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Research Article

Influence of dog-appeasing pheromone on dogs during training

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ABSTRACT

The welfare of working dogs during training is crucial for their health, learning capacity, and long-term performance. This study investigated the efficacy of the Dog Appeasing Pheromone (DAP) in mitigating acute stress responses in Belgian Malinois during a structured 7-day basic obedience training protocol. Thirty-three dogs were randomly allocated to three groups: Group 1 (control, no training or DAP), Group 2 (training without DAP), and Group 3 (training with DAP). Physiological indicators (heart rate, body temperature, salivary cortisol) and behavioral stress markers (ethogram-coded postures) were recorded daily. Dogs in Group 2 exhibited significantly elevated stress responses across all parameters compared to Groups 1 and 3 ($P < 0.001$). Group 3, treated with DAP, showed physiological and behavioral measures not statistically different from the control group, indicating effective stress mitigation. Specific stress behaviors—such as ear retraction, lowered head, and semi-low posture—were markedly reduced in DAP-treated dogs. Strong positive correlations were observed between physiological and behavioral indicators (e.g., cortisol and body posture: $r=0.503$; cortisol and tail position: $r=0.744$), supporting the reliability of behavioral observations in stress assessment. This is the first study to integrate behavioral and physiological metrics to demonstrate the stress-buffering effect of DAP during active training. Given its non-invasive and non-sedative nature, DAP represents a promising tool for improving welfare in working dogs without compromising performance. Incorporating DAP into training protocols may support ethical handling practices and enhance the well-being and functional success of dogs in demanding service roles.

Keywords: Dog behavior, Dog training, Pheromone, Psychophysiology, Stress.

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Introduction

Working dogs trained for critical tasks undergo rigorous processes to ensure reliable performance under diverse and often unpredictable conditions. Both housing conditions (55) and training methods (44) can act as potential stressors during this period. Stress affects attention, memory formation, and recall. According to the Yerkes–Dodson law (54, 61), moderate stress enhances learning, whereas low or excessive stress impairs it.

Selye (47) introduced the term "stress" and emphasized the role of the hypothalamic–pituitary–adrenal (HPA) axis in its physiological regulation. Animals exposed to unfavorable conditions display both behavioral and physiological signs of stress (28). Stress, defined as the requirement to adapt to environmental challenges (18, 32), is one of the main causes of welfare impairment; it reduces productivity, impairs immunity, and shortens lifespan (35). Acute stress in dogs triggers sympathetic activation and HPA axis engagement (3), observable through changes in heart rate, respiratory rate, and stress-related postures (5).

Working dogs, including detection, rescue, and patrol dogs, are exposed to unique environmental and social stressors during training. These include separation from familiar handlers, exposure to novel scents and unpredictable noises, high physical exertion, and demanding problem-solving tasks (24). The Demographic variables of pet owners, such as age, gender, and

education, significantly influence ownership and care behaviours, highlighting that owner-related factors may indirectly shape canine welfare outcomes (57). The way owners and handlers interact with their dogs—including their consistency, emotional tone, and responsiveness—also contributes significantly to how dogs perceive and cope with these stressors (40).

Additionally, the need to maintain accuracy and focus in unfamiliar or distracting environments challenges their emotional regulation and cognitive control (48). Repeated exposure to such conditions may cause cumulative arousal, which, if unmanaged, can affect both welfare and performance (10). Understanding these specific stressors is crucial for developing humane, effective training strategies and assessing interventions such as Dog Appeasing Pheromone (DAP) that may modulate these stress responses (43, 50).

Cannon's "fight or flight" theory (9) describes acute stress responses as increased sympathetic activity and reduced parasympathetic activity, resulting in higher heart rate and blood pressure (36, 42). The HPA axis plays a central role in mediating these processes (26, 39). Cortisol, the dominant glucocorticoid in dogs (62), supports energy mobilization, but chronic exposure can impair hippocampal function and learning (23). Salivary cortisol closely reflects plasma concentrations (6, 30), providing a reliable, non-invasive biomarker of stress (58).

Behavioral observation remains a key non-invasive tool for assessing an animal's emotional state (12). Proactive dogs tend to respond directly to stimuli, whereas reactive dogs show cautious withdrawal (59). Postural and facial cues—such as tail, ear, and mouth positions—offer rapid insight into stress levels (5, 13). A hunched or lowered posture often indicates acute stress and correlates with elevated cortisol (3).

During police and working dog training, stress-related behaviors are of particular concern because excessive stress can impair learning, attention, and handler–dog communication. Feddersen-Petersen and Ohl (16) described the neutral body posture of a relaxed dog as having the body parallel to the ground, straight legs, the head slightly raised, forming a natural angle with the neck and muzzle, the tail hanging loosely, relaxed facial muscles, non-tense lips, and ears aligned with the nose, while the eyes blink softly and in a coordinated manner. In contrast, Schwizgebel (45) reported that harsh or overly demanding training methods lead to lower, crouched postures and more hunched sitting postures, reflecting tension and emotional discomfort. Friend (19) further described behavioral signs of acute fear or pain in dogs as making the body appear smaller, intense vocalizations, escape behaviors, or defensive aggression when avoidance is impossible. Dogs may avert their gaze or turn their heads away from the stressor, frequently defecate, or overreact to neutral stimuli. Beerda et al. (3) experimentally exposed dogs of different breeds to six types of acute stressors and found that a very low body posture is a clear indicator of severe acute stress, accompanied by elevated salivary cortisol concentrations, while a moderately lowered posture is associated with milder stress. These findings emphasize that during police dog training—where animals are frequently exposed to unfamiliar environments, sudden noises, and challenging problem-solving tasks—such postural and behavioral changes should be carefully monitored, as they indicate emotional overload that can interfere with learning efficiency and welfare.

Recent evidence has clarified that the behavioral effects of DAP is mediated through semiochemical communication within the olfactory system. DAP molecules are primarily detected by the vomeronasal organ (Jacobson's organ), which contains chemosensory neurons expressing pheromone-specific receptors. Upon binding, these receptors initiate a cascade of molecular and electrical signals transmitted to the accessory olfactory bulb and subsequently to the medial amygdala and hypothalamic nuclei—regions responsible for emotional processing and the regulation of social behavior (25, 53). Through this neuroendocrine pathway, DAP modulates stress-related hormonal activity, promoting calmness and emotional stability in dogs.

DAP, secreted by lactating females, conveys reassuring chemical cues, detected via the vomeronasal organ, and fosters calmness and social attachment in early life, thereby contributing to the maintenance of emotional stability in adulthood (34, 41, 46). Synthetic analogs of DAP have demonstrated meaningful anxiolytic and autonomic regulatory effects in diverse contexts. Frank et al. (17) systematically reviewed pheromone-based interventions and reported consistent reductions in fear- and anxiety-related behaviors. Hermiston et al. (27) observed that DAP spray effectively reduced vocalization and stress postures in shelter dogs, while Puglisi et al. (43) showed that a novel DAP gel formulation attenuated both behavioral and physiological stress markers during veterinary examinations. Similarly, Minuzzo et al. (38) described cardiovascular modulation indicative of improved autonomic balance in hospital environments, supporting the view that DAP can influence stress resilience through olfactory–limbic interactions.

However, findings across studies are not entirely consistent. Nevertheless, systematic reviews (17) have debated the overall robustness of pheromone effects due to methodological variability, small sample sizes, and inconsistent behavioral endpoints across trials. Such variability underscores the need for context-specific

interpretations rather than assuming uniform efficacy. Some investigations have reported only mild or context-dependent effects, likely due to differences in exposure duration, environmental control, or individual temperament (27, 37, 52). These studies noted that while DAP collars reduced certain anxiety-related behaviors in newly homed rescue dogs, effects were not uniform across individuals. Similarly, Kim et al. (29) and Tod et al. (55) observed that DAP's efficacy was greater under acute stress conditions but was limited in long-term kennelled environments. These results suggest that DAP may not completely suppress stress responses but rather enhance emotional resilience and facilitate faster recovery following stressful events.

In working dogs, sustained emotional control and behavioral focus are crucial for performance. DAP is particularly promising in this context because it promotes calmness without impairing cognitive function, thereby supporting attention, learning, and problem-solving capacity under challenging or unpredictable circumstances. The collar formulation ensures continuous pheromone release and mobile exposure, making it well-suited to dynamic field and training environments (33, 34).

Compared with diffusers and sprays, which provide short-term or localized exposure, collars maintain a stable pheromone concentration for several weeks and are not dependent on environmental conditions such as airflow or spatial confinement (33, 34). This continuous release pattern offers practical advantages for working or training dogs that frequently move between environments.

Although DAP has been extensively investigated in conditions such as separation anxiety, noise phobia, and travel-related stress (37, 52, 55), its effects on acute stress responses elicited during structured training sessions remain unclear. The present study therefore aimed to evaluate the role of DAP in reducing training-induced acute stress, addressing a recognized gap in the literature and offering practical implications for the welfare and training efficiency of working dogs.

Materials and Methods

Study Material: Thirty-three Belgian Malinois dogs (6 months–3 years old) enrolled in training programs for narcotics, tea, and tobacco detection were included. Based on a power analysis using G*Power ($\alpha = 0.05$, power = 0.80, effect size f = 0.5), dogs were randomly assigned to three equal groups (n = 11 per group). All dogs underwent veterinary health screening. Dogs were randomly assigned to three experimental groups: a control group (Group 1; n = 11; 5 females and 6 males), a training group without pheromone exposure (Group 2; n = 11; 5 females and 6 males), and a training group fitted with an Adaptil® pheromone collar (Group 3; n = 11; 5 females and 6 males). Handlers monitored the dogs throughout the study to ensure that the pheromone collars remained intact, and bathing as well as exposure to rain during outdoor activities were avoided in Group 3 to prevent potential interference with pheromone release.

Study Location: All procedures were conducted at the Dog Training Center (General Directorate of Customs Enforcement, Ministry of Trade, Ankara). Salivary cortisol analyses were performed at Algen Diagnostik Laboratory.

Habituation: All dogs in this study were professionally selected adult Belgian Malinois detection dogs with comparable training backgrounds and high adaptability to environmental conditions. Because drive level, motivation, and resilience to novel environments were key selection criteria during detector-dog qualification tests, habituation demands were inherently low and did not differ by age.

The same stress-prevention procedures—systematic desensitization and counter-conditioning, progressively introducing sampling stimuli with positive reinforcement—were applied consistently to all dogs to ensure standardized exposure and minimize stress throughout data collection .

The desensitization protocol was stepwise: dogs were first exposed to the testing area and allowed to explore freely. Subsequently, the sampling equipment (Salivette tubes) was presented visually to the dog, and when no stress signals appeared, the dog was rewarded with sausage. In later sessions, the tube was offered for olfactory inspection, then gently touched to the mouth, and finally held in the mouth for one minute to collect saliva. At each stage, positive reinforcement was used; if stress-related body language was observed, the process was paused and restarted at the previous step (7).

This desensitization process was completed before the formal training protocol began and was maintained until each dog demonstrated behavioral indicators of being fully desensitized to sample collection procedures. Counter-conditioning was incorporated at every incremental exposure step to ensure a positive conditioned emotional response prior to progression. This structured approach ensured consistent habituation, minimized anticipatory stress, and maintained positive associations with handling in all dogs, regardless of age.

Training Procedure: Groups 2 and 3 received 15-minute basic obedience sessions daily for seven days (commands: sit, stay, watch, turn). Chicken-based treats were used for positive reinforcement. Group 1 was placed in the training area for the same duration but did not receive training.

Although Group 1 did not undergo active training, the placement of dogs in the training area itself may have elicited mild arousal or novelty-related stress. Therefore, this condition should be regarded as a low-stimulation control rather than a complete resting state.

Examined Traits: Heart rate, body temperature, and salivary cortisol were measured. Baseline values were recorded after habituation. Heart rate was auscultated over the left pericardial area. Before data collection, the dogs' ear canals were examined for signs of inflammation. Temperature was measured using a Braun ThermoScan IRT 4520 with disposable probes.

Saliva samples were collected 15 minutes after training using Salivette® collection tubes which contains citric acid and no external oral stimulants were administered to the dogs, ensuring a non-invasive and stress-free sampling procedure by researcher. Samples were stored at –20 °C until analysis. Cortisol concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (DiaMetra Cortisol Saliva ELISA, DCM020-9) at Algen Diagnostik Laboratory in Ankara. The sampling and analysis procedures followed established protocols for canine stress assessment, as salivary cortisol reliably reflects plasma cortisol levels and provides a validated, non-invasive indicator of hypothalamic–pituitary–adrenal (HPA) axis activation (3, 15).

Training sessions were video-recorded. Instantaneous sampling (1, 4) at 15-second intervals, based on ethograms (44), was used to score stress behaviors. The study was conducted as a single-blind trial, with the handlers and observers aware of group assignments. To minimize observer bias, physiological stress parameters (heart rate, salivary cortisol, and body temperature) were included alongside behavioral data, ensuring the objectivity and comparability of the results. Complete blinding was not feasible because the DAP collars were visually distinguishable. Although the observers followed a standardized ethogram and scoring system to reduce subjective bias, the potential influence of observer awareness and handler expectations on behavioral scoring was acknowledged. Future studies should adopt a full double-blind design and use independent video analysis to minimize such bias.

Statistical Analysis: Normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively. Because data did not meet parametric assumptions, non-parametric tests were applied. Group differences were analyzed using the Kruskal–Wallis test, followed, where appropriate, by Bonferroni-adjusted pairwise Mann–Whitney U post hoc comparisons. Effect sizes — η^2 for Kruskal–Wallis tests and r for pairwise comparisons — were calculated following Tomczak and Tomczak (56) and interpreted according to Cohen (11): $\eta^2 < 0.06$, small; 0.06–0.14, moderate; > 0.14, large; $r < 0.10$, small; 0.10–0.29, weak; 0.30–0.49, moderate; ≥ 0.50, strong. This approach allows statistical significance to be complemented by the biological relevance of the findings. Correlations among physiological and behavioral stress parameters were evaluated using Spearman's rank correlation. All analyses were performed in SPSS 14.01, with the significance threshold set at $P < 0.05$.

Results

The 7-day mean change in heart rate (post-training minus baseline) differed significantly among groups ($P < 0.001$; $\eta^2 = 0.50$, large effect) (Figure 1, Table 1). The Collar-free Group showed the highest heart rates, while Group 1 and Group 3 did not differ significantly, indicating a comparable stress response.

Post-training temperature was significantly higher in the collar-free group ($P = 0.001$; $\eta^2 = 0.47$), indicating a large effect. Group 3 values were statistically similar to controls (Group 1), indicating a DAP-related mitigation of thermal stress (Figure 2, Table 2). Cortisol levels significantly differed among groups during training ($P < 0.001$; $\eta^2 = 0.27$, moderate–large effect) (Figure 3, Table 3). DAP-treated dogs (Group 3) exhibited cortisol responses comparable to those of controls, suggesting reduced physiological stress. DAP-treated dogs (Group 3) displayed significantly fewer stress-related behaviors than dogs in Group 2 and were similar to controls. Significant group effects were observed for several behavioral variables (Table 4). Specifically, the corner of the mouth pulled back showed a very large effect ($P < 0.001$; $\eta^2 = 0.73$), while head lowered demonstrated a moderate to large effect ($P < 0.01$; $\eta^2 = 0.36$). Similarly, ears back was associated with a large effect size ($P < 0.001$; $\eta^2 = 0.69$), and tail half-low exhibited a very large effect ($P < 0.001$; $\eta^2 = 0.80$). The semi-low posture also showed a very large effect ($P < 0.001$; $\eta^2 = 0.83$). Overall, effect sizes (η^2) for the behavioral parameters ranged from 0.36 to 0.83.

A strong positive correlation was observed between the mean change in heart rate and "corner of mouth pulled back" ($r = 0.737$, $P < 0.001$). Heart rate was also weakly correlated with "head lowered" ($r = 0.395$, $P < 0.05$), moderately correlated with "ears back" ($r = 0.629$, $P < 0.001$), and strongly correlated with "tail half-low" ($r = 0.726$, $P < 0.001$). A moderate positive correlation was found between heart rate and "semi-low posture" ($r = 0.599$, $P < 0.001$) (Table 5). Interpretations of correlation strength were standardized according to Cohen (11), ensuring consistent and accurate descriptions of relationships.

Table 1. Comparison of 7-day mean heart rate changes (bpm) before and after training among groups.

	Group	n	Mean	SE	SD	Median	Min	Max	Range	P	η²	letter
ΔHeart Rate (bpm)	G 1	11	4.21	0.31	1.03	4.00	2.86	6.86	4.00	0.001	0.50	b
	G 2	11	9.97	0.66	2.19	9.71	5.71	14.29	8.58			a
	G 3	11	5.97	1.10	3.65	4.00	3.43	16.00	12.57			b

Table 2. Mean body temperature differences (°C) before and after training among groups over 7 days.

	Group	n	Mean	SE	SD	Median	Min	Max	Range	P	η²	letter
ΔBody Temperature (°C)	G 1	11	0.26	0.08	0.26	0.19	0.04	0.84	0.80	0.001	0.47	b
	G 2	11	0.70	0.06	0.19	0.67	0.46	1.04	0.58			a
	G 3	11	0.31	0.06	0.20	0.26	0.11	0.71	0.60			b

Table 3. Salivary cortisol level changes (µg/dL) before and after training among groups.

	Group	n	Mean	SE	SD	Median	Min	Max	Range	P	η²	letter
ΔSalivary Cortisol (µg/cl)	G 1	11	0.94	0.19	0.64	0.93	0.25	1.90	1.65		0.27	b
	G 2	11	6.26	2.23	7.39	3.58	1.54	26.68	25.14	<0.001		a
	G 3	11	0.99	0.10	0.35	0.99	0.35	1.51	1.16			b

Table 4. Mean frequency of stress-related behavioral indicators observed during training sessions (n=7 days).

	Group	n	Mean	SE	SD	Median	Min	Max	P	η²	letter
Corner of the mouth back	Grup 1	11	2.82	0.26	0.87	3.00	2.00	4.00		0.73	b
	Grup 2	11	8.73	0.86	2.87	8.00	7.00	17.00	<0.001		a
	Grup 3	11	2.55	0.31	1.04	3.00	1.00	4.00			b
Head is lowered	Grup 1	11	0.09	0.09	0.30	0.00	0.00	1.00	0.003	0.36	b
	Grup 2	11	0.82	0.18	0.60	1.00	0.00	2.00			a
	Grup 3	11	0.18	0.12	0.40	0.00	0.00	1.00			b
Ears are back	Grup 1	11	2.00	0.27	0.89	2.00	1.00	4.00	<0.001	0.69	b
	Grup 2	11	7.09	0.85	2.81	7.00	3.00	14.00			a
	Grup 3	11	1.27	0.33	1.10	1.00	0.00	3.00			b
Tail is half low	Grup 1	11	2.91	0.21	0.70	3.00	2.00	4.00	<0.001	0.80	b
	Grup 2	11	10.55	0.91	3.01	10.00	8.00	19.00			a
	Grup 3	11	3.27	0.27	0.90	3.00	2.00	5.00			b
Semi-low posture	Grup 1	11	2.09	0.28	0.94	2.00	1.00	3.00	<0.001	0.83	b
	Grup 2	11	8.55	0.68	2.25	9.00	4.00	11.00			a
	Grup 3	11	1.45	0.31	1.04	1.00	0.00	3.00			b

Table 5. Spearman correlation coefficients between physiological stress indicators (heart rate, body temperature, salivary cortisol) and stress-related behaviors in dogs (n=33).

		Heart Rate		Body Temperature		Salivary Cortisol
Corner of the mouth back	r	0.737	r	0.663	r	0.670
	P	<0.001	P	<0.001	P	<0.001
	n	33	n	33	n	33
Head is lowered	r	0.395	r	0.345	r	0.332
	P	<0.05	P	<0.05	P	>0.05
	n	33	n	33	n	33
Ears are back	r	0.629	r	0.483	r	0.412
	P	<0.001	P	<0.005	P	<0.05
	n	33	n	33	n	33
Tail is half low	r	0.726	r	0.590	r	0.744
	P	<0.001	P	<0.001	P	<0.001
	n	33	n	33	n	33
Semi-low posture	r	0.599	r	0.578	r	0.503
	P	<0.001	P	<0.001	P	<0.005
	n	33	n	33	n	33

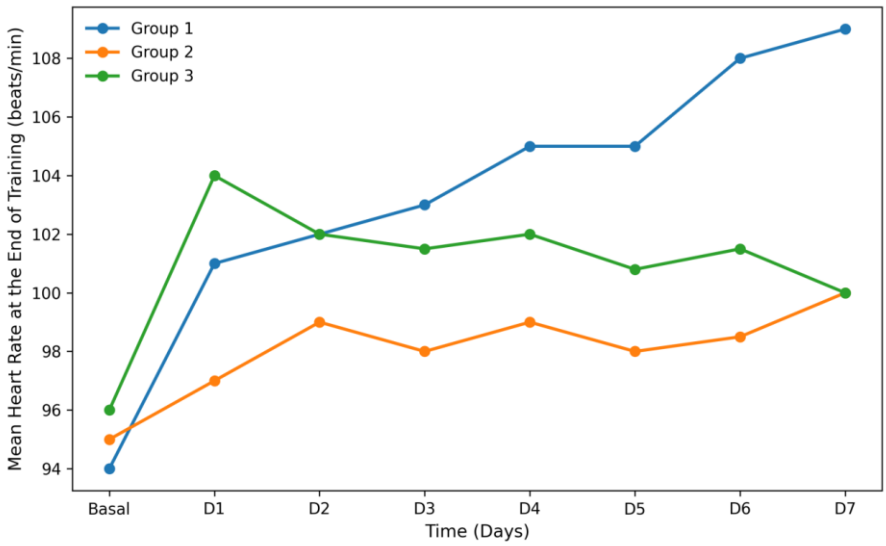


Figure 1. Mean heart rate (bpm) after training in three experimental groups over 7 days (mean ± SD).

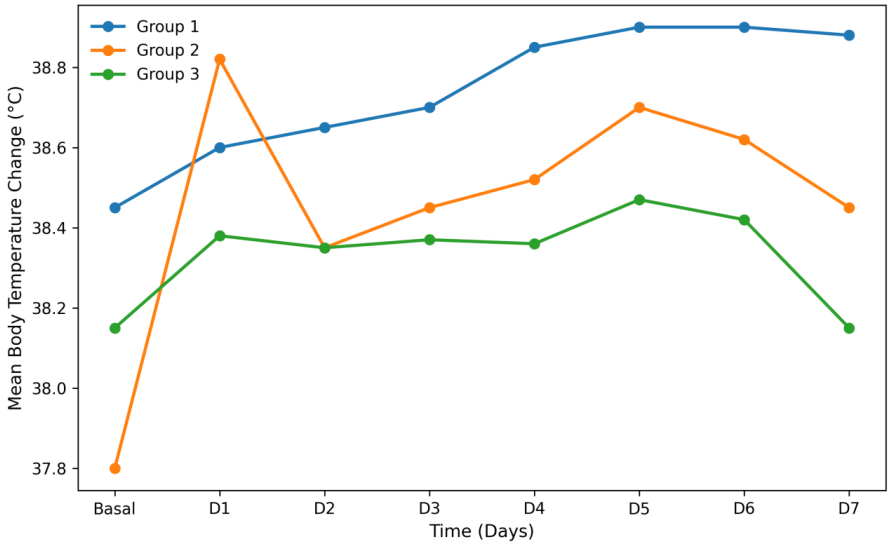


Figure 2. Mean body temperature (^{0}C) after training in three experimental groups over 7 days (mean ± SD).

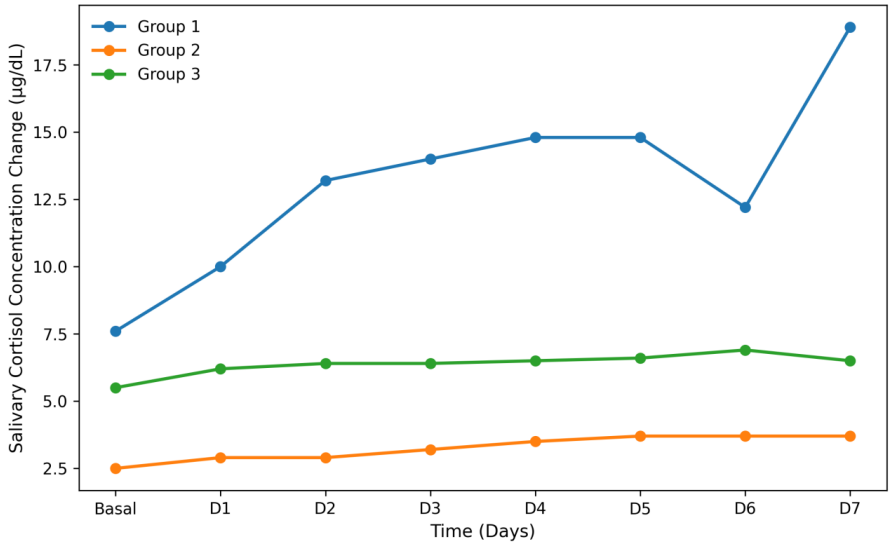


Figure 3. Salivary cortisol concentration (µg/dL) after training in three experimental groups over 7 days (mean ± SD).

Discussion and Conclusion

Stress-induced physiological adjustments are primarily mediated through activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis (3). In the present study, dogs exposed to DAP (Group 3) exhibited smaller post-training increases in heart rate compared with untreated training groups, suggesting reduced sympathetic activation and faster autonomic recovery. This finding is consistent with previous reports describing heart rate elevation as a reliable indicator of acute stress (3, 8). The attenuation of this response under DAP exposure indicates that pheromonal communication may support autonomic stability, allowing dogs to remain alert yet less physiologically over-aroused during training. Reported baseline heart rate values vary among breeds (21, 22), underscoring the importance of breed-specific reference ranges in interpreting stress reactivity. Similarly, body temperature increases were markedly lower in DAP-treated dogs, aligning with evidence that hyperthermia accompanies acute stress responses (51, 60). From a physiological perspective, increased body temperature during training may arise from both muscular thermogenesis due to physical exertion and sympathetic activation resulting from psychological stress. The reduced temperature rise in DAP-treated dogs therefore suggests that pheromone exposure may specifically attenuate stress-induced hyperthermia rather than suppressing normal thermoregulatory responses to exercise. These results collectively suggest that DAP modulates autonomic activity, maintaining physiological balance under training-induced stress without suppressing necessary arousal. Unlike earlier studies that examined stress in passive or environmental contexts, this study demonstrates DAP's modulatory effect during actively performed tasks, extending its relevance to training-associated stress.. Unlike earlier studies that examined stress in passive or environmental contexts, this study demonstrates DAP's modulatory effect during actively performed tasks, extending its relevance to training-associated stress.

Salivary cortisol is a key hormonal indicator of acute stress, reflecting activation of the hypothalamic–pituitary–adrenal (HPA) axis (3, 24). In this study, salivary cortisol levels in DAP-treated dogs remained comparable to those of untrained controls, suggesting that pheromone exposure attenuated the endocrine stress response during training. Similar patterns have been reported in previous studies, in which pheromone treatment or reduced environmental stress was associated with lower cortisol secretion and calmer behavioral states (24, 50). Saliva saliva sampling, as validated in earlier research (2, 31), provided a non-invasive and reliable measure of physiological stress reactivity. In this study, saliva was collected using Salivette® devices containing citric-acid–impregnated cotton. Although citric acid was not applied directly to the oral cavity, this embedded stimulant facilitates collection of a sufficient sample volume for ELISA. Because citric acid can lower salivary pH and artificially elevate cortisol concentrations (15), using the same tube type for all dogs minimized systematic bias. Taken together, these findings indicate that DAP may help regulate HPA axis activation under training conditions, moderating cortisol output while preserving normal adaptive responses essential for learning and focus However, unlike in those studies, cortisol changes in the present study were observed under controlled training stress, suggesting that DAP can mitigate physiological activation even during performance-related challenges.

Non-DAP-trained dogs exhibited more stress-related postures, such as lowered ears, tucked tails, and crouched body positions—behaviors well-documented as markers of acute emotional tension in dogs (14). In contrast, DAP-treated dogs maintained more neutral or relaxed postures, comparable to those of untrained controls, indicating improved emotional regulation under training-related pressure. These behavioral patterns suggest that pheromonal exposure enhances coping capacity and social confidence, mitigating fear-related responses without diminishing attentiveness or motivation. Collectively, the alignment between behavioral and physiological results reinforces DAP's potential role in stabilizing arousal and supporting welfare during obedience training The replication of these behavioral patterns in a structured training context confirms the ethogram's reliability across environments and supports DAP's ability to maintain behavioral stability under task-related stress.

The strong positive associations observed between physiological parameters and behavioral indicators highlight the interconnected nature of stress responses. For example, the close relationship between tail position, heart rate, and cortisol concentrations suggests that both autonomic and endocrine components of stress are reflected in observable postural cues. Such integration between physiological and behavioral data supports a holistic assessment of welfare and emotional state, consistent with the framework proposed by Beerda et al. (3). These correlations confirm that non-invasive behavioral observations can serve as reliable, real-time proxies for underlying physiological arousal, providing valuable insight for evaluating stress and welfare in working dogs.

Overall, DAP appears to effectively reduce acute stress in working dogs undergoing obedience training. These findings align with previous studies demonstrating the anxiolytic effects of DAP in dogs exposed to separation, fireworks, transport, or shelter environments (20, 34, 41, 49). However, unlike prior work, this study is among the first to investigate DAP's impact during active training with controlled stress induction. The close similarity of stress levels between DAP-treated and

untrained control dogs suggests that DAP can moderate the physiological stress load without affecting responsiveness or learning opportunities. These results imply that by stabilizing physiological arousal, DAP may indirectly enhance learning efficiency and attention during training, thereby supporting both welfare and performance outcomes in working dogs.

The observed correlations between behavioral indicators (e.g., "tail half-low" posture) and physiological parameters (e.g., cortisol) support the validity of noninvasive behavioral assessments as reliable welfare indicators. This reinforces the importance of integrating ethological tools into working dog welfare monitoring protocols.

While the sample size was adequate, the study lacked a double-blind design, a limitation that should be addressed in future research. The study population was intentionally limited to Belgian Malinois dogs to reduce inter-breed variability in physiological and behavioral stress responses. Previous research has shown that baseline body temperature, resting heart rate, and salivary cortisol concentrations vary significantly among dog breeds, reflecting differences in autonomic tone and hypothalamic–pituitary–adrenal (HPA) axis activity (3, 24, 31). Smaller or more reactive breeds often demonstrate higher sympathetic activity and cortisol reactivity compared to larger working breeds, which tend to show lower basal values and more stable thermoregulatory responses (15, 26). Such breed-dependent differences could confound interpretation of stress-related outcomes and obscure the specific effects of DAP. Therefore, using a single, behaviorally homogeneous working breed was considered essential to control physiological variation and ensure internal validity. Nevertheless, we acknowledge that this choice limits the generalizability of the findings, and we recommend that future studies include multiple breeds and training intensities to improve generalizability.

This study demonstrated that exposure to DAP significantly reduced physiological and behavioral indicators of acute stress in Belgian Malinois dogs during basic obedience training. Dogs treated with DAP showed minute changes in heart rate, body temperature, and salivary cortisol that approached those observed in unstressed control animals, indicating partial rather than complete normalization of stress responses. These results imply that by stabilizing physiological arousal, DAP may indirectly enhance learning efficiency and focus during training, supporting both welfare and performance outcomes in working dogs. From a practical standpoint, incorporating DAP collars into training protocols may help trainers and handlers maintain dogs' emotional stability and attention during demanding tasks. Future studies should explore long-term effects, breed-specific responses, and placebo-controlled designs to strengthen causal interpretation and assess DAP's role under real operational conditions.

Strong correlations between physiological and behavioral parameters confirmed the reliability of using both in parallel for stress assessment. The findings highlight the utility of DAP as a welfare-enhancing intervention in training programs for working dogs. From a practical standpoint, incorporating DAP collars into training protocols may assist trainers and handlers in maintaining dogs' emotional stability and attention during demanding tasks. Future studies should explore long-term effects, breed-specific responses, and placebo-controlled designs to strengthen causal interpretation and assess DAP's role under real operational conditions. Incorporating DAP into training routines may enhance performance and welfare, particularly in demanding roles such as detection work, by minimizing stress-related disruptions.

This study demonstrated that exposure to DAP significantly reduced physiological and behavioral indicators of acute stress in Belgian Malinois dogs during basic obedience training. These results imply that by stabilizing physiological arousal, DAP may indirectly enhance learning efficiency and focus during training, supporting both welfare and performance outcomes in working dogs. From a practical standpoint, incorporating DAP collars into training protocols may assist trainers and handlers in maintaining dogs' emotional stability and attention during demanding tasks. However, the study did not include a placebo collar group, which limits the ability to fully exclude potential placebo-related behavioral effects. Previous research has indicated that, under stress, younger dogs tend to exhibit higher locomotor activity (e.g., pacing) and increased panting, whereas older dogs may show more stable postural responses and lower reactivity (24). These age-related behavioral variations suggest that training programs should be adapted to individual stress sensitivity and coping capacity. In the present study, using professionally trained adult Belgian Malinois effectively minimized potential age-related variability, because all dogs had comparable habituation and learning experiences, ensuring uniform stress responsiveness across subjects. Nevertheless, future studies could investigate age-dependent physiological and behavioral responses to stress to determine whether the modulatory effects of DAP differ across developmental or aging stages. Future research should explore long-term outcomes, breed-specific responses, and placebo-controlled designs to strengthen causal inference and evaluate DAP's applicability under real operational conditions.

Overall, these findings suggest that DAP represents a promising, welfare-oriented adjunct in working-dog training, capable of reducing acute stress without impairing focus or task performance.

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Ethical Statement

This study was approved by Ankara University Local Ethics Committee (Protocol No: 2014-16-96).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

CEŞ and NS conceived and planned the experiments. CEŞ carried out the experiments. CEŞ and NS contributed to the interpretation of the results. CEŞ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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