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Stress-related behaviors play a crucial role in **canine well-being**. As we come together for this **symposium on Advancements in Canine Behavior and Welfare**, our goal is to explore the latest insights into stress-related behaviors, behavioral diagnostics, and the multifaceted factors influencing canine well-being. Additionally, we will learn about the role of **probiotics in behavior modulation** and the responsibilities of pet food companies in promoting animal welfare.

Throughout this symposium, we have had the privilege of bringing together **esteemed specialists** in veterinary medicine, behavior science, and nutrition. Their expertise allows us to delve into canine stress responses, interspecies differences in stress perception, and evidence-based strategies for recognizing and managing stress-related behaviors. Attendees will also gain insights through the review of real-life case reports and practical applications that enhance the human–dog bond.

Our mission throughout this event is to advance **canine welfare** by integrating scientific research with practical behavioral and nutritional strategies. Through ongoing collaboration and knowledge-sharing, we strive to promote innovative approaches that contribute to healthier, happier, and more resilient canine companions.

We extend our gratitude to all attendees and speakers of this event. We truly believe that through global collaboration and the dissemination of expert knowledge, we can make a meaningful difference in the lives of dogs and their human companions. Together, we have the power to transform canine welfare and enrich the journey of pets and owners alike.

We hope you enjoy this **Symposium on Advancements in Canine Behavior and Welfare** and find inspiration in the knowledge shared today.

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Dr Sarah Heath, FRCVS - RCVS and EBVS® European Veterinary Specialist in Behavioural Medicine

Sarah qualified from Bristol and spent four years in mixed general practice before setting up Behavioural Referrals Veterinary Practice in 1992. In 2018 she was made a Fellow of the Royal College of Veterinary Surgeons in recognition of her work in establishing Behavioural Medicine as a veterinary discipline. She is an RCVS and EBVS® European Veterinary Specialist in Behavioural Medicine. Sarah is an External Lecturer in small animal behavioural medicine on the veterinary undergraduate courses at Liverpool University and the University of Lancashire School of Veterinary Medicine. She is a Certificated Clinical Animal Behaviourist and sees clinical cases across North West England. Sarah is the author of the Heath Model and promotes the concept of Comprehensive Veterinary Healthcare. She has a special interest in the interplay between emotional and physical illness in dogs and cats and particularly in the role of pain. She promotes the recognition of emotional health issues in companion animals and the role of the veterinary profession in safeguarding the welfare of animals in this context. Sarah lectures at home and abroad and is an author, co-author and editor of several books and papers.

UNDERSTANDING CANINE STRESS AND ANXIETY: CAUSES AND IMPACTS ON OUR DOGS

Dr Sarah Heath, FRCVS - RCVS and EBVS® European Veterinary Specialist in Behavioural Medicine

Introduction

The words anxiety and stress are often used in combination, but it is important to define what is being discussed. Both of these words relate to normal biological responses, emotional and physiological, which have the ultimate aim of ensuring the survival of the individual. They are distinct but related and an appreciation of how they arise, and the impact they have on our dogs, is crucial to enable us to support our canine companions as they live in our human domesticated world. It is important to differentiate between anxiety when it is justified and appropriately responded to, with the aim of achieving resolution and the emotional response when it is either unjustified or unresolvable or sometimes both. Likewise stress, both physiological and psychological, can be beneficial to the individual but when it is chronic in nature and cannot be resolved it can have considerable detrimental impact. The terms distress and eustress are sometimes used to distinguish between the two.

What causes anxiety and stress in dogs?

Adaptive anxiety and resulting physiological stress are triggered by situations of uncertainty or exposure to contexts in which there have been previous experiences related to potential for danger and threats to survival. The trigger activates the amygdala leading to the emotional response of anxiety which in turn leads to triggering of the SAM and HPA axis components of the stress response. The emotional activation leads to behavioural responses which are designed to ensure survival through increasing distance and decreasing interaction with the trigger or through finding out more about it in order to limit the damage it can cause. Alongside the behavioural response physiological changes, such as increased heart rate and blood pressure and increased availability of glucose, enhance the physical ability of the individual to resolve the problematic situation. Negative feedback via the hippocampus and glucocorticoid receptors return the individual to baseline and a state of resolution. However, when exposure to the problematic stimulus or context is prolonged or repeated and the individual is unable to use natural behavioural responses to provide a sense of safety and security there is a risk that this adaptive response can become maladaptive. For domestic dogs, the challenges of living in a human dictated environment can be a risk factor for development of anxiety and stress responses that become detrimental rather than beneficial. These challenges are many and varied and their impact on an individual will depend on range of factors including genetics, in utero environment, maternal and early environmental influences, post-natal experiences, availability of predictability and control within their environment and the ability to express natural behavioural responses in ways that support emotional recovery. When some or all of these factors are compromised anxiety can become triggered in contexts where potential for harm is either not present at all or is mild and should be easily resolvable. The unresolved emotional state is associated with chronic and potentially detrimental physiological stress as well as psychological stress. The latter can be defined as the "emotional and cognitive response that occurs when an individual perceives a situation as threatening, unpredictable, uncontrollable, or exceeding their coping resources – even in the absence of immediate physical danger".

What are the impacts of anxiety and stress in dogs?

When considering the potential impact of anxiety and physiological and psychological stress it is helpful to take a comprehensive veterinary healthcare approach. From an emotional health perspective anxiety is a protective emotion and can lead to a variety of behavioural responses which are designed to achieve a state of safety and security for the individual. These include repulsion and avoidance, which increase distance from any perceived threat and decrease the potential for interaction with it. Both of these responses can cause difficulty for a dog living in a domestic environment and lead to behaviours which can be classed by caregivers, veterinary professions and even wider society as undesirable and problematic. The protective behavioural responses can also be detrimental to the individual and increase risk of injury, through confrontational interactions or through avoidance responses which put them in danger such as bolting leading to road traffic accidents. Anxiety can also be associated with behavioural responses which aim to acquire more information about the potential threat and inhibition and appeasement are common responses in a socially obligate species like a dog, particularly in the context of potential social threat. Protective behavioural responses occur in situations where the anxiety is justified as well as when it is maladaptive. In situations where the anxiety is unjustified or out of proportion to the context it is possible that the behavioural responses can have more complex consequences for the individual but also for others. In addition, if the behavioural responses are not successful in achieving recovery from the anxious state the individual may move between protective responses and the emotion of frustration can also be triggered leading in intensification and acceleration of responses and an increase in confrontational interactions. All of this can increase the impact for those involved. Cognitive impact of anxiety and stress is also an important consideration. Through classical conditioning associations between the protective emotional bias and particular contexts, both physical and social, can impact the behavioural responses of the individual when they encounter the same contexts in the future. This can be particularly relevant in the context of the veterinary visit where protectional emotional bias can become associated with the veterinary environment as well as with veterinary personnel. Learning through operant conditioning also needs to be considered. When a dog experiences a consequence following an action this can alter the probability that the action will be performed in the future. The presence of anxiety and resulting stress can have a direct impact on the individual's perception of such a consequence and thereby either increase or decrease the likelihood of them responding to a cue with a particular action in the future. Misinterpretation of this change in behaviour can lead to dogs being considered to be stubborn or "disobedient" and result in interactions which detrimentally impact the individual and can have implications for their relationship with caregivers or veterinary professionals. The third health domain to consider is that of physical health. Both the behavioural responses and the physiological changes associated with anxiety have the potential to impact on the physical health of the dog. This may happen within the context of justified anxiety and acute stress and impact on physiological parameters which are traditionally used in a veterinary context to assist in diagnosis of disease. For example, altered heart rate, respiratory rate or biochemical markers can make interpretation of clinical examination and blood tests more challenging. In cases where anxiety is unjustified or ongoing the resulting chronic physiological impact and allostatic load can have significant impact on the physical health of the individual. This impact can be related to a range of physical health presentations. These include but are not limited to gastrointestinal, dermatological, cardiovascular, endocrine and metabolic conditions as well as sleep disturbance, reproductive impact and limitations to recovery from illness and surgery.

Conclusions

The role of the veterinary profession is to safeguard the health and welfare of non-human animal species. In order to fulfil this role, we need an understanding of the emotional and cognitive health of our patients as well as their physical health. The interplay between the protective emotion of anxiety and the physiological response of stress can lead to significant impact on physical health as well as behavioural responses, both of which can challenge the welfare of the individual. Recognition of protective emotions which are justified can help us to appreciate the importance of the use of

appropriate environmental adjustments, both social and physical, in order to optimise the environments in which non-human animals live. The aim is to improve their sensations of safety, autonomy and agency so that the protective emotions are no longer necessary. It is equally important to identify situations where anxiety is unjustified or unresolvable and to give appropriate cognitive, nutraceutical, pheromonal and pharmacological support to these individuals. Similarly distinguishing physiological or psychological stress which is adaptive and beneficial from chronic and detrimental stress is an important step in providing appropriate interventions.

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Recognizing and Diagnosing Stress and Anxiety in Dogs

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RECOGNIZING AND DIAGNOSING STRESS AND ANXIETY IN DOGS

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Stress, fear, anxiety and animal welfare

Stress has been defined as a response to a challenge or threat to survival or homeostasis. The stress response includes an emotional reaction and a set of behavioural and physiological changes. Behavioural changes are the most commonly used indicators of stress in clinical settings, and will be described in more detail below. Physiological changes -which are often used to assess stress in research settings- result mainly from the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Ultimately, the activations of the HPA axis causes an increase in the plasma concentration of cortisol, and blood concentration of cortisol has been one of the most widely used measures of stress.

A reduction of stress is usually interpreted as resulting in better welfare. However, the stress response is not always associated with an unpleasant emotional state and actually highly motivated and rewarding behaviours -such as play, exploration or mating- can also result in the activation of the HPA axis and the sympathetic nervous system (Koolhaas et al., 2011). In these circumstances, however, such physiological changes are by no means associated with a decrease in animal welfare. Therefore, in the context of this paper and following Pfaff et al. (2007) and several other authors, stress is defined more narrowly as a state of high arousal and negative emotional valence. According to this definition, it is clear that changes in plasma cortisol levels -and for the same reason in several other physiological markers- are related to the state of arousal rather than to the valence of the animal's emotion, and therefore their relevance for animal welfare should be interpreted with caution.

Stress can be conveniently divided into acute and chronic. Acute stress in dogs has been studied mainly in three contexts: when dogs are taken to the veterinarian, when they are exposed to loud noises and when suffering separation-related problems. In all these cases, fear (i.e. an emotional response to an actual threat) and / or anxiety (i.e. an emotional response to an anticipated threat) are the main causes of stress. Chronic stress has been mainly studied in relationship to housing and husbandry conditions, for example in dogs kept in shelters. In addition, some other studies have assessed stress in dogs used in animal-assisted therapies.

Recognizing and diagnosing stress: importance and difficulties

Stress, fear and anxiety are emotional reactions with a negative valence and have therefore a direct impact on animal welfare. Additionally, long-lasting, repeated or intense stress can have negative effects on dogs' physical health. Stress, fear and anxiety often cause behavioural problems, which have a negative impact on human-dog bond and on dog welfare. Finally, negative emotional reactions when dogs are taken to the veterinarian can result in safety issues for veterinarians and technicians, and can ultimately reduce the quality of veterinary care.

Despite their major impact on dog welfare, recognizing stress, fear and anxiety in dogs is fraught with difficulties. Some of these difficulties simply result from the fact that some signs of stress are rather subtle and can be easily overlooked. Furthermore, several behavioural changes that may be indicative of stress can also occur in other contexts which are unrelated to stress. Also, individual dogs differ in their temperament. The term "temperament" refers

to individual differences in behaviour which are not accounted for by sex or age and are consistent over time and across situations (Manteca and Deag, 1993). To a large extent, differences in temperament relate to how individual dogs respond to stress. For example, some dogs may respond to a frightening stimulus with obvious signs of aggression or attempts to escape, whereas other may respond by "freezing".

Several studies have tried to identify the main factors that affect our ability to identify stress, fear and anxiety in dogs. As could be expected, the more subtle behavioural signs -such as lip licking or yawning, for example- appear to be more difficult to recognize even by veterinarians than more obvious signs such as trembling or "low-body" posture. A study by Menor-Campos et al. (2022) found that a good knowledge of even basic stress physiology results in veterinary students being better at identifying stress in dogs. Previous or current pet ownership also improves the students' ability to identify stress in dogs. This is a rather interesting result that could be interpreted in two different -although not mutually exclusive- ways: it could be that pet ownership increases familiarity with dog behaviour, or it could also be argued that pet ownership is linked to a higher degree of empathy with animals. Indeed, there are reasons to believe that lack of empathy -even in otherwise knowledgeable persons- can result in their being less apt at identifying signs of stress.

Acute stress, fear and anxiety can be assessed using scales such as the Lincoln Canine Anxiety Scale (LCAS) and the Spectrum of Fear, Anxiety and Stress (Fear Free®, 2022). The Lincoln Anxiety Scale consists of a 16-item owner-report measure of anxiety signs and is based on the content of the instrument used to determine noise phobia (Lincoln Sound Sensitivity Scale). The LCAS has been subjected to validation studies and is a reliable and valid measure of anxiety in dogs that has also been developed into a short form for clinical use (Mills et al., 2020).

The Spectrum of Fear, Anxiety and Stress is an eight-item scale, graduating from 0 to 5, designed to assess canine fear, stress and anxiety during veterinary visits. A recent study by Gatehouse et al. (2025) identified areas for improvement -including adequate training- but it also concluded that "the FAS Spectrum" is one of the few viable options for assessing behavior within a practice setting.

Displacement behaviours such as lip licking, yawning and paw lifting have been frequently suggested as subtle indicators of fear and stress. Some studies have indeed shown an increase in the frequency of these behaviours when dogs are exposed to threatening stimuli. However, it appears that displacement behaviours are very dependent on the context and other variables, and hence their interpretation may be difficult (e.g. Pedretti et al., 2023). Displacement behaviours as potential indicators of fear and stress will be discussed in the presentation. Similarly, facial expressions could also be useful to identify emotions in dogs (Mota-Rojas et al., 2021).

Chronic stress has a major impact on dog welfare, and many welfare problems are associated with chronic stress. Indeed, dog welfare assessment tools -although not specifically intended to identify stress and anxiety- can provide relevant information on stress, fear and anxiety. Animal welfare assessment tools usually combine output-based (also called animal-based indicators) and input-based indicators (also called resource or environment-based indicators). The set of indicators that are included in a welfare assessment tools should cover all the domains of welfare, i.e. feeding and nutrition, environment, health, behavior and mental state, and particularly those related to behaviour and mental state are useful to identify negative emotions including stress and fear (e.g. Barnard et al., 2016)

Pacing and other abnormal repetitive behaviours or stereotypies have been widely used to identify both chronic and acute stress. Although such behaviours can indeed provide useful information on stress and welfare, there are several elements that should be considered before drawing any firm conclusion on their relevance (Mason and Latham, 2004). Additionally, one major difficulty when identifying chronic stress is that it can result in dogs developing a state of reduced physical activity and apparently low arousal, which can be misleading or easily overlooked.

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A Holistic Overview of Probiotics and Their Influence on Canine Behavior

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Dr. Ragen McGowan is the Director of Digital Product Development at Purina. She has over 25 years of experience in animal behavior research, the last 16 of which have been with Nestlé Purina using a holistic approach incorporating behavior, physiology and endocrinology to quantify affective states in dogs and cats. She aims to develop products that cater to different behavioral needs of pets, including focus on the impact of nutrition on behavior via the gut-brain axis, new insights into the emotional lives of pets and understanding the human-animal bond from the pet's perspective. Ragen earned a B.S. in Zoology, a B.A. in Foreign Language & Literature and a PhD in Applied Ethology from Washington State University. Her PhD research focused on Contrafreeloading, in grizzly bears, laying hens and mice. Prior to joining Nestlé Purina, Ragen held a post-doctoral research position at the Swedish University of Agricultural Sciences in Uppsala, Sweden, where her research focused on the study of emotionality in dogs. This included exploring Cognitive Bias and the 'Eureka Effect' in dogs. She is now bringing her knowledge of animal behavior and emotions into the digital space to better connect owners with their pets. Ragen has a passion for sharing her vast knowledge, speaking at a variety of events and contributing as a scientific expert for congresses, roundtables and podcasts.

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A HOLISTIC OVERVIEW OF PROBIOTICS AND THEIR INFLUENCE ON CANINE BEHAVIOR

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Introduction

Behavioral responses indicative of stress in dogs manifest in various forms and affect a significant portion of the canine population, with studies reporting up to 86% of dogs exhibiting owner-reported stress-related behaviors.[1,2,3] Such stress-related behaviors can harm the pet-owner relationship and are among the leading causes of dog relinquishment to shelters.[4,5]

Mounting evidence suggests that the gut microbiota may influence behavior through the gut-brain axis. Studies demonstrate that anxious behavior transfers with gut microbes[6] or that anxious states can be influenced by the composition or absence of gut microbiota.[7,8,9] Prior work has demonstrated that the probiotic strain BL999 can reduce depression scores and emotional responding in people with irritable bowel syndrome (IBS)[10] and reduce perceived psychological stress in healthy people.[11]

Here we discuss two studies evaluating the effect of BL999 on stress responses in dogs, both behavioral and physiological, in various ethologically relevant situations. The efficacy of BL999 was assessed during typical and potentially stressful experiences that pets may encounter, such as novel situations, brief separations from caregivers or transport in a car. Evidence is also presented demonstrating the safety of BL999 as a probiotic for dogs.

Methods

Study 1: Twenty-four reactive Labrador Retrievers participated in a blinded crossover design where each dog acted as his or her own control. Dogs were fed a complete and balanced diet and supplemented with either the probiotic BL999 (1×10^9 CFU/dog/day) or a placebo for six weeks, followed by a three-week washout period, before crossing over to the opposite treatment for an additional six weeks. During each phase, the dogs' typical behavior in response to normal day-to-day stimuli was observed using a scan sampling method. In addition, each dog experienced a formal behavioral challenge (i.e. visiting novel environments, hearing strange noises, meeting strangers or brief periods of separation from their main caretaker) to assess his or her response to non-social, social, and separation-related stressors at the end of each supplementation phase. Non-invasive physiological measures were assessed throughout the evaluation. Heart rate (HR) and heart rate variability (HRV) were recorded through the entirety of the formal behavioral challenge, and salivary cortisol concentrations were assessed prior to and following the formal behavioral challenge.

Study 2: A blinded two-phase crossover design was used with 20 adult dogs of varying breeds and sizes, each serving as his or her own control. Dogs were fed a complete and balanced diet and received either BL999 (1×10^9 CFU/dog/day) or a placebo for six weeks. Dogs were challenged with a car ride as a commonly encountered stressor for pet dogs. On the day of the car ride, dogs were taken to the veterinary clinic followed by a 10-minute car ride and a 30-minute recovery period. Physiological (HR, HRV, tympanic membrane temperature, and blood cortisol) measures were collected at predefined time points, and behavioral responses during the car ride were video recorded and scored using a blinded ethogram analysis. After a 23-day washout, treatments were switched and the procedure was repeated.

Safety Assessment:

The strain was submitted to both genomic and phenotypic assessments according to EFSA guidance.[13,14] Whole genome sequencing was performed to confirm the absence of acquired antimicrobial resistance (AMR), virulence, and toxigenic genes. Additionally, the absence of AMR was assessed phenotypically.

Results

Study 1: BL999 significantly impacted day-to-day behavior (scan samples), with dogs showing improved behavior when supplemented with BL999 as compared to when they were supplemented with the placebo. This included significant reductions in barking ($P < 0.0001$), jumping ($P < 0.006$), spinning ($P = 0.014$), and pacing ($P = 0.026$). During the formal behavioral challenge, dogs supplemented with BL999 showed increased exploratory behavior in a novel environment compared to when they were supplemented with the placebo ($P<0.05$). In addition, dogs had reduced salivary cortisol concentrations in response to both exercise and stressors when supplemented with BL999 ($P < 0.001$). When considering cardiac activity, dogs showed a decrease in HR ($P < 0.001$) and an increase in HRV (HF: $P < 0.03$; RMSSD: $P < 0.001$) indicating a more positive response to common stressors when supplemented with BL999.

Study 2: Dogs supplemented with BL999 showed significantly lower tympanic membrane temperature ($p<0.05$) and reduced Low frequency (LF)/High frequency (HF) ratio ($p<0.05$) in the veterinary setting before the car ride, compared to when supplemented with the placebo. During the car ride, BL999 supplementation resulted in significantly reduced lip licking frequency score ($p<0.05$) and panting frequency score ($p< 0.1$) and time spent standing ($p<0.1$). During the car ride, no treatment differences were observed in cardiac function (HR or HRV), or in post-ride blood cortisol and tympanic temperature. However, during the recovery period (30 mins post car ride), dogs receiving BL999 demonstrated significantly higher HRV parameters (HF, RMSSD, and pNN50%), reflecting enhanced parasympathetic activation and a calmer post stress state.

Strain Safety:

Comparison of the whole genome of strain BL999 to publicly available sequences, including type strains, unambiguously identified it as belonging to the *Bifidobacterium longum* subsp. *longum* taxon commonly known as *Bifidobacterium longum,* previously evaluated by EFSA positively and included in the "Qualified Presumption of Safety" (QPS) list.[14] Analysis of the BL999 whole genome demonstrated the absence of known toxin production genes and that BL999 was free of AMR. Phenotypic testing further confirmed absence of antibiotic resistance against the antibiotics required to be tested by EFSA.

Discussion

Using a holistic approach incorporating both behavioral and physiological measures we have demonstrated that dogs supplemented with BL999 are less reactive (lower salivary cortisol, less barking), more calm (lower HR, higher HRV), and potentially in an improved affective state (higher HRV) when experiencing common stressors than when they were supplemented with a placebo. In addition, and possibly most noticeable to an owner, dogs supplemented with BL999 exhibited less daily problem behavior like barking and jumping than when supplemented with a placebo.

Furthermore, dogs entering a veterinary setting had evidence of reduced body temperature and a modulation in one HRV measure, indicating dogs experienced a less reactive state when supplemented with BL999. Despite no difference in physiological responses—likely due to the highly arousing experience—dogs supplemented with BL999 exhibited significantly fewer negative behavioral responses during the car ride. Stress can persist even after the stressor is removed. Post-car ride measurements of HRV indicated that dogs supplemented with BL999 exhibited a better affective state compared to those given a placebo, suggesting a faster recovery from the car ride experience.

In conclusion, from both a behavioral and physiological standpoint, the probiotic BL999 had a positive effect on the stress response of dogs and could serve as a useful and safe tool to improve the well-being and stress resilience in dogs.

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Real-Life Applications: Case Reports on Calming Care

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She is bachelor in Veterinary Medicine from the School of Veterinary Medicine of the Autonomous University of Barcelona, where she also completed a Master's Degree in Veterinary Research and Food Sciences and the Phd. Diplomate by the European College of Animal Welfare and Behavioral Medicine since 2008. Head of the Clinical Ethology Service of the Veterinary Clinical Hospital of the UAB and researcher in the Department of Animal Science and Food of the Faculty of Veterinary Medicine of the UAB.

REAL-LIFE APPLICATIONS: CASE REPORTS ON CALMING CARE

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Stress-related disorders are highly prevalent in dogs. In addition to their detrimental effects on animal welfare, these conditions can significantly impair the human–animal bond. A variety of strategies are available for their management. In some cases, alongside behaviour modification programmes, environmental enrichment and improved owner handling, adjunctive products aimed at reducing stress levels may be administered. Recently, a probiotic formulation known as Calming Care has been developed and is proposed to contribute to stress reduction in dogs.

The aim of performing these case reports was to evaluate the effectiveness of Calming Care in dogs presenting with stress-related behavioural problems. Cases were recruited at the Faculty of Veterinary Medicine of the Autonomous University of Barcelona during the month of November. Three dogs meeting the predefined inclusion criteria were enrolled. These criteria included being of the same sex, of similar age, living in comparable environments and having no recent dietary changes.

A structured interview with the owners was conducted by a veterinary behaviour specialist to collect information regarding the dogs' behavioural profiles, daily routines, handling practices and environmental conditions. Additionally, behavioural changes following probiotic administration were assessed using a validated instrument, the Canine Behavioural Assessment and Research Questionnaire (C-BARQ). The questionnaire was completed prior to the initiation of probiotic supplementation and repeated after six weeks of administration. The probiotic was administered once daily in accordance with the manufacturer's instructions.

Behavioural improvements were observed in all three cases. During the presentation, the methodological considerations and limitations inherent to this type of study will be discussed, and the results obtained will be presented.

Real-Life Applications: Case Reports on Calming Care

Dr. Clara Palestrini, PhD, Diplomate, Professor of the Department of Veterinary Medicine and Animal Sicence, University of Milan

Clara graduated in Veterinary Medicine in 1995 from the Faculty of Veterinary Medicine of the University of Milan. In 2000 she obtained with Laude the title of Specialist in Applied Ethology and Pet Welfare and in 2003 the title of PhD. She is currently Associate Professor at the Department of Veterinary Medicine and Animal Sciences of the University of Milan and conducts behavioral examinations at the Behavioral Medicine service of the University Veterinary Hospital of Lodi. Author of numerous scientific publications as well as speaker at conferences and courses at international level, in 2005 she obtained the Diploma from the European College of Behavioral Medicine (ECAWBM) and the title of European Specialist in Behavioral Medicine. The research areas of particular interest are the behavioral pathologies of dogs and cats and the evaluation of the welfare of shelter dogs.

REAL-LIFE APPLICATIONS: CASE REPORTS ON CALMING CARE

Clara Palestrini, Austin: effect of stress related behaviours on welfare. A combined behavioural and nutritional approach

Introduction

Behavioural disorders associated with stress represent a major cause of reduced welfare in dogs and constitute an increasingly relevant clinical challenge in veterinary practice. Although often overlapping with clinical anxiety, stress-related behaviours require specific assessment and a multimodal approach based on behavioural evaluation, investigation of possible concurrent medical conditions, and appropriate management and nutritional interventions. Many manifestations traditionally attributed to anxiety are, in fact, responses to persistent environmental stressors that can significantly affect the quality of life of the animal and its caregivers. Clinical signs are frequently nonspecific and may overlap with painful or medical conditions, making an integrated evaluation essential for distinguishing between primary causes, comorbidities, or consequences of the behavioural picture.

In recent years, growing scientific evidence has highlighted the role of the bidirectional communication between the gut–brain axis (GBA) and stress regulation in dogs. The intestinal microbiota, consisting of a complex community of resident microorganisms, contributes to maintaining enteric homeostasis and influences several physiological, immune, and neuroendocrine functions. Its composition can modulate emotional and behavioural responses through neural, metabolic, endocrine, and immune-mediated pathways. Conditions of dysbiosis—qualitative or quantitative disruptions of the microbial population—have been associated with inflammatory states, gastrointestinal disorders, and behavioural alterations, including amplified stress responses.

It has been demonstrated that chronic activation of the hypothalamic–pituitary–adrenal (HPA) axis, typical of prolonged stress, can induce changes in intestinal microbial composition, increase gut permeability, and contribute to systemic and neuroendocrine inflammatory phenomena. These changes can, in turn, influence behaviour, creating a feedback loop between stress, microbiota alterations, and further behavioural modification. These findings support the growing interest in innovative strategies based on nutritional and microbiome-focused interventions, not intended as treatments for pathological anxiety, but rather as tools to support calmer behaviour and modulate the stress response.

The present report describes the case of Austin, an adult dog showing persistent stress-related signs with a significant impact on his overall well-being. His clinical pathway offers an opportunity to discuss the main diagnostic challenges, nutritional considerations, and behavioural management strategiesinvolved in the integrated management of stress-related conditions in dogs.

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Presentation

Patient description
Austin is a GOLDEN RETRIEVER 2-year-old intact male, 28 kg

Reason for consultation
Austin was presented for evaluation due to behaviours consistent with persistent stress. His stress-related behaviours leads to weight loss and prevents proper weight gain. The onset of stress was associated with the persistent presence of unformed faces.

Case history
Austin lives in a breeding facility and alternates his daily routine between the kennel environment and a family and office setting. He is an active stud dog, mounts only in solitary contexts.
He receives regular preventive vaccinations and antiparasitic treatments. No past or current medical conditions have been reported. He is not currently taking any medications.

Austin has a normal sleep pattern, normal drinking behaviour, normal exploratory behaviour. He does not destroy or steal objects, engage in pica or coprophagia. No inappropriate elimination has been ever reported.

Austin completed a three-month training program starting at four months. He plays appropriately with other dogs and shows no aggression.

At 10 months of age, the patient experienced a significant startle episode. Suddenly, he heard the intense barking of a highly agitated dog housed in a nearby kennel. The conspecific repeatedly attempted to escape confinement, producing loud, rattling noises that contributed to a marked fear response in the patient. Since that episode, Austin has begun to show generalized stress-related behaviours both in familiar and unfamiliar situations, along with weight loss and persistent diarrhoea.

Owner describes Austin as frequently agitated and reactive. Loud noises trigger stress responses. He pants frequently, has often diarrhoea, hides, trembles, seeks physical contact. He shows environment-dependent mood fluctuations. He demonstrates improved behavioural regulation in his own kennel and in familiar environments, as well

as when accompanied by behaviourally stable and confident dogs, consistent with a marked gregarious attitude. He is frightened by the sudden arrival of unfamiliar people or by doors slamming; in these situations, he may bark, although he does not bark often.

Nutritional evaluation
- Austin is 28 kg
- Body condition score, BCS: 3
- Diet history:

Since weaning, Austin had been fed Pro Plan Sensitive Digestion Medium Puppy. He remained on this diet until around 10–12 months of age, when he began to exhibit recurrent episodes of diarrhoea and signs of generalized stress-related behaviours.

Following the onset of these clinical manifestations, Austin was taken for a consultation and underwent an examination by a veterinary nutritionist. A complete clinical examination and a full set of laboratory tests were performed. These included a complete clinical chemistry panel, a complete blood count, and full capillary protein electrophoresis. A complete urinalysis and urinary chemistry were also conducted. An ELISA test for Giardia was performed and resulted negative. A faecal parasitological examination was carried out and was also negative. Serum canine trypsin-like immunoreactivity (TLI) was measured and fell within the normal reference range. Additionally, vitamin B9 (folate) and vitamin B12 levels were evaluated and found to be within normal limits.

At 12 months of age, a gastroenteric diet was prescribed, consisting of EN Gastroenteric Purina (wet and dry), Fortiflora, honey, and flaxseed oil. Purina Pro Plan Relax+ was also added. The diet was prescribed to address chronically soft stools and, consequently, to promote weight gain. Over the course of one year, he gained 3 kg, the episodes of diarrhoea have been significantly reduced. However, despite this, there was no reduction in the symptoms attributable to a persistent state of stress.

Behaviour assesment and clinical plan

Behaviour assessment
A behavioural assessment was conducted by a European specialist at the University of Milan. During the clinical examination Austin exhibited signs of stress-related behaviours. The tutor was asked to fill out a behavioural assessment questionnaire before starting the probiotic and every 5 days. At 6 weeks, the questionnaire should be completed again to see if there are any changes.

Clinical Plan
A behavioural modification was suggested and consisted of ignoring undesirable behaviours (stress-related behaviours, barking), reward desirable behaviour, use the more stable dog in potentially critical situations.

No medication was prescribed.

Nutritional feeding plan

Product choice
- Diet/s
  The diet with Purina EN twice daily, was maintained.
- Supplements
  Fortiflora was maintained and Calming Care was added to the diet
- Treats
  No treats were included in the plan
- Feeding management and feeding methods.
  No other feeding recommendation was given

Follow up

Timeline
After six weeks, owner reported that the patient not only maintained the 3 kg previously gained but also demonstrated an additional 2 kg weight increase since starting Calming Care. Faecal consistency has remained consistently normal throughout the period.

Patient status evolution and nutrition changes
While his overall health status has remained largely unchanged, a notable improvement in behavioural stability has been observed both within the breeding facility in the office environment has improved, both when he is alone and when interacting with other dogs. His generalized stressed emotional state has shown partial improvement. When exposed to novel stimuli or to the sudden arrival of individuals, the patient presents increased behavioural stability, does not exhibit agitation, and shows more regular and controlled behavioural responses.

During this period, he also completed a mating. Previous matings had resulted in very small litters, as his intestinal dysbiosis had negatively affected fertility. The most recent mating produced six puppies, indicating an improvement in reproductive performance.

The owners are willing to continue Calming Care long-term, provided it can be incorporated into his regular diet, as they believe prolonged administration may lead to significant additional clinical benefits.

Implications and Conclusions

Discussion

In some forms of stressed emotional state, particularly when psychological signs are accompanied by somatic manifestations, the use of a supplement capable of modulating both components—the psychological and the somatic—represents an ideal management option. The simultaneous reduction of both stress-related behaviours and gastrointestinal signs further supports the need to address these two dimensions—organic and behavioural—in parallel.

Moreover, because the product is not a pharmaceutical agent, it can be administered easily and safely by the owner, while also offering the additional advantage of being fully compatible with the introduction of pharmacological treatment if needed. It can certainly be recommended as a long-term, continuous solution—rather than a periodic one—for the management of stress-related presentations associated with concurrent somatic symptoms.

Conclusions

The animal must always be considered as an integrated organism, in which soma and psyche constitute a single functional entity

Behavioural signs should therefore be interpreted within the broader context of the animal's overall physiological condition, and a comprehensive evaluation of the patient is always required.

The use of supplements becomes particularly relevant when multiple domains—organic, behavioural, and emotional—need to be addressed simultaneously. Such interventions can be incorporated while still allowing, when indicated, the implementation of behavioural modification protocols and/or pharmacological treatment.

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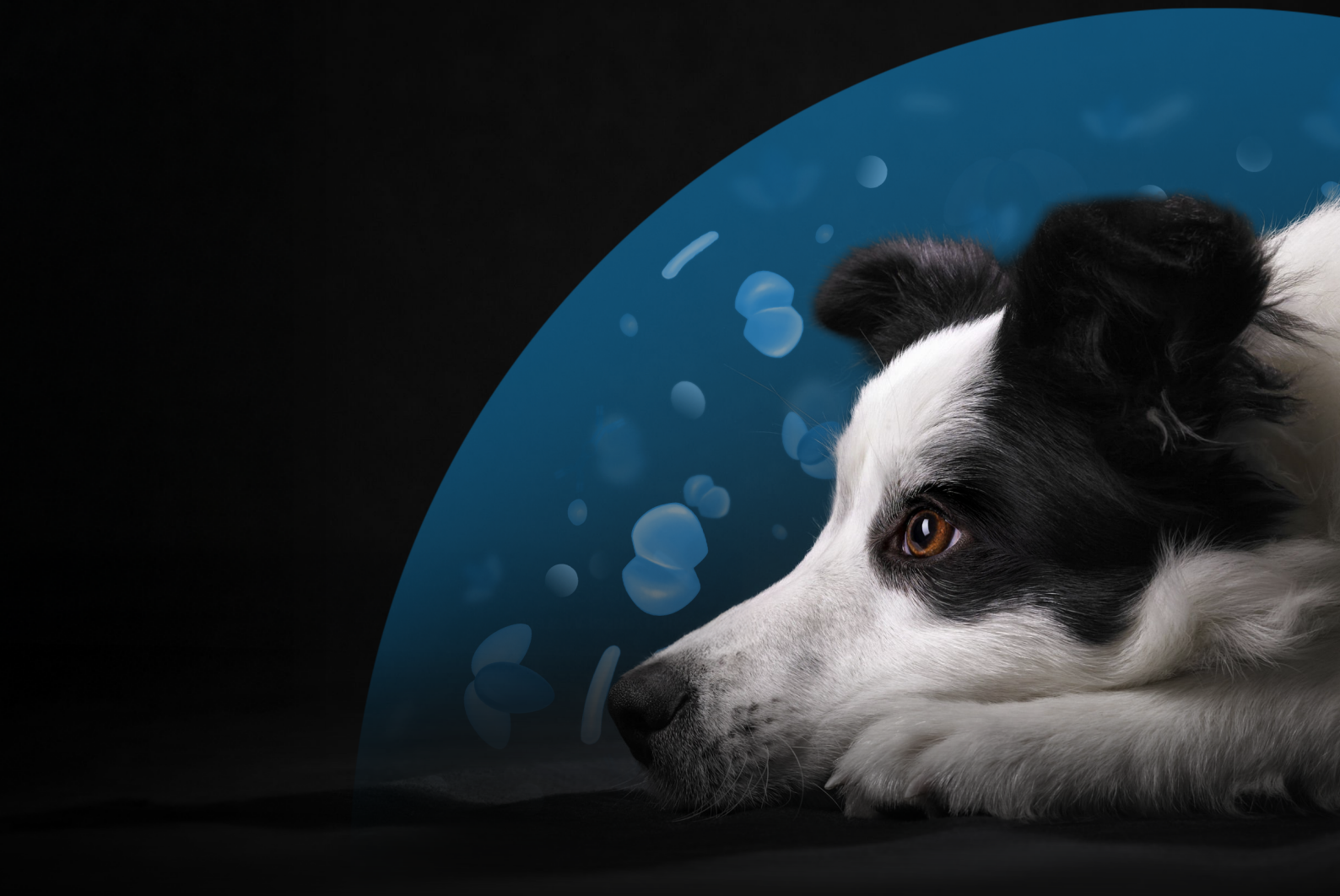

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