cortisol stimulates the breakdown of proteins (proteolysis) in the muscles. The amino acids released from this process are used to synthesize glucose in the liver. Although this helps maintain energy balance, prolonged proteolysis can lead to muscle atrophy and a decrease in overall muscle mass (Markovszky et al., 2020). Cortisol also promotes lipolysis, the breakdown of fats, which leads to the release of free fatty acids that can be used as a source of energy.

One marker of acute stress in dogs is salivary cortisol. However, its use has some drawbacks that can lead to misinterpretation of the data. The key aspect is a standardized sampling method and subsequent processing before immunoassay. In addition, circadian changes and individual variability in cortisol levels should be consistently taken into account in the preparation of the experimental design, statistical data processing, and subsequent interpretation of the measurements (Chmelíková et al., 2020).

The stage of resistance can last for a long time, but if the stressor continues to act, the body may become exhausted, leading to the transition to the third stage of stress — the stage of exhaustion.

The third stage, known as the exhaustion stage, occurs when the body is exposed to a stressor for an extended period. At this stage, the adaptive functions of the adrenal glands, despite their hypertrophy and other body systems decline, leading to exhaustion. Despite the initial hypertrophy of the adrenal glands, their functional capacity gradually decreases. This leads to a decrease in the production of glucocorticoids, especially cortisol, which makes it impossible to further support stress adaptation (Viena et al., 2012). A decrease in cortisol levels means that the body can no longer maintain the required level of gluconeogenesis and energy mobilization. Lack of sufficient cortisol and depletion of glycogen stores leads to a decrease in blood glucose levels, which can lead to hypoglycemia. This makes it difficult to provide energy to cells, especially neurons, which depend on a stable supply of glucose.

Due to the prolonged protein breakdown during the previous phase, amino acid stores are depleted, leading to significant catabolism of muscle tissue and other protein structures. Muscle atrophy becomes pronounced, reducing physical strength and recovery.

Lipolysis may be impaired at this stage and the free fatty acids released may not be used effectively due to reduced metabolic activity. This can lead to an accumulation of lipids in the blood and impaired cell membrane function.

Prolonged exposure to cortisol in the previous stages suppresses the immune system. In the exhaustion stage, the immune system is further weakened, making the body susceptible to infections and other diseases. The body's specific and non-specific resistance is significantly reduced. The body can no longer effectively use energy reserves, leading to general exhaustion, weakness, weight loss, and organ failure. The ability to regenerate tissues is significantly reduced, making it difficult to recover from injury or illness. This can contribute to the development of chronic diseases and irreversible changes in organs and tissues (Feldsien, 2010).

Chronic stress can cause serious damage to the lives of animals, affecting their health, behavior, and overall well-being, underscoring the importance of creating conditions that minimize stressors and promote adaptation and recovery. Animals, especially dogs and cats, in human care should experience as little stress as possible, so it is necessary to measure and quantify stress levels. Stress parameters that can be measured non-invasively can help identify poor animal welfare.

The study aims to quantify stress levels and identify effective methods to help animals cope with stressful conditions.

Materials and methods. The study was conducted on 12 dogs and 14 cats evacuated from the combat zone. Inclusion criteria included animals without visible injuries. The examination of the animals began with the study of the general clinical condition of the dogs and cats by determining the TPR indicators, and general condition of the animals.

Blood samples were collected for biochemical and hematological studies; to assess any changes related to blood components.

Biochemical analysis was performed in the laboratory to determine serum cortisol and glucose levels. Blood samples were collected from the jugular vein or saphenous vein (external for dogs and medial for cats) into vacuum blood collection tubes (Vacusera) with a clotting activator inside using a sterile needle and syringe. The animals were handled carefully and without excessive stress during collection. The collected blood samples were allowed to clot and then centrifuged to separate the serum from the blood cells.

Hematologic analysis was performed on an automatic HTI microCC-20Plus analyzer with the determination of the total number of leukocytes and eosinophils as the main indicators of stress in animals. Cortisol levels were determined by a Bionote Vcheck V200 fluorimeter, and glucose levels by an HTI BioChem SA biochemical analyzer.

Statistical processing of the results was performed using the Statgraphics software package (Manugistic Inc.; STATistical GRAPHICsystem, USA). Data were presented as M ± SE (mean ± standard error); $p < 0.05$ was considered statistically significant.

Results and discussion. Stress is a complex physiological response to an anticipated threat or challenge, and it can have several negative effects on the physiology and behavior of pets. Stress can weaken the immune system, making animals more vulnerable to disease (Rom and Reznick, 2015).

The data obtained in the course of the studies allow us to conclude that there was a significant increase in serum cortisol and glucose levels observed in dogs and cats (Table 1).

Table 1 — Cortisol and glucose levels in blood serum of dogs and cats

Indicator	Dogs		Cats	
	Result obtained	Reference limits	Result obtained	Reference limits
Cortisol, nmol/l	241.9 ± 16.5	28–170	276.5 ± 17.2	28–140
Glucose, mmol/l	11.8 ± 1.21	4.3–6.6	15.2 ± 2.11	3.2–7.9

The results show significant statistical changes in the values of cortisol and glucose in the blood serum during chronic stress in which the animals were exposed compared to the reference values ($p < 0.05$).

The increase in cortisol levels indicates increased stress in these animals in response to changes in their daily lives. Cortisol is a well-established biomarker of stress response in both humans and animals, and its increased secretion is a physiological response to stressors. Our results are in line with studies that emphasize the role of cortisol as a sensitive indicator of stress in the study of biological mechanisms of animal behavioral laterality (Salgirli Demirbas et al., 2023).

The increase in cortisol levels during prolonged stressors (under combat conditions) indicates that the dogs and cats perceived changes in the environment as stressful. These changes included increased noise levels, frequent explosions, vibrations, changes in living conditions, lack of familiar routines and social contact, and possible difficulty in accessing food and water. The fact that both species (dogs and cats) showed this response underscores the impact of chronic stress on the physiological functions and behavior of companion animals.

Based on our observations, high cortisol levels cause increased anxiety, fear, and nervousness in animals. This manifests itself as avoidance, trembling, or hiding. Animals may also react aggressively to normal stimuli. In general, prolonged elevation of cortisol levels due to chronic stress can significantly impair the quality of life of dogs and cats, which underscores the importance of creating favorable conditions to reduce the effects of stress.

An increase in glucose levels by an average of three times (compared to reference values) may indicate an increase in the process of gluconeogenesis stimulated by excess cortisol (Table 1).

Stress hyperglycemia (also called physiological hyperglycemia) is the most common condition observed in cats. An increase in blood glucose levels occurs due to prolonged exposure to stressors. Chronic stress leads to constant activation of the sympathetic nervous system and the adrenal glands, which secrete stress hormones, especially cortisol. This hormone promotes the breakdown of glycogen in the liver and the release of glucose into the bloodstream to provide the body with energy.

Prolonged hyperglycemia due to chronic stress can have negative consequences: consistently elevated glucose levels lead to the development of insulin resistance, which increases the risk of diabetes; chronic stress and concomitant hyperglycemia can reduce the effectiveness of the immune response, making animals more susceptible to infection and disease.

Hematological studies of serum have also shown an increase in the total number of leukocytes in both dogs and cats during chronic stress, indicating an increased immune system response to stressors. An increase in the number of leukocytes is consistent with the idea that stress can trigger an immune response as the body prepares to defend itself against potential threats (Beerda et al., 1999). As shown in Table 2, an increase in neutrophil count is an indicator of a non-specific defense response in dogs and cats.

Table 2 — Total and differential leukocyte counts in the dogs and cats studied

Indicator	Dogs		Cats	
	Result obtained	Reference limits	Result obtained	Reference limits
Leukocytes, $\times 10^3$/mm^3	18.17 ± 2.12	5.0–14.1	22.21 ± 3.38	5.5–19.5
Neutrophils, %	85.90 ± 9.35	61–88	70.01 ± 11.12	47–66
Eosinophils, %	0.10 ± 0.12	0–9	0.50 ± 0.15	0–4
Basophils, %	0.20 ± 0.10	0–1	0.15 ± 0.08	0–1
Monocytes, %	6.75 ± 1.32	2–10	3.91 ± 0.42	0–5
Lymphocytes, %	7.05 ± 0.91	8–21	25.43 ± 5.94	27–36

The number of eosinophils, a component of the immune system, showed species-specific variation in response to stressors. A significant decrease in the number of eosinophils was observed in dogs, which may indicate suppression of immunity in response to acute stress.

The differential response of eosinophils in dogs and cats underscores the complexity of the immune system's response to stress. Dogs, as social animals, may experience more pronounced immune modulation in response to stressors, potentially making them more sensitive to fluctuations in immune cell numbers (Salgirli Demirbas et al., 2023). Cats, with their more solitary and independent nature, may maintain a more stable immune response under similar circumstances (Westropp, Kass and Buffington, 2006).

The results of the study show that during chronic stress, the dogs studied had an increase in the total number of leukocytes, indicating an immediate activation of the immune system in response to stressors. This observation is consistent with the idea that dogs are highly sensitive to the emotional state of their owners and may respond more dynamically to changes in human behavior (Brooks et al., 2018). In contrast, cats may have a somewhat delayed white blood cell response. This delay is indicative of the more self-sufficient nature of cats and their ability to cope with stress independently (Amat, Camps and Manteca, 2016).

Behavior is often the most visible indicator of an animal's emotional well-being. Changes in behavior, including anxiety, hiding, vocalization, and altered social interaction, can reflect the emotional and psychological effects of stress (Alho, Pontes and Pomba, 2016). Thus, several changes were observed in animals under chronic stress, the most common of which was increased anxiety (55.9%) and attention-seeking behavior (44.1%) in dogs. Cats, on the other hand, hid more (55.2%), groomed less (48.3%), and became more territorial (41.4%).

Conclusions. 1. Chronic stress has created unique stressors for dogs and cats, with both species experiencing elevated cortisol levels and behavioral changes.

2. The difference in eosinophil counts between these two species highlights their different immune responses to stressors during prolonged stress.

3. This study highlights the importance of understanding the specific needs of dogs and cats in emergencies and adapting interventions to reduce stress and promote well-being.

4. Further research is needed to elucidate the underlying mechanisms that drive these species-specific stress responses.

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