DOI 10.32900/3083-7987-2026-137-182-193
UDC 636.7:612.8:591.1:612.018

CORTISOL LEVELS IN THE BLOOD OF DOGS WITH DIFFERENT AUTONOMIC NERVOUS SYSTEM VEGETATIVE STATUS AND THEIR CORRECTION

Vladyslav REDKO, PhD student, https://orcid.org/0009-0008-8124-749X
Olga BOBRYTSKA, Doctor of veterinary sciences, professor,
https://orcid.org/0000-0002-5368-8094
Oleksandr KHAVIN, PhD student, https://orcid.org/0009-0001-5660-663X
Larysa VODOPIANOVA, Candidate of Biological Sciences, Associate Professor,
https://orcid.org/0000-0002-9331-1689
State Biotechnological University, Kharkiv, Ukraine
Kateryna SOVIK, DVM, https://orcid.org/0009-0001-1974-702X
Incline veterinary hospital, Incline village , NV, USA

Received: April 27, 2026
Revised: May 1, 2026
Published: May 29, 2026

This is an Open Access article under the **CC BY-NC-ND 4.0 license**

The problem of stress resistance in dogs is highly relevant in modern veterinary medicine, as stressors significantly affect the physiological state, behavior, and adaptive capacity of animals under various housing and use conditions. The autonomic nervous system plays a key role in shaping the stress response by determining the intensity and duration of neuroendocrine reactions. The aim of this study was to determine the tonus-dependent hormonal response of dogs to an acute acoustic stressor and to evaluate the possibilities for its correction using nano-compounds of trace elements. The study was conducted on 30 German Shepherd dogs with different types of vegetative tone. Acute stress was induced using the open field test with thunder sound playback, and organism reactivity was assessed by measuring plasma cortisol levels before and after stress exposure. The results showed that dogs with sympathicotonia exhibited the highest baseline cortisol levels and the greatest amplitude of the hormonal response with delayed recovery, indicating increased reactivity and reduced adaptive reserves. Dogs with a normotonic type of autonomic regulation demonstrated a more balanced and controlled cortisol dynamics, whereas vagotonic dogs showed a less intense acute response accompanied by high individual variability. The use of nano-compounds of trace elements was associated with a tendency toward a reduced amplitude of the cortisol response to stress, particularly in dogs with sympathicotonic autonomic profiles, suggesting their potential as a promising means of modulating stress reactivity. The obtained results confirm the decisive role of vegetative tone type in shaping the neuroendocrine stress response in dogs and may be applied to predict individual stress resistance and to develop approaches for its correction in veterinary practice.

Keywords: dogs; autonomic nervous system; vegetative tone; cortisol; sympathicotonia; vagotonia; normotonia; stress; stress resistance; nano-compounds of trace elements; adaptation.

ISSN 3083-7987 print

РІВЕНЬ КОРТИЗОЛУ В КРОВІ СОБАК З РІЗНИМ ВЕГЕТАТИВНИМ СТАТУСОМ АНС ТА ЙОГО КОРЕКЦІЯ

Владислав РЕДЬКО, асп., https://orcid.org/0009-0008-8124-749X
Ольга БОБРИЦЬКА, д. вет. н., професор, https://orcid.org/0000-0002-5368-8094
Олександр ХАВІН, аспірант, https://orcid.org/0009-0001-5660-663X
Лариса ВОДОП'ЯНОВА, к. б. н., доцент, https://orcid.org/0000-0002-9331-1689
Державний біотехнологічний університет, Харків, Україна
Катерина СОВІК, лікар ветеринарної медицини,
https://orcid.org/0009-0001-1974-702X
Інклайн ветеринарний шпиталь, Інклайн вілледж, Невада, США

Дата надходження до редакції: 27.04.2026 р.
Дата прийняття до друку після рецензування: 1.05.2026 р.
Дата публікації: 29.05.2026 р.
Це стаття відкритого доступу за ліцензією **CC BY-NC-ND 4.0**

Проблема стресостійкості собак є актуальною у сучасній ветеринарній медицині, оскільки стресові впливи істотно позначаються на фізіологічному стані, поведінці та адаптаційних можливостях тварин у різних умовах утримання та використання. Автономна нервова система відіграє ключову роль у формуванні стресової відповіді, визначаючи інтенсивність і тривалість нейроендокринних реакцій організму. Метою дослідження було встановити особливості тонусзалежної гормональної відповіді собак на дію гострого звукового стресового подразника та оцінити можливості її корекції за застосування наносполук мікроелементів. Дослідження проведено на 30 собаках породи німецька вівчарка з різним типом вегетативного тонусу. Модуляцію гострого стресу здійснювали за допомогою тесту «відкрите поле» із відтворенням звуку грози, а оцінку реактивності організму проводили за динамікою рівня кортизолу в плазмі крові до та після дії стресора. Встановлено, що у собак із симпатикотонією спостерігається найвищий базальний рівень кортизолу та максимальна амплітуда гормональної відповіді з уповільненим відновленням показників, що свідчить про підвищену реактивність і знижені адаптаційні резерви. Нормотонічний тип автономної регуляції характеризується більш збалансованою та контрольованою динамікою кортизолу, тоді як у ваготонічних собак відзначається менша інтенсивність гострої відповіді за високої індивідуальної варіабельності. Застосування наносполук мікроелементів супроводжувалося тенденцією до зниження амплітуди кортизолової відповіді на стрес, особливо у собак із симпатикотонічним типом АНС, що дозволяє розглядати їх як перспективний засіб модуляції стрес-реактивності. Отримані результати підтверджують визначальну роль типу вегетативного тонусу у формуванні нейроендокринної відповіді собак на стрес і можуть бути використані для прогнозування індивідуальної стресостійкості та розробки підходів до її корекції у ветеринарній практиці.

Ключові слова: собаки; автономна нервова система; вегетативний тонус; кортизол; симпатикотонія; ваготонія; нормотонія; стрес; стресостійкість; наносполуки мікроелементів; адаптація.

Introduction. Stress is a universal neuroendocrine and behavioral response that ensures the mobilization of the body's resources in response to threatening or biologically significant stimuli and determines the level of welfare and performance of dogs under various housing and utilization conditions (Miller et al., 2025). In veterinary medicine and applied fields of cynology, this issue has substantial practical relevance, as both acute and chronic stressors are associated with changes in behavior, functional status, and key animal welfare markers (Teo et al., 2022). In particular, it has been shown that stress exposures are capable of modifying behavioral patterns in dogs, emphasizing the need for objective assessment of stress reactivity and adaptive mechanisms (Salgirli Demirbas et al., 2023).

A key role in the implementation of the stress response is played by the autonomic nervous system (ANS) in interaction with the hypothalamic–pituitary–adrenal (HPA) axis (Tóth & Dobolyi, 2025). The sympathetic branch provides a rapid mobilization response of the "fight-or-flight" type through activation of the cardiovascular system and neurohumoral mechanisms, whereas the parasympathetic branch promotes recovery, limits excessive activation, and stabilizes homeostasis (Somppi et al., 2022). Syntheses in ANS physiology highlight that an imbalance between sympathetic and parasympathetic influences can generate both hyperreactive and prolonged response patterns and may potentially reduce adaptive capacity (Wehrwein et al., 2016).

In recent years, the objectification of dogs' psycho-emotional responses has increasingly relied on the combined use of hormonal markers (cortisol, ACTH) and cardiac indicators (heart rate, HRV), which are sensitive to emotional valence and arousal level (Mârza et al., 2024). Experimental data demonstrate that negative emotional scenarios in dogs are accompanied by pronounced HRV changes and elevated hormonal stress markers, and that psychological factors, particularly frustration, may affect the physiological state and performance of working dogs more strongly than physical load itself (Flint et al., 2024). The social component of regulation is also important, as a phenomenon of coordinated responses within the dog–owner dyad has been demonstrated based on HRV and activity during interaction (Koskela et al., 2024).

In this context, it is particularly relevant to investigate how vegetative tone (normotonia, vagotonia, sympathicotonia) modulates the amplitude and duration of the hormonal response to an acute stressor. To achieve this objective, the following tasks were set: To determine the baseline cortisol levels in dogs with normotonia, vagotonia, and sympathicotonia; To evaluate changes in these levels 15, 30, and 60 minutes after exposure to the acoustic stimulus; To establish the influence of the autonomic tone type on the intensity and duration of the stress response; To analyze the interaction between the acoustic stressor and ANS tone.

Materials and research methods. The experiment was conducted on 30 dogs (Canis familiaris) of the German Shepherd breed aged 2 to 5 years, with an average body weight of 34.0 ± 3.1 kg. Based on anamnesis data, clinical examination, and general blood analysis, all animals were classified as clinically healthy; no electrocardiographic or echocardiographic abnormalities were detected.

Assessment of autonomic regulation in the dogs was performed using the method of variational pulsometry. According to the obtained results, three experimental groups were formed: dogs with normotonia (sympatho-vagal balance), dogs with sympathicotonia (predominance of the sympathetic nervous system), and dogs with vagotonia (predominance of the parasympathetic nervous system).

Acute stress in dogs was induced using the open field test. For this purpose, a test arena (room) with an area of 16 m² was equipped with a video camera to monitor the dogs' behavior. In the first experiment, the animal was placed in the room and left alone;

during the first three minutes the environment was silent, followed by a 3-minute playback of thunder sounds (average sound level 90–100 dB), after which silence was maintained for another 3 minutes.

To determine the effectiveness of nano-compounds of biogenic metals in correcting stress resistance, a second series of experiments was conducted two months later on the same animals that participated in the first experiment. All dogs received a mixture of trace element citrates with drinking water (Mg – 1000 mg/dm³, Zn – 200 mg/dm³, Fe – 10 mg/dm³, Cu – 40 mg/dm³, Co – 4 mg/dm³, Mo – 4 mg/dm³, Cr – 2 mg/dm³, Ge – 2 mg/dm³, Se – 0.4 mg/dm³; TU U 15.8-35291116-014:2011) at a dose of 0.2 mL/kg body weight once daily for ten days. The nano-compounds were provided by LLC "Nanomaterials and Nanotechnologies". During this period, the general condition of the animals was monitored. Starting from day 11 of the experiment, acute stress was induced again using the open field test, as in the previous experiment.

The biological material consisted of blood samples collected on the day of the experiment before its onset and at 15 and 60 minutes after exposure to the acoustic stimulus. Plasma cortisol levels were measured using the "Cortisol-KS-ELISA" kit (HEMA, Ukraine). The assay procedure followed the K210CFI instruction manual. Measurements were performed using an ELx800 universal microplate reader (Bio-Tek Instruments, USA).

Biometric processing of the obtained data was carried out using MS Excel software with the built-in "Data Analysis" function. Statistical analysis included descriptive statistics (to determine mean values and standard deviations) and one-way analysis of variance. Differences between mean values were assessed using Student's t-test to evaluate statistical significance. The significance of changes was determined at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, ensuring reliable interpretation of the results.

All experimental procedures involving animals were conducted in accordance with the principles of humane treatment of laboratory and farm animals, the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the provisions of EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The research protocol was reviewed and approved by the local bioethics committee of the institution where the study was conducted. All manipulations were performed with minimization of stress load, pain, and discomfort for the animals. No invasive or traumatic procedures were used, and the intensity and duration of the stressor were limited to the minimum necessary to achieve the scientific objectives. Throughout the study, continuous monitoring of the animals' general condition was performed, and in case of signs of excessive stress or deterioration of well-being, the experimental procedures were discontinued.

Research results. Table 1 presents the results of the statistical analysis of plasma cortisol levels in dogs with different types of autonomic nervous system (ANS) tone under exposure to an acoustic stressor. The study was conducted in three groups of animals—normotonic, vagotonic, and sympathicotonic—with assessment of indicators over the course of the experiment: before exposure to the stimulus and at 15, 30, and 60 minutes after its application (n = 5; ng/mL). The obtained data allow analysis of both the intensity of the acute hormonal response and the features of its temporal dynamics depending on the dogs' vegetative profile.

In normotonic dogs, the baseline cortisol level averaged 34.3 ng/mL (range 25.3–44.0 ng/mL). Following exposure to the acoustic stimulus, a sharp increase in hormone concentration was observed at 15 minutes, reaching 162.2 ng/mL, indicating a pronounced acute stress response. At 30 minutes, cortisol levels significantly decreased

to 89.5 ng/mL, and by 60 minutes further declined to 47.1 ng/mL, approaching baseline values, which indicates effective adaptive mechanisms. Variability of the indicators was moderate (SD ranged from 7.65 to 21.9).

In the group of vagotonic dogs, the initial cortisol level was lower (28.1 ng/mL; 20.3–31.8 ng/mL). After stimulation, cortisol concentration increased to 122 ng/mL at 15 minutes, but to a lesser extent than in normotonic dogs, indicating a less intense acute response. At 30 minutes, cortisol levels remained relatively high (92.7 ng/mL), and at 60 minutes decreased ($P < 0.001$) to 50.1 ng/mL. Notably, high variability was observed in this group (SD up to 57.34 ng/mL), reflecting substantial individual differences in the stress response among vagotonic dogs.

Dogs with sympathicotonia exhibited the highest baseline cortisol levels (37.9 ng/mL; 31.3–41.5 ng/mL). After exposure to the acoustic stimulus, the maximum increase in cortisol concentration was observed at 15 minutes, reaching 230.7 ng/mL, which markedly exceeded levels in the other groups. At 30 minutes, hormone levels remained high (178.4 ng/mL), and at 60 minutes decreased to 74.9 ng/mL, but still remained approximately twice the baseline level. The low degree of dispersion (SD 9.61–24.60 ng/mL) indicates a homogeneous response and a more stable stress-response pattern in sympathicotonic dogs.

Table 1

Statistical analysis of plasma cortisol levels in dogs with different ANS tone under exposure to an acoustic stressor (n = 5; ng/mL)

Statistical parameters	Study period			
	Before exposure	After exposure		
		15 min	30 min	60 min
Normotonia				
M *(min.–max.)*	34,3 *(25,3–44,0)*	162,2 *(140,6–177,1)*	89,5 *(80,3–101,2)*	47,1 *(24,3–77,4)*
SD	8,08	15,66	7,65	21,96
SEM	3,62	7,0	3,42	9,82
S^2	65,35	245,32	58,48	482,33
CI (95%)	10,04	19,45	9,5	27,27
Vagotonia				
M *(min.–max.)*	28,1 *(20,3–31,8)*	122 *(81,8–158,9)*	92,7 *(44,2–163,5)*	50,1 *(27,6–91,2)*
SD	4,52	29,98	57,34	27,42
SEM	2,02	13,41	25,64	12,26
S^2	20,4	898,95	3288,13	751,59
CI (95%)	5,61	37,23	71,20	34,04
Sympathicotonia				
M *(min.–max.)*	37,9 *(31,3–41,5)*	230,7 *(205,1–258)*	178,4 *(145,3–199,3)*	74,9 *(60,2–85,5)*
SD	4,01	24,60	24,28	9,61
SEM	1,79	11,00	10,86	4,30
S^2	16,10	605,06	589,67	92,41
CI (95%)	4,98	30,54	30,15	11,94

Note. Mean – arithmetic mean; min–max – minimum and maximum values; SD – standard deviation; SEM – standard error of the mean; S^2 – sample variance; CI (95%) – 95% confidence interval.

ISSN 3083-7987 print

Thus, the results demonstrate a clear dependence of both the intensity and duration of the stress response in dogs on ANS tone. The most pronounced and prolonged response to the acoustic stressor was observed in dogs with sympathicotonia, which exhibited both the highest peak of cortisol secretion and a slower return of cortisol levels to physiological baseline values. In normotonic dogs, the stress response was less pronounced and normalized relatively rapidly. The weakest, yet at the same time the most variable response was detected in vagotonic dogs, indicating substantial individual differences in sensitivity and functional reserve capacity within this group.

Figure 1 illustrates the dynamics of differences in plasma cortisol levels in dogs with vagotonia and sympathicotonia compared with normotonic dogs under exposure to the acoustic stressor (n = 5; ng/mL). These indicators reflect the degree of deviation of cortisol concentrations in the experimental groups from the reference values of normotonic dogs at each stage of the experiment.

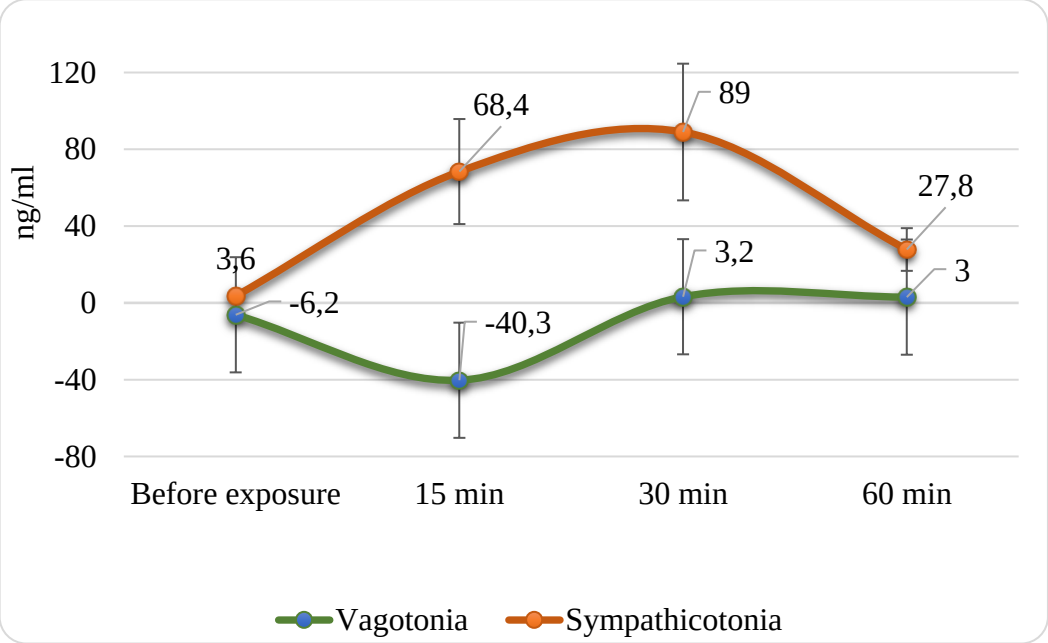

Fig. 1. **Dynamics of differences in plasma cortisol levels in dogs with vagotonia and sympathicotonia compared with normotonia under exposure to an acoustic stimulus (n = 5; ng/mL).**

In dogs with vagotonia, the baseline cortisol level was 6.2 ng/mL lower than in normotonic dogs, indicating a lower initial activity of the hypothalamic–pituitary–adrenal (HPA) axis. At 15 minutes after exposure to the acoustic stimulus, the difference decreased to −40.3 ng/mL, demonstrating a less intense acute hormonal response compared with normotonic dogs. However, at 30 and 60 minutes, cortisol levels in vagotonic dogs nearly equaled those of normotonic dogs (differences of +3.2 and +3.0 ng/mL, respectively), indicating a slower return to physiological baseline values and a more prolonged effect of stress on the organism.

Dogs with sympathicotonia exhibited an elevated baseline cortisol level, which was 3.6 ng/mL higher than that in normotonic dogs. At 15 minutes after exposure, cortisol levels exceeded normotonic values by 68.4 ng/mL, and at 30 minutes by 89.0 ng/mL, indicating an excessively strong and sustained stress response. Even at 60 minutes, the difference remained substantial (27.8 ng/mL), reflecting prolonged maintenance of an elevated hormonal background.

Table 2 presents the results of the analysis of variance assessing the effects of the acoustic stimulus and autonomic nervous system (ANS) tone on plasma cortisol levels in dogs. The effect of the acoustic stimulus was highly statistically significant (F = 29.8; P < 0.001), with a sum of squares (SS) of 46,425.8 and a mean square (MS) of 23,212.9. The obtained F value markedly exceeded the critical F value (FU = 3.26), indicating a significant impact of acoustic stress on cortisol levels in the blood of dogs.

Table 2

Effects of the acoustic stimulus and autonomic nervous system tone on plasma cortisol levels in dogs

Factors	Statistical parameters					
	SS	df	MS	F	P	FU
Effect of the acoustic stimulus	46425,8	2	23212,9	29,8	< 0,001	3,26
Effect of ANS tone	98250,7	2	49125,3	63,1	< 0,001	3,26
Interaction between factors	11567,1	4	2891,8	3,7	0,013	2,63
Within-group (error)	28047,8	36	779,1	–	–	–
Total	184291,3	44	–	–	–	–

Note. SS – sum of squares; df – degrees of freedom (number of factor levels minus 1); MS – mean square; F – Fisher's criterion for assessing the effect of the factor; P – level of significance; FU – critical F value.

The effect of autonomic nervous system (ANS) tone proved to be even more pronounced (F = 63.1; P < 0.001), with a sum of squares of 98,250.7 and an MS of 49,125.3. The interaction between the factors – the acoustic stimulus and ANS tone – was also statistically significant (F = 3.7; P = 0.013), with a sum of squares of 11,567.1 and an MS of 2,891.8. This indicates that the nature of the stress response depends not only on the strength of the external stimulus or ANS tone individually, but also on their combined effect. The within-group variation (error) amounted to 28,047.8 with 36 degrees of freedom (df), which was used to calculate the main statistical criteria. Thus, the analysis of variance confirmed that both the acoustic stimulus and ANS tone exert strong and significant effects on plasma cortisol levels in dogs, while their interaction shapes the individual pattern of the hormonal stress response.

In dogs with a normotonic ANS profile, administration of microelement nanocompounds resulted in a significant increase in plasma cortisol levels 15 minutes after exposure to the stimulus – by 365.7% (P < 0.001) compared with baseline values (Table 3). After 60 minutes, cortisol concentration decreased by 69.7% (P < 0.001) from the peak level, remaining 41.2% higher than baseline. In vagotonic dogs, cortisol levels increased by 328.4% at 15 minutes after stimulation (P < 0.001), which was lower than in normotonic dogs. After 60 minutes, cortisol levels decreased by 58.7% (P < 0.001) from the peak concentration but remained 76.7% above baseline (P < 0.001). Dogs with a sympathicotonic autonomic profile exhibited the strongest response: cortisol levels increased by 453.0% (P < 0.001) at 15 minutes after stimulation, exceeding those of normotonic dogs by 33.9% (P < 0.05) and vagotonic dogs by 61.9% (P < 0.01). After 60 minutes, cortisol levels decreased by 63.5% (P < 0.001) but remained 101.7% above baseline (P < 0.001), which was 61.1% higher than in normotonic dogs (P < 0.05) and 43.2% higher than in vagotonic dogs (P < 0.05).

Table 3

Plasma cortisol levels in dogs with different ANS tone under the influence of microelement nanocompounds (n = 5; ng/mL)

Statistical parameters	Study period		
	Before exposure	After exposure	
		15 min	60 min
Normotonia			
M *(min.–max.)*	30,6 (22,8–45,3)	142,5 (107,2–194,3)	43,2 (26,7–77,4)
SD	9,15	34,83	20,22
SEM	4,09	15,58	9,04
S^2	83,72	1212,91	408,74
CI (95%)	11,36	43,24	25,1
Vagotonia			
M *(min.–max.)*	27,5 (22,9–34,2)	117,8 (95,4–156,6)	48,6 (37,6–63,8)
SD	4,39	23,32	11,26
SEM	1,96	10,43	5,04
S^2	19,27	543,85	126,82
CI (95%)	5,45	28,96	13,98
Sympathicotonia			
M *(min.–max.)*	34,5 (28,2–45,7)	190,8 (175,6–204,9)	69,6 (51,4–83,3)
SD	7,67	13,38	13,69
SEM	3,43	5,98	6,12
S^2	58,81	179,02	187,53
CI (95%)	9,52	16,61	17

Note. SS – sum of squares; df – degrees of freedom (number of factor levels minus 1); MS – mean square; F – Fisher's criterion for assessing the effect of the factor; P – level of significance; FU – critical F value.

Thus, the dynamics of cortisol levels indicate a pronounced dependence of the hormonal stress response in dogs on the type of autonomic tone.

The description of the differences (Δ) in plasma cortisol levels in dogs with different autonomic nervous system (ANS) tone between animals administered microelement nanocompounds and the control group is presented in Figure 2. In dogs with a normotonic ANS profile, administration of microelement nanocompounds contributed to a reduction in cortisol levels compared with the control group by 3.7 ng/mL before exposure to the stressor, by 19.7 ng/mL at 15 minutes, and by 3.9 ng/mL at 60 minutes after stress exposure.

In vagotonic dogs, the effect of the nanocompounds was less pronounced: cortisol levels were lower than in the control group by 0.6 ng/mL before the stressor, by 4.2 ng/mL at 15 minutes, and by 1.5 ng/mL at 60 minutes after exposure. In dogs with a sympathicotonic profile, the most pronounced differences between the experimental and control groups were observed: 3.4 ng/mL before exposure, 39.9 ng/mL at 15 minutes ($P<0.05$), and 5.4 ng/mL at 60 minutes.

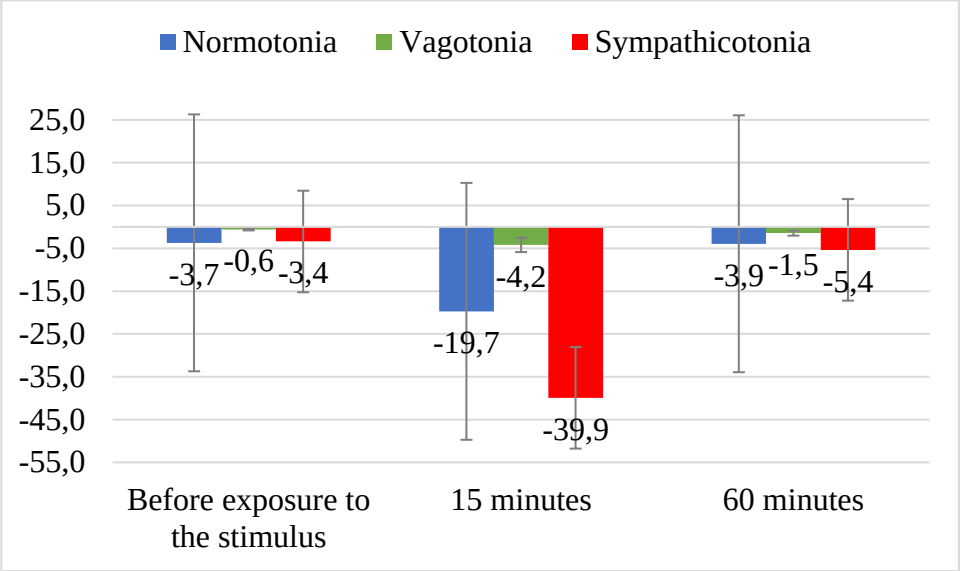

***Fig. 2*. Difference (Δ) in plasma cortisol levels in dogs with different ANS tone before and after administration of microelement nanocompounds (n=5; ng/mL).**
*Note. Significant differences: * – P≤0.05; ** – P≤0.01; *** – P≤0.001.*

Discussion. The obtained results demonstrate that the cortisol response to an acoustic stressor has a distinctly tone-dependent character and confirm the integrated role of the ANS and the hypothalamic–pituitary–adrenal (HPA) axis in shaping the acute stress response (Roy et al., 2025; von Majewski et al., 2023). In all groups, a rapid increase in cortisol was observed at 15 minutes after exposure, which is consistent with the concept of rapid neuroendocrine mobilization during acute stress. At the same time, intergroup differences were evident both in peak magnitude and in recovery rates, corresponding to the concept of different autonomic regulatory "profiles", where sympathetic influences determine the strength of initial mobilization, while parasympathetic mechanisms govern recovery efficiency and limitation of excessive activation (Wehrwein et al., 2016).

Dogs with sympathicotonia were characterized by the highest basal cortisol levels, the maximal post-stressor peak, and a delayed return to baseline values. This profile can be interpreted as a state of increased readiness of stress-realization systems, with a greater amplitude of hormonal response and potentially prolonged neuroendocrine load. From the perspective of ANS physiology, this may reflect dominance of the sympathetic branch and relatively weaker inhibitory recovery mechanisms, which is important for understanding individual risks of transition from an acute response to prolonged strain under repeated stressors (Kim et al., 2018; Sahar et al., 2001).

In normotonic dogs, a pronounced but more controllable response was observed, with a tendency for the parameters to approach baseline values by 60 minutes, which can be interpreted as a more balanced interaction between sympathetic and parasympathetic mechanisms. In vagotonic dogs, basal cortisol levels were lower; however, marked individual variability during the response phase was evident, which may indicate heterogeneity in reserve capacities and different "costs of adaptation" within the same autonomic profile. Such variability is important for practice, as it complicates the prediction of reactivity based solely on autonomic tone and underscores the need for a comprehensive assessment (e.g., combining cortisol with HRV) (Khavin et al., 2025; Somppi et al., 2022). In line with contemporary approaches, multicomponent indicator batteries are considered the most informative for assessing acute emotional states in dogs,

where hormonal markers are interpreted together with cardiac and behavioral measures (Flint et al., 2024).

Two-factor analysis of variance confirmed that both the acoustic stressor itself and the type of ANS tone exert a highly significant effect on cortisol levels, and a significant interaction between the factors was also established. This indicates that the stressor effect is not realized as a uniform response, but rather as the result of a combined influence of the external stimulus and internal regulatory organization, which is consistent with current views on the contextual and individually specific nature of stress responses. Additionally, evidence of high sensitivity of physiological parameters to emotional factors in working dogs highlights the applied importance of considering individual regulatory profiles during selection and training (Dickinson & Feuerbacher, 2025).

Of particular interest are the results of correction with microelement nanocompounds. Overall, compared with the control, a tendency toward reduced cortisol levels before and after the stressor was observed, with the most pronounced effect in sympathicotonic dogs at the peak response (15 minutes). This may indicate a potential modulatory action on stress-realization pathways (Merino del Portillo et al., 2024); however, mechanistic interpretation should be made cautiously. Based on the available data, it is more appropriate to speak of a hormonal effect (reduction in response amplitude) rather than a proven influence on specific ANS branches or the HPA axis. Nevertheless, given that stress states in dogs in experimental models are often accompanied by concurrent changes in cortisol, ACTH, and HRV, expanding the protocol to include parallel HRV and behavioral measurements appears promising for a more comprehensive explanation of the corrective effect (Flint et al., 2024).

Hypothetically, the reduction in the amplitude of the cortisol response after administration of microelement nanocompounds may be associated with a modulatory effect on central stress-realization pathways, particularly on regulation of the hypothalamic–pituitary–adrenal (HPA) axis and adrenal sensitivity to ACTH (Sharan et al., 2024; Nunez et al., 2025). Such a mechanism may manifest predominantly in animals with sympathicotonia, in which baseline stress-system reactivity is higher and neuroendocrine responsiveness to acute stressors is more pronounced (Nunez et al., 2025). In addition, it cannot be excluded that the observed effect of microelement nanocompounds is partially mediated through improvement of the prooxidant–antioxidant balance and reduction of oxidative load accompanying the acute stress response (Patani et al., 2023; McEwen et al., 2020). Indirectly, this could attenuate the intensity of neuroendocrine activation and promote a more controlled hormonal dynamics through redox-dependent regulation of glucocorticoid signaling and adrenal responsiveness (Patani et al., 2023; Sharan et al., 2024).

Among the study limitations, the small sample size (n = 5 per group) should be noted, which necessitates caution in extrapolating the findings to a broader population and increases the need for replication. A practically important next step would be to increase the sample size, include additional time points (e.g., 5 and 120 minutes), and standardize pre-experimental conditions affecting cortisol and HRV (circadian rhythms, feeding, physical activity, and the context of human interaction). Consideration of the social context is also essential, as dog–owner interaction may modulate physiological responses, which is important both for interpretation and for practical recommendations (Koskela et al., 2024).

Conclusions. The obtained results indicate that the acute stress response of dogs to an acoustic stimulus has a clearly expressed tone-dependent character and is determined by the integrated interaction of the autonomic nervous system and the hypothalamic–pituitary–adrenal axis.

1. Dogs with a sympathicotonic autonomic profile are characterized by the highest basal cortisol levels, the greatest amplitude of hormonal response, and delayed recovery, indicating increased reactivity and a potentially higher "cost of adaptation."

2. A normotonic ANS profile обеспечивает a more balanced and controlled hormonal response with a tendency toward faster cortisol normalization, whereas vagotonic dogs exhibit a lower intensity of the acute response but high individual variability and a more prolonged pattern of changes.

3. Two-factor ANOVA confirmed a significant effect of both the acoustic stressor and autonomic tone, as well as their interaction, on plasma cortisol levels.

4. Administration of microelement nanocompounds was associated with a tendency toward reduced amplitude of the hormonal stress response, particularly in sympathicotonic dogs, suggesting their potential as a promising means of modulating stress reactivity; however, further experimental confirmation with expanded sample sizes and inclusion of additional physiological markers is required.

References

Dickinson, S., & Feuerbacher, E. N. (2025). Frustration and its impact on search and rescue canines. *Frontiers in Veterinary Science*, 12, 1546412. https://doi.org/10.3389/FVETS.2025.1546412/BIBTEX

Flint, H. E., Weller, J. E., Parry-Howells, N., Ellerby, Z. W., McKay, S. L., & King, T. (2024). Evaluation of indicators of acute emotional states in dogs. *Scientific Reports*, 14(1), 6406. https://doi.org/10.1038/s41598-024-56859-9

Khavin, O., Bobrytska, O., & Sovik, K. (2025). Short−term heart rate variability in dogs with different temperament characteristics. *Theoretical and Applied Veterinary Medicine*, 13, 9–15. https://doi.org/10.32819/2025.13012

Kim, H. G., Cheon, E. J., Bai, D. S., Lee, Y. H., & Koo, B. H. (2018). Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. *Psychiatry Investigation*, 15(3), 235–245. https://doi.org/10.30773/PI.2017.08.17

Koskela, A., Törnqvist, H., Somppi, S., Tiira, K., Kykyri, V.-L., Hänninen, L., Kujala, J., Nagasawa, M., Kikusui, T., & Kujala, M. V. (2024). Behavioral and emotional co-modulation during dog–owner interaction measured by heart rate variability and activity. *Scientific Reports*, 14(1), 25201. https://doi.org/10.1038/s41598-024-76831-x

Mârza, S. M., Munteanu, C., Papuc, I., Radu, L., Diana, P., & Purdoiu, R. C. (2024). Behavioral, Physiological, and Pathological Approaches of Cortisol in Dogs. *Animals :* An Open Access Journal from MDPI, 14(23), 3536. https://doi.org/10.3390/ANI14233536

McEwen, B. S., & Akil, H. (2020). Revisiting the stress concept: Implications for affective disorders. *Journal of Neuroscience*, 40(1), 12–21. https://doi.org/10.1523/JNEUROSCI.0733-19.2019

Merino del Portillo, M., Clemente-Suárez, V. J., Ruisoto, P., Jimenez, M., Ramos-Campo, D. J., Beltran-Velasco, A. I., Martínez-Guardado, I., Rubio-Zarapuz, A., Navarro-Jiménez, E., & Tornero-Aguilera, J. F. (2024). Nutritional Modulation of the Gut–Brain Axis: A Comprehensive Review of *Dietary Interventions in Depression and Anxiety Management. Metabolites*, 14(10), 549. https://doi.org/10.3390/METABO14100549

Miller, J., Cavalli, C., Azadian, A., & Protopopova, A. (2025). Exploring the impact of a brief positive experience on dogs' performance and stress resilience during a learning task. *PLOS ONE*, 20(6), e0326368. https://doi.org/10.1371/JOURNAL.PONE.0326368

Nunez, S. G., Rabelo, S. P., Subotic, N., Caruso, J. W., & Knezevic, N. N. (2025). Chronic Stress and Autoimmunity: The Role of HPA Axis and Cortisol Dysregulation. *International Journal of Molecular Sciences*, 26(20), 9994. https://doi.org/10.3390/ijms26209994

Patani A, Balram D, Yadav VK, Lian K-Y, Patel A and Sahoo DK (2023) Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress. Front. *Endocrinol.* 14:1271521. https://doi.org/10.3389/fendo.2023.1271521

Roy, S., Fan, Y., Mosayebi-Samani, M., Claus, M., Mutlu, N., Kleinsorge, T., & Nitsche, M. A. (2025). Multimodal assessment of acute stress dynamics using an aversive video paradigm (AVP). *International Journal of Clinical and Health Psychology*, 25(3). https://doi.org/10.1016/J.IJCHP.2025.100607

Sahar, T., Shalev, A. Y., & Porges, S. W. (2001). Vagal modulation of responses to mental challenge in posttraumatic stress disorder. *Biological Psychiatry*, 49(7), 637–643. https://doi.org/https://doi.org/10.1016/S0006-3223(00)01045-3

Salgirli Demirbas, Y., Isparta, S., Saral, B., Keskin Yılmaz, N., Adıay, D., Matsui, H., Töre-Yargın, G., Musa, S. A., Atilgan, D., Öztürk, H., Kul, B. C., Şafak, C. E., Ocklenburg, S., & Güntürkün, O. (2023). Acute and chronic stress alter behavioral laterality in dogs. *Scientific Reports*, 13(1), 4092. https://doi.org/10.1038/s41598-023-31213-7

Sharan, P., Chauhan, A., Gupta, P., & Singh, V. (2024). Hypothalamic-Pituitary-Adrenal (HPA) Axis: Unveiling the Potential Mechanisms Involved in Stress-Induced Alzheimer's Disease and Depression. *Cureus* 16(8): e67595. https://doi.org/10.7759/cureus.67595

Somppi, S., Törnqvist, H., Koskela, A., Vehkaoja, A., Tiira, K., Väätäjä, H., Surakka, V., Vainio, O., & Kujala, M. V. (2022). Dog-Owner Relationship, Owner Interpretations and Dog Personality Are Connected with the Emotional Reactivity of Dogs. *Animals :* An Open Access Journal from MDPI, 12(11). https://doi.org/10.3390/ani12111338

Teo, J. T., Johnstone, S. J., Römer, S. S., & Thomas, S. J. (2022). Psychophysiological mechanisms underlying the potential health benefits of human-dog interactions: A systematic literature review. *International Journal of Psychophysiology*, 180, 27–48. https://doi.org/10.1016/J.IJPSYCHO.2022.07.007

Tóth, A., & Dobolyi, Á. (2025). Prolactin in sleep and EEG regulation: New mechanisms and sleep-related brain targets complement classical data. *Neuroscience & Biobehavioral Reviews*, 169, 106000. https://doi.org/https://doi.org/10.1016/j.neubiorev.2024.106000

von Majewski, K., Kraus, O., Rhein, C., Lieb, M., Erim, Y., & Rohleder, N. (2023). Acute stress responses of autonomous nervous system, HPA axis, and inflammatory system in posttraumatic stress disorder. *Translational Psychiatry*, 13(1), 36. https://doi.org/10.1038/s41398-023-02331-7

Wehrwein, E. A., Orer, H. S., & Barman, S. M. (2016). Overview of the Anatomy, Physiology, and Pharmacology of the Autonomic Nervous System. *Comprehensive Physiology*, 6(3), 1239–1278. https://doi.org/10.1002/J.2040-4603.2016.TB00714.X