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Stress Biomarkers in Companion Animals: An Integrative Review

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Abstract: Stress in companion animals is a multifaceted physiological and behavioural response driven primarily by activation of the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic–adrenal–medullary (SAM) system, often resulting in compromised welfare, disrupted homeostasis, reduced fitness, and impaired alignment with owner and societal expectations. This review provides current knowledge on recognising different signs of stress and diverse biomarkers used to assess acute and chronic stress in companion animals—highlighting long-term endocrine measures such as cortisol accumulation in hair and nails during dog maternity, neuromodulators including prolactin and serotonin for evaluating stress-associated behavioural and lifestyle influences, oxytocin linked to sociality despite lower circulating levels in assistance dogs, metabolic indicators from neonatal blood gas changes reflecting intrapartum hypoxia or asphyxia risk, non-invasive skin temperature shifts measured via infrared thermography, facial expression–based tools like FACS adaptations and Grimace/Grimace-type scales for emotional and welfare evaluation, and oxidative-stress biomarker panels combined with enzymatic and non-enzymatic antioxidant defences (SOD, CAT, GPx, TAC, GSH, vitamins, trace-element ratios, and oxidative damage products such as MDA, 8-OHdG, protein carbonyls, isoprostanes, and myeloperoxidase). Evidence across studies remains heterogeneous due to influences of diet, age, sex, breed, lifestyle, environment, and pre-analytical sampling variability, reinforcing that no single biomarker can comprehensively define stress across contexts, and that integrative multi-biomarker frameworks, standardized sampling protocols, and longitudinal validation are critical to improve diagnostic reliability and advance clinical and welfare-centric stress assessment in dogs and other companion species.

Keywords: Stress, Companion animals, Biomarkers, Animal welfare.

Introduction

Animal stress is defined as an environmental effect on an individual that overtaxes its control systems, leading to adverse consequences and reduced fitness. It involves physiological responses that occur when an animal must adapt to changes to maintain homeostasis, primarily driven by the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis.

Stress experienced by companion animals could impair their physical and psychological welfare, impacting their social relationships in domestic environments [1].

Stress in companion animals (such as dogs, cats, and small mammals) is a physiological and behavioural response to environmental, physical, or emotional challenges that exceed the animal's ability to cope. It can be caused by various factors including separation from owners, loud noises, unfamiliar environments, poor handling, illness, or lack of social interaction.

In general, stress can be strictly detrimental to the emotional as well as physical wellbeing of the animal. More particularly, in companion animals (dogs and cats), this welfare infringements can produce considerable constraints on the affected animal's behaviour and its ability to behave in a way that is consistent with an owner's. and. Such failures results in hurting owners as well as the general public's expectations.

Biomarkers of stress are measurable physiological or biochemical indicators that reflect an animal's response to stressors. In companion animals like dogs and cats, these biomarkers help assess both acute and chronic stress and are useful in clinical, behavioural, and welfare evaluations. Therefore, identifying reliable biomarkers which are quantifiable indicators of biological processes [2] that has long been a key objective in veterinary behavioural medicine and animal welfare.

This review article This article provides information on different biomarkers that that can be measured to indicate normal biological functions, pathological processes, or biological responses to various exposures or interventions, including therapeutic treatments [3].

Recognising signs of stress in companion animals:

Animals express stress through exhibiting various behavioural, physiological, and physical signs. However, these signs vary depending on the species, type of stressor, and duration of exposure. Some of the important recognising signs and symptoms of stress in companion animals, more specifically in dogs [4] are given below.

Well -known signs of stress encountered in dogs (From Hargrave, 2015)

1. Turning body away
2. Walking away from stressor
3. Yawing
4. Blinking
5. Nose licking
6. Staring
7. Stiffening
8. Tail lowered or tucked
9. Scratching body
10. Full body shake
11. Ignoring owner requests for cooperation or responding in a distracted manner
12. Shaking as though following a bath or swim
13. Showing the whites of eyes or closing eyes
14. Raised hackles
15. Scanning visual field by rapidly looking from side to side
16. Muscle tension
17. Trembling
18. Inappetence for treats or snatching treats
19. Involuntary urination or defecation
20. Mounting behaviour
21. Lunging
22. Barking
23. Growling
24. Snapping
25. Biting

Subtle- signs of stress encountered in dogs (From Hargrave, 2015)

1. Sitting close to owners
2. Pawing of owners
3. Turning head away from stressor
4. Staring at an item (e.g. the second hand on a clock or a spot on a wall)
5. Raised fore leg
6. Lying down
7. Ears back and/or flattened
8. Creeping — slow deliberate movement

9. Body arching away
10. Rolling over to expose undercarriage
11. Sniffing ground or engaging in a seemingly meaningless displacement behaviour
12. Shedding hair
13. Higher tail carriage, tail vibrating in short, sharp bursts
14. Crouching
15. Increased sensitivity to other stimuli
16. Sweaty paws
17. Moving in slow motion
18. Panting
19. Excessive salivation
20. Mounting behaviour Biting/chewing of lead or owner's hands/clothing
21. Furrowed brow/raised eyebrows
22. Urine marking
23. Tightened muzzle
24. Appearing sleepy/tired
25. Ducking, cowering or backing away to avoid contact from hands

Biomarkers of Stress in Companion Animals:

These biomarkers are crucial for understanding animal welfare, behaviour, and health, especially in clinical, shelter, training, and research settings

1. **Possible Biomarkers of Stress during Maternity of Dogs**

   Cortisol, dehydroepiandrosterone (DHEA), and its form *dehydroepiandrosterone sulfate (DHEA-S)* are hormones produced when the hypothalamic–pituitary–adrenal (HPA) axis is activated. Earlier studies have measured these hormones in blood, urine, faeces, and saliva. However, these samples are not very useful for studying long-term changes in the body. To solve this problem, researchers now use materials like hair and claws, which can show hormone levels over a longer period [5]. Therefore, these hormone levels were measured in samples like claws and hair in order to overcome these issues. Although the oxytocinergic system is activated during mother–infant interactions [6], which in turn releases oxytocin that could exert inhibitory action on the HPA axis [7], maternity seems to play a vital role in HPA axis activation. In dogs, during pregnancy and postpartum cause a long-term increase in cortisol levels, but not in DHEA-S, in their hair and nails [5].

2. **Neuro-hormone as Possible Biomarkers of Different Lifestyles in Dogs**

   i) Prolactin (PRL) is a polypeptide neurohormone with multiple homeostatic roles that operates in close interaction with the dopaminergic system [8]. Circulating PRL levels increase during both acute and chronic stress induced by either psychological or physiological stimuli and elevated PRL has been associated with emotional disorders and anxiety-related behaviours in dogs along with other mammalian species [9]. In one study it is reported that in sheltered castrated male dogs, PRL concentrations do not correlate with behavioural stress scores or fear-related behaviours, although a weak negative correlation between cortisol and PRL was observed [10]. In our earlier research, we found that anxious dogs (AD) exhibited higher circulating PRL levels than pet dogs under controlled conditions [11], further supporting the need to investigate this neuromodulator in greater depth. Increased levels of prolactin have been associated with acute and chronic stress in dogs. However, the findings remain inconsistent, likely due to substantial individual variability in this neurohormone's response [12].

   ii) Serotonin (5-hydroxytryptamine; 5-HT) is a monoamine present in the central nervous system, gastrointestinal tract, and blood, where it exerts broad physiological effects as a neuromodulator [13]. In dogs, 5-HT has emerged as an important neuromodulator for understanding behavioural and emotional responses. Low 5-HT levels have been linked to distress and several behavioural disorders, including impulsivity, impaired motor control, and certain forms of aggression [14]. Indeed, aggressive dogs have been shown to exhibit significantly lower serum 5-HT concentrations compared with non-aggressive dogs [14,15]. Moreover, L-tryptophan which is a precursor of serotonin [16] and serotonin reuptake inhibitors [17,18] are widely used in the treatment of behavioural disorders. However, a clear association between this monoamine and lifestyle factors in dogs has not been established [12], possibly because peripheral serotonin levels appear to be strongly influenced by dietary patterns [19]. In comparison with prolactin, serotonin seems to be a more reliable biomarker.

iii) Oxytocin (OT) is a nonapeptide made in the hypothalamus and released into the bloodstream from the posterior pituitary, mainly supporting parturition and lactation [20]. It is also transported to several brain regions, where it acts as a neuromodulator involved in social behaviours, including social memory, bonding, parental and sexual behaviours, and certain forms of aggression [21, 22]. Oxytocin (OT) is involved in positive social interactions both within a species as well as between species [23, 24], including human–animal relationships [25, 26]. In dogs, OT research often examines how interactions with humans [27], or social stress [28] affect OT levels, as well as how OT administration influences cognition and behaviour. Intranasal OT has been shown to enhance positive expectations [29], improve responsiveness to human cues, reduce aggression toward threatening humans, increase affiliation with owners and other dogs, promote play, and increase visual contact [23, 30]. In fact, even though oxytocin has been frequently linked with more friendliness or sociability, free and total blood oxytocin were found to be surprisingly lower in assistance dogs than pet dogs [12].

3. Possible Biomarkers of Intrauterine Asphyxia in Newborn Canines

Vassalo et al., 2015 states that “Intrapartum hypoxia/asphyxia negatively impacts newborn puppies' adaptation to extrauterine life” which literally means hypoxia or asphyxia during birth can make it harder for newborn puppies to adjust to life outside the womb [31]. Therefore, Blood gas analysis helps to check oxygen levels, metabolism, and acid–base balance in newborns. Even during normal births, changes in blood gases and metabolites can show signs of breathing or metabolic problems caused by a shortage of oxygen during delivery. The mother's body weight also affects the size of her puppies. Heavier puppies are more likely to face problems during birth because they are harder to pass through the birth canal, which increases the risk of oxygen deprivation before birth [32].

4. Facial Expressions as Possible Biomarkers of Emotions in Dogs

Facial expressions, a form of nonverbal communication, convey both positive and negative emotions and play a crucial role in regulating and de-escalating social interactions among dogs [33]. Although extensive literature exists on the development of standardized Facial Action Coding Systems (FACS), objectively linking specific facial movements to underlying emotional states remains challenging [34]. This difficulty largely arises from interspecies and interbreed variability in facial mimicry, which complicates standardization efforts [34]. Nonetheless, several veterinary adaptations of the FACS, such as the Grimace Scale, have been developed to assist clinicians in recognizing pain and distress in various domestic animals [18]. These tools are essential for improving the interpretation of animal body language and enhancing veterinarians' understanding and attitudes toward animal welfare [35].

5. Skin Temperature as Possible Biomarker of Stress in Companion Animals

Stressful events are known to activate the sympathetic nervous system, leading to an increase in body temperature. Infrared thermography (IRT) utilizes specialized cameras to detect emitted radiation and visualize changes in skin surface temperature, which can be correlated with health or stress conditions in companion animals [36]. Although IRT is a safe and non-invasive technique that does not induce additional stress, current evidence regarding its reliability remains inconclusive, primarily due to the potential influence of environmental factors [36].

6. Possible Biomarkers of Oxidative stress in Companion Animals

Oxidative stress is defined as an imbalance between oxidants such as oxygen-derived free radicals and the antioxidant defence system, in favour of the oxidants. This imbalance disrupts normal redox signalling and can lead to molecular damage involving lipids, DNA, and proteins [37]. oxidative stress (OS) biomarkers can be assessed in fluids like blood, serum, plasma, urine, saliva etc. Levels of oxidative stress (OS) biomarkers in canines may vary with diet [38]; age [39,40]; sex [39,41], and even the duration and quality of time the dog spends in the veterinary clinical facility before sampling [42].

Oxidative stress in dogs is commonly assessed using a combination of enzymatic and non-enzymatic biomarkers that reflect the balance between oxidant production and antioxidant defenses.

Key enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which help neutralize reactive oxygen species. Non-enzymatic antioxidants include reduced glutathione (GSH), total antioxidant capacity (TAC), vitamins C and E; along with race element like copper, zinc, Copper and zinc ration, and Selenium provide additional insight into the antioxidant status. Copper and zinc are mineral micronutrients acting as cofactors of certain endogenous antioxidant enzymes [43].

Indicators of oxidative damage include Malondialdehyde (MDA) and Isoprostanes for lipid peroxidation, Myeloperoxidase as inflammatory markers, protein carbonyls for protein oxidation, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) for DNA damage. Together, these biomarkers are valuable for evaluating health status, monitoring disease progression, and assessing stress-related or metabolic conditions in dogs.

Conclusion

Stress in companion animals is a complex, multidimensional response mediated mainly by the HPA and SAM axes, affecting physiological homeostasis, behaviour, emotional wellbeing, and overall fitness while also disrupting harmony with owner expectations and public perception. A broad spectrum of stress biomarkers—ranging from long-term endocrine indicators such as hair and nail cortisol, neurohormones like prolactin and serotonin, childbirth-associated oxytocin changes, neonatal blood-gas metabolic shifts reflecting intrapartum hypoxia/asphyxia risk, non-invasive thermal responses measured through infrared thermography, facial-expression-based scales for emotional and welfare assessment, and oxidative stress markers alongside enzymatic and non-enzymatic antioxidant defenses (SOD, CAT, GPx, TAC, GSH, vitamins, trace-element ratios, and oxidative damage indices such as MDA, 8-OHdG, and protein carbonyls)—provides valuable insight into both acute and chronic stress states, though findings show variability across lifestyles, diets, breeds, and sampling conditions. No single biomarker can reliably capture stress across all contexts; therefore, a combined multi-biomarker panel approach offers the most accurate, welfare-friendly, and clinically meaningful assessment, and future research must prioritize longitudinal validation, environmental standardization, and harmonized methodologies to strengthen diagnostic precision and companion-animal welfare monitoring.

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