



Beneficial effects of a novel nutraceutical supplement, Calmina® (BUONAPET) upon mood-related disorders in dogs: 5 case reports

Luigi Sacchettino¹, Viviana Orsola Giuliano¹, Anna Terracciano², Federica Manunta³, Luigi Avallone¹, Danila d'Angelo^{1*}, Francesco Napolitano^{1,4*}

¹ *Department of Veterinary Medicine and Animal Production, University of Naples Federico II, 80137 Naples, Italy*

² *Veterinary Behaviorist, 04020 Itri, Italy*

³ *Veterinary Behaviorist and Consulting Service at "Free Interdisciplinary Italian Dog Academy" (LAICI), 25126 Brescia, Italy*

⁴ *CEINGE-Biotecnologie Avanzate Franco Salvatore, 80145 Naples, Italy*

Abstract: Behavioral disorders in pets, including aggression, separation anxiety or noise phobia are increasingly common over the recent years, reach up to more than 80% worldwide, thus representing one of main concerns in animal wellbeing and human-animal relationship as well. Despite the availability of conventional therapies, based on the use of the quite effective medication, pet parents are often reluctant to use it, rather preferring natural compounds. Therefore, the aim of the present study was to describe the effects of a novel nutritional supplement, namely Calmina®, in five family dogs, affected by dysfunctional behaviors. Depending on the symptom's severity, the animals were treated from one to four months. We observed an improvement of behavioral symptoms, and an easier management by their owners. Overall, our preliminary data suggest that the administration of Calmina® might be used to reduce anxiety-related behaviors and facilitate rehabilitation program. Further studies are mandatory to better analyze potential effects in a larger sample of dogs with behavioral disorders and in long-term treatment, as well as tolerance and safety.

Key Words: integrated approach, dog behavior, animal well-being, behavioral therapy, stress, anxiety.

* *Corresponding Author:* francesco.napolitano3@unina.it; danila.dangelo@unina.it

Introduction

Animals are employed in many aspects of human life, including maintenance and agriculture, as well as companionship and research. Significant changes in human-animal relationships over time occurred, so that pets are nowadays employed not only for work, house security, and mouse hunting, but also, for social interaction and development of long-lasting friendships among humans (Overgaauw et al., 2020). However, alongside with anthropomorphism and animal hoarding, the close link between dogs and humans may be sometimes the trigger for interspecies aggression events, thus turning into fear, anxiety, or compulsive behaviors (d'Angelo et al., 2022; Mota-Rojas et al., 2021; Sacchettino et al., 2023). Unfortunately, these behavioral issues very often cause the pet parents to move their dogs to shelters, where they are likely to remain for their lifetime, unless they're included in behavioral rehabilitation programs (d'Angelo et al., 2022; Siracusa et al., 2017). Thus, deeper investigations about dysfunctional behaviors in dogs are still regarded as an unmet need. Behaviors arise from an orchestrate activity of chemical messengers (neurotransmitters and hormones) within the central nervous system (CNS), whose alterations might be related to dysfunctional attitudes. In this respect, lower serotonin levels have been linked to aggression in many species, including dogs (Bosch et al., 2007). Anxiety disorders are often considered the most frequently reported problem, with separation anxiety being the most commonly problem in this category (Bamberger & Houpt, 2006). An estimated 29% of pet dogs were

reported to show signs of anxiety-related behaviors, whereas 17% to 49% of all dogs have been estimated to demonstrate an aversion to noise (Blackwell et al., 2013; Landsberg et al., 2015). Anxiety is a reaction to the anticipation of potential or imagined danger or uncertainty, whereas fear is a state of alarm and agitation produced by the present or imminent danger (Sherman, 2008). As a result of separation-related disorders, anxiety, and behaviors associated with fear and phobia, dogs may also exhibit destructive behaviors, such as digging into furniture, chewing on doors, windows, or owners' clothing. However, young pets are generally prone to destructive patterns of behavior, since its alterations might stem from a desire to play or simply explore their surroundings (González et al., 2011). Behavioral issues are generally influenced by previous experiences, particularly during the sensitive phases of puppies, which cause an imbalance in nervous or hormonal transmission as a consequence of environmental and stressful experiences (Bacque-Cazenave et al., 2020; Howell et al., 2015; Sacchetti et al., 2023). In fact, early contact deprivation with their own mother, and lack of proper context-dependent stimuli during development predispose dogs for to the sensory deprivation syndrome, which generally brings about deficits in the management of sensory information within that environment. Patients experiencing such a condition generally suffer from anxiety and phobia, the persistence of which eventually can fall into a depressive state (Giussani et al. 2013; Riggio, 2017). The lack of the mother during the first months of life is also considered one of the predisposing factors to the Hypersensitivity-Hyperactivity Syndrome (HSHA), as defined in the French veterinary psychiatry model; the puppy has not learned self-controls, developing a deficient sensory filter and is unable to modulate stimuli. This occurs with a dysfunctional level of arousal (hyperactivity/hyperkinesis), an inability to modulate one's emotions (impulsivity) and learning (memory deficit), emotional and cognitive overflow (Pageat, 1998). Psychoactive drugs, including benzodiazepines, azapirones, tricyclic antidepressants, and selective serotonin reuptake inhibitors, are commonly prescribed in the treatment of behavioral disorders, particularly those associated with fear and anxiety (Landsberg et al., 2015). Among them, fluoxetine and clomipramine are the only drugs allowed to be used in dogs, although they both have some limitations, since they can take much time to achieve their full therapeutic effects. Nevertheless, some owners are reluctant or unwilling to administer these medications, because of their potential side effects or personal bias against psychotropic drug use (Mills & Cracknell, 2005). This makes it necessary to investigate new therapeutic aids, which can assist the behavioral rehabilitation program, and achieve compliance with the owner. To date, several studies supported a potential anxiolytic effect of some complementary supplements in dogs, such as alpha-casozepine, L-theanine, dog appeasing pheromone, tryptophan, cannabidiol, cannabis, essential oil, melatonin and probiotics (Araujo et al., 2010; Beata et al., 2007; Corsato Alvarenga et al., 2023; Dramard et al., 2018; Kim et al., 2010; Palestini et al., 2010; Sacchetti et al., 2023; Templeman et al., 2018). Thus, natural products might be regarded as a valid alternative to conventional therapy to face mood-related dysfunctions, being able to overcome potential toxicity or refractory events associated to the available drugs affecting CNS (Bódizs et al., 2020; Landsberg, 2005). An integrated approach is needed to address all issues that impact the pet's behavior and well-being (i.e., health, nutrition, environment, and behavioral management), whether the therapy relies on pharmacological or complementary approaches. Indeed, mood-related disorders can be faced by adopting a scheduled program, based on a tailored behavioral program, together with a proper environmental management techniques, that identify and address both the underlying cause of behavioral dysfunctions, as well as perpetuating factors (Landsberg et al., 2023). Therefore, in the present work we sought to explore the effects of a novel nutraceutical supplement (Calmina®, BuonaPet), enriched with natural anti-stress compounds (PEPTIDYSS®, Zinc, Vitamin B6, L-theanine, Griffonia simplicifolia, Lactobacillus helveticus HA-122, Magnesium), as an adjuvant to the rehabilitation treatment, in five dogs carried out for behavioral examination at the Veterinary Teaching Hospital (Department of Veterinary Medicine and Animal Production, University of Naples- Federico II). We hypothesize that these compounds, thanks to

their synergistic action, may help patients relax in cases of stress, fear, and discomfort, thus facilitating the behavioral rehabilitation program.

Case 1 Presentation

This case describes a 13-month-old entire male dog, 21 kg of weight, Dalmatian breed, adopted when he was two months from a box kennel, where he lived with his mother and six siblings. The pet parent requested a behavioral examination following ingestion of a cloth, and that was the reason why he ended up in surgery for an emergency gastrotomy.

History and Clinical Signs

The pet parent was a 32-year-old woman, who lived alone with the dog, in a house of downtown with terrace. She reported that the patient was thin and malnourished at the time of adoption, as well as an exuberant puppy, with a tendency to chew on different objects, such as pillows, stuffed animals, and furniture. He used to go for a walk three times a day with the caregiver since he was three months, and he was recognized to pull on the leash from the beginning. Each walk lasted more than an hour, mainly when the dog was free from the leash and allowed to socialize with other dogs and people. He used to eat commercial dry food three times a day, sleep with pet parent, with no night-time awakenings reported. The games usually played by the patient with the pet owner consisted of throwing a ball (predatory play, as chasing balls) and pull/spring (tug play) as well. He would spend more than 8 hours alone in the house, when chewing activities and destruction behaviors become more frequent. The patient used to jump over the people he met, as a way of greeting and catch the attention for soliciting play, or interaction.

Physical and Laboratory Evaluation

In the present case, diagnosis was based on the results of clinical examination and behavioral life history, to rule out any causes of these pica episodes; complete blood count and chemistry were normal, and no parasites in the faeces were found. In addition, examination with the gastroenterologist did not detect gastrointestinal disease.

Diagnosis and Treatment

After the behavioral examination, we prescribed the therapeutic intervention for destructive behaviors (Negar et al., 2020) based on an interdisciplinary approach, including a nutraceutical treatment together with behavioral treatment. To facilitate the achievement of a calm state and assist the behavioral program, we prescribed Calmina[®] at the dose of 3.5 ml/SID, administered one hour before the pet parent left him alone in the house.

The behavioral program was aimed at:

- enhancing the pet parent's awareness of the dog's developmental and ethological needs;
- increasing environmental enrichment;
- improving chewing activities;
- promoting independent activities.

In particular, his caregiver was given primary instructions to adequately manage the patient, which relied on reward behaviors and provide proper outlets, to accomplish breed and age typical needs, since young pets are generally prone to destructive behavior patterns because misbehavior might stem from a desire to play or just explore their surroundings (González Martínez et al.,

2011). Environmental enrichment was based on food toys and chew (such as Kong® with gastrointestinal wet food), either in the presence of the owner or not, restoring the state of calm during the daily routine. The dog was trained to daily perform olfactory research, involving finding a treat in the house, by following the verbal signal of “Search,” with the aim of lowering the state of emotional activation since olfactory research increases concentration and leads the dog to an intermediate level of arousal (d’Angelo et al., 2022). These olfactory activities were gradually made more complicated by changing settings or hiding the treat on the terrace. Additionally, the patient was resting on a mat, or chewing on an appropriate chew toy, as well as improving independent activities every day, such as puzzle solving. Furthermore, the owner was trained to a proper management of the environment, removing reinforcement, and preventing undesirable behaviors (for example, lift the curtains off the ground, remove tea towels, socks, and cloth objects in the environment where the dog was resting when supervision was not possible).

Follow-Up

Within a day of taking the supplement, the dog experienced an episode of self-limiting vomiting. Three days after integrated therapy- nutraceutical plus behavioral program-, the pet parent reported a reduction in arousal, a much calmer welcoming ritual, and no episodes of destruction when left alone in the house. After three months of integrated treatment, the patient had no recurrence of pica, the frequency of chewing behaviors was greatly reduced and directed toward food toys dedicated to him.



Figure 1. The dog was chewing a food toy, during the last follow up, while pet parent was talking to the behaviorist.

Case 2 Presentation

The case here reported describes a 12-year-old spayed female dog, 32 kg of weight, mixed breed, adopted one month before from a shelter, where she stayed 1.5 years. Pet parents, namely a 45-year – old woman, with her son and daughter – had no further information about the dog, since remote medical history was mute. They requested a behavioral examination for the dog, following disruptive behaviors she experienced, especially when left alone at home.

History and Clinical Signs

From the first week after adoption, the patient showed signs of anxiety, characterized by increased motor activity, constant or frequent reactivity, alertness, lack of focus, hypervigilance, and agitation at home and during the walk (Notari et al., 2022). The first disruptive episode occurred when she was alone at home, ten days after adoption. In that situation, she destroyed doors and windows. When the caregivers went back home, the patient was extremely agitated, showing tachypnoea, need for more attention and intense greeting behavior. The next day the dog was again left alone, and she damaged all other home stuff and furniture. Therefore, the pet parents found necessary to use a metal crate. Unfortunately, the solution didn't work at all because she was able to break even that. The dog was used to eat commercial dry food twice a day (with added steamed chicken) and sleep well in the pet parent's room. They used to take the dog for a walk three times a day, both in urban and suburban areas, also those equipped for dogs, where she was allowed to be free from the leash. However, she was generally reluctant to play, either alone or in company. The pet parents reported that their presence makes the dog feel heartened.

Physical and Laboratory Evaluation

In the present case, diagnosis was based on the results of clinicopathologic tests and behavioral life history; physical and behavioral evaluation to rule out any causes of disruptive behaviors. No parasites in the faeces were found. The complete blood count and chemistry were normal.

Diagnosis and Treatment

After the behavioral examination, we prescribed the therapeutic intervention for separation-related disorders and generalized anxiety, based on an integrated treatment to be addressed in the patient. Diagnosis of a canine separation-related problem generally relies on indirect evidence, based on the reported destruction or barking events by neighbors, since the behavior occur primarily in the absence of the owner (Sargisson, 2014). In addition, it is well known that dogs adopted from shelters, rescues, veterinary institutions, or show also other fears, phobias, and anxiety disorders are more likely to exhibit separated-related behavior (Bradshaw et al., 2002). To reduce the state of anxiety and avoid worsening of symptomatology, we prescribed, on the daily base, Calmina® at the dose of 5 ml/SID, administered one hour before when she was left alone in the house.

The behavioral program was aimed at:

- protecting the patient and home;
 - increasing environmental enrichment;
 - relaxing therapy;
 - modifying pet parents' departure and greeting behavior;
 - giving the patient time to create an attachment bond, and housing, without overexposing him.
- In particular, we invited the pet parents to coat the windows with cardboard and leave the dog

alone at home gradually. We prescribed to reinforce calm independence and reward it; administer food toys, since are helpful to incentivize the independent activities, since they are nourished with food that motivates the dog. Provided attention at the time of departures, take care to the dog with caresses and verbal greetings without amplifying them, thus helping to make owners a secure base for their dog (Teixeira et al., 2021).

Follow-Up

Within four days of initiating treatment, the patient was improved and capable of being alone in the house for about an hour; she slightly scratched the protections on the windows but no damage to the home nor self- injuries. The duration of treatment was two months, in which the patient had no recurrence of destructiveness, showed a calmer greeting behavior (no more overarousal) and, during the walk appeared more explorative and less inhibited. Even at home also began to express play engagement with family.

Case 3 Presentation

Here we described the case of an eight-month-old entire male dog, 11 kg of weight, mixed breed, adopted when he was two months from an animal volunteer. The pet parent requested a behavioral examination, since the dog demonstrated frenetic energy, overactivity, and separation anxiety.

History and Clinical Signs

The patient lived with a young couple (in their early 30s) in a house with garden and swimming pool, in the countryside. Unfortunately, the patient's history is substantially missing, in that the adopter did not provide any information neither about litter, nor parents who the patient belonged to. However, on the first clinical evaluation, the veterinarian assumed that he was just 4-5 weeks old, by looking at both deciduous incisors and canines erupted, and no presence of premolars (Modina et al., 2019). The patient had been showing exuberant himself since he was puppyhood, almost never rested during the day, used his mouth too much when cuddled, with a tendency to jump on both strangers and family members when engaged. He could not tolerate separation from his caregivers. Indeed, when he was left alone at home (even for few minutes) he would bark and howl in a compulsive way, hardly getting quiet. As reported by the neighbors, he could bark for up to 4 hours at a time. From the age of three months, he went out one time a day with the pet parents, for socializing with urban environment and people, whereas he spent the remaining time playing in the garden with the neighbors' dogs. He used to pull on the leash during the walk. At the age of five months, he became intrusive and impulsive, making him even more difficult to manage, hence he did not interact with other dogs, or even obeyed to stop signs by the pet parents. He ate commercial dry food three times a day, in a quiet corner of the kitchen, although he used to tip over the bowl, stare at it or rasp constantly, as well as prefer to eat off the floor. The patient slept with pet parents at night, while during the day he hardly ever, and they reported that after hours of activity he would suddenly collapse into a sleep lasting about half an hour. The dog tended to come up with dynamic games such as predatory play (chasing balls) and pull/spring (tug play) as well. The patient jumped over the people as a sign of greeting and soliciting play, attention, or interaction.

Physical and Laboratory Evaluation

In the present case, diagnosis was based on the results of clinicopathologic testing and behavioral life history, such as physical, neurological, and thyroid evaluation, to rule out any other alterations related to the mood-related disorders. During behavioral examination at home, the patient was continuously in a state of overactivity (pacing, barking, chewing), distractibility, short attention spans, impulsiveness, repetitive behavior on the bowl, lack of trainability, and failure to settle in calm, along with somatic signs associated to tachycardia, tachypnoea, and salivation. When a chewing toy was proposed, he kept steering it for a few seconds, and then moved on to propose a new activity.

Diagnosis and Treatment

After the behavioral examination, we diagnosed the hypersensitivity–hyperactivity syndrome (as defined by the French veterinary psychiatry community) and prescribed an interdisciplinary approach based on rehabilitation plan together with a SSRI (selective serotonin reuptake inhibitors) treatment, fluoxetine (Pageat, 1998; Bleuer-Elsner et al., 2021). Pet parents were reluctant to administer him with SSRI treatment, and that was the reason why we decided to administer Calmina® (3,5 ml/SID) every day, as helping for anxiety and psychological stress. In particular, the supplement was given to the patient one hour before just he was left alone at home, or in the morning if the parents stayed at home for work, without giving attention to him.

The behavioral program was aimed at:

- enhancing the pet parent’s awareness of the dog’s communication and ethological needs;
- improving the state of calm and self-control;
- promoting independent and olfactory activities.

In particular, the caregiver was given instructions to adequately manage the patient, relying on reward positive behaviors, and provide adequate outlets necessary to address age-dependent needs. In addition, pet parents were trained to have structured interactions with the dog, using as minimally excitatory communication as possible, restrained, and essential gestures, voice control and proper physical contact (Giussani et al., 2013). Remove reinforcement and prevent unwanted behavior (for example, they learned not to involve the dog when he was playing with the Kong® or did not propose exciting games, rather they avoided forms of punishment and ignored attention seeking behaviors). Moreover, the dog was trained to perform olfactory research, involving finding a treat in the house and garden, by following the verbal signal of “Search”, with the aim of lowering the state of emotional activation (d’Angelo et al., 2022). Pet parents were instructed on the graduality of these olfactory activities and short working times, followed by a rest period in which the dog could reframe the experience. Independent activities were based on food toys and chew (i.e., Kong®), either in the presence or absence of the owners; rewarded the state of calm during the daily routine (resting on a mat or chewing on an appropriate chew toy), such as puzzle solving, ensuring that it is not difficult or impossible to manage, which would lead to increased frustration and reduced self-efficacy.

Follow-Up

Seven days from Calmina® treatment, the pet parents reported a reduction in separation anxiety when left alone in the house, even up to four hours (caregivers monitored him through video cameras and noticed that after a few minutes of crying he curled up on the bed). The treatment lasted four months, in which the patient had calmer separation and welcoming ritual, as well as an easier management at home during daily routine. However, symptoms were getting worse when the dog faced exceptional events (i.e., a pool party), so he still showed up very exuberant. The not

easy handling on walks, and the interaction with other dogs were unchanged. Therefore, thanks to the fully compliance of the owners, after four-month-Calmina® treatment, the patient was administered fluoxetine, together with the rehabilitation program.

Case 4 Presentation

We documented the case of a nine-year-old male dog, 18 kg of weight, mixed breed hunt dog, adopted when he was 2 years old from a shelter, in which he lived for a few months. He lives with a woman and his elderly mother in a downtown apartment, close to city parks. The pet parents asked for a behavioral examination, because of a bite event against the younger pet parent, who strived to contain him when his harness slipped off during a walk on holiday.

History and Presenting Signs

Before getting into the shelter, the patient was owned by a hunter, who kept him in a kennel with other dogs. Since the time of adoption, the dog appeared shy and showed a lot of reactivity in being caressed, manipulated, making even difficult to put the harness, that was left on his body night and day. The aggression episode happened while on holiday; in particular, the owners were waiting for the lift to go out, while the younger pet parent realized that the harness was coming off. Thus, she got scared, raised her voice, and made chaotic movements, forcibly leading him in the room. From that episode on, she wasn't allowed to approach him anymore, because he ran away and growled, thus turning into inability to wear the harness and take him out for a walk. Consequently, house soiling was one of the main concerns that the owners had to be faced. The patient ate dry commercial food with wet ones twice a day. He was very interested in food but did not show aggression in defending the food. He slept on the sofa or on the bed with the pet parent; however, if the owners tried to interact with him, he walked away. The animal did not have a particularly deep sleep, waking up easily and having everything under control. Before the aggression episode, the dog and the younger pet parent woke up early and went out for a walk, then stayed at home with the older pet parent. Two more walks during the day, one in the afternoon and one in the evening. After the aggression episode, the patient is no longer wanted to go out, not showing need for. To manage this reluctance, the caregivers attempted to lead him on a walk without a leash to the shared garden, that has the access directly from the building; although it occurred without physical contact, is not welcomed by the dog. The caregivers reported that patient didn't play much, he had one or two of his own games and requested the pet parents to throw to him, for a few repetitions. After the aggression episode, he reduced gaming activity.

Physical and Laboratory Evaluation

In the present case, diagnosis was based on the results of life history and behavioral examination; no physical examination, complete blood count, chemistry and thyroid levels were performed, considering the dog's handling difficulties. The previous veterinary examination happened a few months earlier. During behavioral examination at home, the patient showed reduced exploratory behavior and disengage from the social interaction with avoidance behavior, not any threatening behavior. Moreover, he became anxious whenever he saw the leash, and reacted by hiding and growling. When invited to walk in the shared garden, the patient looked insecure, and every time he tried to get back home.

Diagnosis and Treatment

After the behavioral examination, we diagnosed a situational fear in a patient affected by Sensory Deprivation Syndrome (as defined by the French Veterinary Psychiatry Community), due to the hypo-stimulating environment in which he grew up (Pageat, 1998; Landsberg et al., 2023). The traumatic triggering event occurred when he was taken in a new environment, with different exposure from the daily routine, even making difficult the pet parent management. Thus, such unpleasant stimuli, together with the poor database of experience and emotional state of the owners, contributed to the trigger of situational fear (Landsberg et al., 2015). The sensitizing event brought about a rift in the bond, so that both dog and pet parents lost trust each other. The patient did appear irritated and scared when he was touched by his parent, who was afraid of being attacked. Therefore, we prescribed him 3.5 ml/SID Calmina[®] treatment, with the aim of reducing the activation of the biological stress and fear response and facilitating the behavioral rehabilitation plan by the family.

In particular, we prescribed him:

- To restore a predictable daily routine;
- A gradual exposure to fearful situations, to help break the pattern of avoidance and fear.

Pet parents were advised to offer him to go out into the shared garden, with a joyful and welcoming voice, and for a short time. Once the previous experience was consolidated, a fixed collar was put on to the dog, as the owners stood at the door, to access the shared garden. Finally, they were suggested to take him out for a walk, by attaching the leash in front of the exit gate.

Follow-Up

Seven days following the integrated treatment, the dog appeared calmer at home and showed again play activity. He went out regularly in the shared garden at least once a day. After 14 days, the pet parent managed to put the fixed collar on the dog and then attached the leash. Three weeks later from the nutraceutical and behavioral prescriptions, the patient let the pet parent hook the leash without any anxiety signs, welcoming the ritual for the walk.

Case 5 Presentation

We documented the case of a 2-year-old entire male dog, 10 kg of weight, mixed breed, adopted 10 days before the behavioral examination. He lived with a 40-year-old couple in a country house, equipped with a garden. The pet parents asked for a behavioral examination because the patient was freezing with emotional urination, whenever he had to wear the harness and leash, as well as he woke up welcoming the pet parents or guests who got in the house.

History and Clinical Signs

The pet parents reported that the dog was found by an animalist volunteer in a semi-rural context, disoriented, immobile and frightened of car noise. He looked for protected spaces (under hedges or under trees), to take refuge and stay there unmoving. The caregivers had to grab him to take him in the house, where he even remained motionless for a while. Since the adoption, the dog had been feeling scared by the home context, cars, and manipulations. Although the remote behavioral and medical history was missing, we hypothesized that he grew up in a rural area with limited exposure to the outside world. The patient went out to elimination urine and faeces four times a day, carried by his pet parent's arms on a piece of land just outside the house, without a leash, because once the leash was attached, he froze. When he was unleashed, he moved not ex-

hibiting any issues, sometimes running away, and going towards the nearby fields, returning only after a few minutes. He spent almost the whole day outside in the garden, in which he did not eliminate faeces and urine, except on outside of it. The pet parents reported that they were only able to cuddle him if they sat on the ground and wait for the dog to approach. When pet parents went at work, the dog stayed inside the house alone, not showing signs of discomfort. He was worried and scared in the car, drooling and vomiting. He ate dry industrial food twice a day. He was very interested in food, which he reached out only when the pet parents went away from the bowl, and he did not show aggression in defending the food. He slept well outside. At home he woke up more easily, especially if humans moved. He never tried to get on the couch or go upstairs. According to what reported by the caregivers, he did not spend much time in playing, in that seven days from the adoption he begun to play alone with a rope ball, but he didn't pursue it when the pet parents tried to play with him by throwing it. When he found things on walk, like branches, he took them into the garden and chewed them. During behavioral examination at home, the patient showed reduced exploratory behavior and disengage from the social interaction with avoidance behavior refuging under the hedge; he just approached with food.

Diagnosis and Treatment

The patient was affected by Sensory Deprivation Syndrome, due to the hypo-stimulating environment in which he grew up in the early stages of development, as well as the change of environment and the loss of points of reference (Riggio, 2017). We prescribed administration of Calmina® (2 ml/SID), helping for anxiety and psychological stress, together with a specific rehabilitation program. In this respect, during the first examination we gave primary instructions to the pet parents how to adequately manage the patient, mainly based on:

- providing time enough to create an attachment bond, and adapting to his home and context, without overexposing him. In addition, they were advised to keep the front door open, to allow him to freely get in and out the garden, and serve him the exit towards the neighboring land, without forcing him and avoiding picking him up;
- cuddle session from the ground at his pleasure, introducing the harness by placing it on the ground with treats, taking care of raising it on the neck progressively and, by the way, giving him space enough to lower the emotional pressure;
- reward and playing activities soon after the stroll (or short trips by car), to allowing him to feel more comfortable towards external stimuli, and more manageable by the owners.

Follow-Up

The patient looked like calmer at home, was much less resistant to the collar and leash for longer walks just after one week of the combined treatment. He started interacting with balls, playing the “toss and fetch” with his caregivers. He also asked for cuddle in other situations than before the therapy, although he still ran away when he came across unfamiliar. Two weeks later, the dog was able to wear the harness even on walks, which was even easier for him. To enter the house, the owner used the leash and harness, without picking him up. After a month, in the presence of the pet parents welcomed them allowing being cuddled. The pet parents introduced a dog sitter, who soon managed to take him for a walk. The dog started going upstairs. He very often looked for contacts with pet parents and accepted manipulation in all areas of his body, followed them at home and outdoors and increased his play activities even with “push-and-pull” and chasing. After 45 days the dog entered and leaved the house alone and was much less alert, except for noisy objects (such as the vacuum cleaner which, if turned off, he could get closer with the pet parents help). To go out on walk, he wagged his tail and inserted his head by himself to put on the harness. After two months of treatment, he was still afraid of the car but tolerated short trips without vomiting.

Discussion

The present study detailed the effects of Calmina[®], together with rehabilitation plan in five cases of behavioral issues, managing stress and anxiety, and assisting dogs in the changes induced by behavioral rehabilitation. Complementary medicine is often utilized to address, in an integrated approach, all the issues that impact the pet's behavior and well-being. The composition of Calmina[®] is based on PEPTIDYSS[®], L-theanine, Griffonia simplicifolia, Lactobacillus Helveticus, Vitamin B6, Zinc, Magnesium.

Peptidyss is a protein hydrolysate from the by-products of sardine processing, made by state-of-the-art biotechnological processes, whose molecular weight is mostly concentrated below 1,000 Daltons. This is a major determinant for the absorption of Peptidyss. The use of natural resources, such as fish hydrolysates, may be an interesting strategy to improve stress management (Dinel et al., 2021). Protein hydrolysis enhances the functional properties of the product, thus allowing the release of low-molecular-weight peptides with diverse bioactivities, which have various effects, as antioxidant, antimicrobial, antihypertensive, anti-inflammatory, antihyperglycemic, skin anti-aging and anxiolytic ones (Bernet et al., 2000; Carrera et al., 2013; Gevaert et al., 2016; Le Faouder et al., 2022; Zamora-Sillero et al., 2018). Beyond the anxiolytic effect, the anti-inflammatory and antioxidant effects should not be underestimated in patients with behavioral alterations found in our five cases, since a recent review highlighted the links between inflammation and behavioral diseases in dogs and cats (Piotti et al., 2024). In fact, cytokine storm may impact brain functioning, resulting in a mind disease (Susan et al., 2001).

L-theanine is an amino acid naturally present in green tea (*Camellia sinensis*), and recent clinical studies have proposed adjuvant effects of L-theanine, helping for anxiety and psychological stress. Indeed, given its structural similarity with glutamic acid, L-theanine modulates the excitatory neurotransmission, by inhibiting neuronal glutamine uptake which, in turn, suppresses the conversion of glutamine to glutamate, thus highlighting its potential role in counteracting anxiety-related disorders (Nathan et al., 2006). Several studies in pets reported an effect of L-theanine in reducing noise fears and phobia, storm-related anxiety fear of unfamiliar people (Berteselli & Michelazzi, 2007; Dramard et al., 2018; Pike et al., 2015). In a previous study, Nathan and colleagues documented that L-theanine showed highest concentrations in blood and liver one hour after administration, and enter the CNS after 5 hours, with a significant decrease within 24 hours thus suggesting a rapid onset (Nathan et al., 2006). This rapid anxiolytic effect was needed in cases 2, 3, 4 and 5 in which anxiety sustained the dysfunctional behaviors of the patients; in fact, the pet parents reported a reduction in anxious behaviors as early as a few days after administration of the supplement, improving confidence in therapeutic intervention and facilitating behavioral rehabilitation activities, on which anxiety negatively impacts (Küçük et al., 2008).

A different compound in Calmina[®] is the leguminous plant *Griffonia simplicifolia* Baill, perennial woody shrub that grows in the tropical rainforest of central and West Africa. The chemical constituents of the seeds have been studied intensively since 1960, and they were found rich in 5-hydroxy-1-tryptophan (5-HTP), a direct precursor in the synthesis of serotonin (5-HT) (Carnevale et al., 2011). Thus, dietary supplementation of L-tryptophan has been found to increase 5-hydroxyindoleacetic acid (5-HIAA) to serotonin ratio in the brain, inducing an inhibitory effect on aggressive behavior, most likely increasing brain serotonergic activity, and improving fear and competitive behaviors and territorial aggression as well (DeNapoli et al., 2000; Robinson et al., 2020). This effect was beneficial in cases 2, 3, and 4, by mimicking the action of serotonergic-acting psychotropic drugs. Accordingly, during the follow-up, occurred few weeks later, the owners reported that their dog was calmer and more pleasant. This was particularly evident in the case 3, where the pet parents previously refused fluoxetine-based therapy, willingly accepting the administration of Calmina[®], rather. Thanks to the improvements with this supplement, the family have solidified their therapeutic alliance with the veterinary behaviorist, basically relying on therapy

(rehabilitation program plus fluoxetine). In fact, we noticed an improvement in all symptoms, most likely mediated by serotonergic tone. In recent years, much emphasis is being placed on the connection between brain, behavior, and gut microbiota, which is regarded as one of the crucial players in the onset and severity of stress-related disorders, through different mechanisms, such as the enteric nervous system, the sympathetic nervous system, the HPA-axis, immune signaling and gut bacterial metabolites (Dinan & Cryan, 2017). Several studies highlighted benefits of probiotics to preserve or restore a healthy condition, through the microbiota-gut-brain axis (Mondo et al., 2019). It seems that probiotics alleviate psychological disorders by acting on multiple fronts, namely protecting the gut barrier, improving mucosal and systemic immunity, balancing the function of the hypothalamic-pituitary-adrenal axis, influencing neurotransmitters and levels of neurotrophic factors from the brain (de Punder & Pruimboom, 2015; Dhaliwal et al., 2018; Galley et al., 2014). Indeed, Calmina® is enriched with *Lactobacillus helveticus*, a strain capable to defeat stress, anxious or depressive-like behaviors in a mouse model of subchronic mild social (Maehata et al., 2019). Yu-Min Yeh and collaborators, in a recent study reported that the canine aggression and separation anxiety were likely to be ameliorated by effects of *Lactiplantibacillus plantarum* and decreased plasma serotonin turnover ratio with separation anxiety (Yeh et al., 2022). Moreover, administration of a single *Lactobacillus* strain improves stress-induced behaviors in (Bharwani et al., 2017). This was especially helpful in cases 1, 2 and 5, as the patients were young and the addition of a *Lactobacillus*-based probiotic has been able to improve microbiota health and, consequently, reduce the emotional discomfort. We believe that *Lactobacillus* genus would have positive effects on ameliorating behavioral issues through the intestinal neuromodulation. Indeed, *Lactobacillus* levels decrease after stress (Marin et al., 2017). Behavioral treatment plans should adopt an integrated approach that incorporates medications, environmental management, and behavioral modification. While psychotropic compounds may be beneficial in many instances, they may not be the most popular choice for some patients and may not be suitable in every clinical case. In fact, Italian pet parents often are worried using psychotropic drugs on their pets and may prefer supplements administration along with other interventions (Riggio, 2017). Of note, these compounds are likely to be safe and have minimal side effects, compared to pharmacologic agents, and relatively palatable, thus even making treatment easier for the caregivers. Furthermore, it is important to point out that supplements based on the aforementioned compounds take advantage of the synergistic effect, due to formulation with poly components, providing greater efficacy but also greater safety (compared to the one component), as reported by Crayhon (2001). In an integrated approach, treatment of dogs affected by behavioral issues should be considered in a holistic way, with an overview that considers the patient's personality and diseases, family relationship, and environment.

Conclusions

According to these case studies, the administration of Calmina® can reduce anxiety, stress and may facilitate rehabilitation therapy in treating behavioral disorders. Further studies are needed to better address the potential effects arising from long-term Calmina® treatments, as well as tolerance and safety. Although the analyzed reports can't offer conclusive a proof, they can be helpful in emphasizing observations crucial to the behavioral field.

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References

- Araujo J.A., Riveraa C., Ethierb J.L., Landsbergb G.M., Denenbergc S., Arnold S., Milgram N. ANXI-TANE tablets reduce fear of human beings in a laboratory model of anxiety-related behavior. *J. Vet Behav.* 5:268-275; 2010.
- Bacque-Cazenave J., Bharatiya R., Barriere G., Delbecque J.P., Bouguiyou N., Di Giovanni G., Cattaert D., De Deurwaerdere P. Serotonin in Animal Cognition and Behavior. *Int J Mol Sci.* 21(5):1649; 2020.
- Bamberger M., Houpt K.A. Signalment factors, comorbidity, and trends in behavior diagnoses in cats: 736 cases (1991-2001). *J Am Vet Med Assoc.* 229:1602-1606; 2006.
- Beata C., Beaumont-Graff E., Coll V., Cordel J., Marion M., Massal N., Marlois N., Tauzin J. Effect of alpha-casozepine (Zylkene) on anxiety in cats. *J Vet Behav.* 2:40-46; 2007.
- Bernet F., Montel V., Noel B., Dupouy J.P. Diazepam-like effects of a fish protein hydrolysate (Gabolysat PC60) on stress responsiveness of the rat pituitary-adrenal system and sympathoadrenal activity. *Psychopharmacology (Berl).* 149:34-40; 2020.
- Berteselli G.V., Michelazzi M. Use of L-theanine tablets (Anxitane™) and behavior modification for treatment of phobias in dog: A preliminary study. *J. Vet. Behav.* 2(3):101; 2007.
- Bharwani A., Mian M.F., Surette M.G., Bienenstock J., Forsythe P. Oral treatment with *Lactobacillus rhamnosus* attenuates behavioral deficits and immune changes in chronic social stress. *BMC Med.* 15:7; 2017.
- Blackwell E.J., Bradshaw J.W.S., Casey R.A. Fear responses to noises in domestic dogs: Prevalence, risk factors and co-occurrence with other fear related behavior. *Appl. Anim. Behav. Sci.* 145 (1-2):15-25; 2013.
- Bleuer-Elsner S., Muller G., Beata C., Zamansky A., Marlois N. Effect of fluoxetine at a dosage of 2-4 mg/kg daily in dogs exhibiting hypersensitivity-hyperactivity syndrome, a retrospective study. *J Vet Behav.* 44:25-31; 2021.
- Bódizs R., Kis A., Gácsi M., Topál J. Sleep in the dog: comparative, behavioral and translational relevance. *Curr Opin Behav Sci.* 33:25-33; 2020.
- Bosch G., Beerda B., Hendriks W.H., van der Poel A.F., Verstegen M.W. Impact of nutrition on canine behavior: current status and possible mechanisms. *Nutr Res Rev.* 20:180-194; 2007.
- Bradshaw J.W., McPherson J.A., Casey R.A., Larter S. Aetiology of separation-related behavior in domestic dogs. *Vet Rec.* 151:43-46; 2002.
- Carnevale G., Di Viesti V., Zavatti M., Zanolini P. Anxiolytic-like effect of *Griffonia simplicifolia* Baill. seed extract in rats. *Phytomedicine.* 18:848-851; 2011.
- Carrera M., Canas B., Gallardo J.M. The sarcoplasmic fish proteome: pathways, metabolic networks and potential bioactive peptides for nutritional inferences. *J Proteomics.* 78:211-220; 2013.
- Corsato Alvarenga I., Panickar K.S., Hess H., McGrath S. Scientific Validation of Cannabidiol for Management of Dog and Cat Diseases. *Annu Rev Anim Biosci.* 11:227-246; 2023.
- Crayhon, R., 2001. The Synergistic Approach: The Future of Nutrition Therapy. *Townsend Letter for Doctors and Patients*, (213), 38.
- d'Angelo D., Sacchettino L., Carpentieri R., Avallone L., Gatta C., Napolitano F. An Interdisciplinary Approach for Compulsive Behavior in Dogs: A Case Report. *Front Vet Sci.* 9:801636; 2022.
- d'Angelo D., Sacchettino L., Quaranta A., Visone M., Avallone L., Gatta C., Napolitano F. The Potential Impact of a Dog Training Program on the Animal Adoptions in an Italian Shelter. *Animals (Basel).* 12(14):1759; 2022.
- de Punder K., Pruimboom L. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front Immunol.* 6:223; 2015.
- DeNapoli J.S., Dodman N.H., Shuster L., Rand W.M., Gross K.L. Effect of dietary protein content and

- tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in dogs. *J Am Vet Med Assoc.* 217:504-508; 2000.
- Dhaliwal J., Singh D.P., Singh S., Pinnaka A.K., Boparai R.K., Bishnoi M., Kondepudi K.K., Chopra K. *Lactobacillus plantarum* MTCC 9510 supplementation protects from chronic unpredictable and sleep deprivation-induced behavior, biochemical and selected gut microbial aberrations in mice. *J Appl Microbiol.* 125:257-269; 2018.
- Dinan T.G., Cryan JF. Gut-brain axis in 2016: Brain-gut-microbiota axis - mood, metabolism and behavior. *Nat Rev Gastroenterol Hepatol.* 14:69-70; 2017.
- Dinel A.L., Lucas C., Le Faouder J., Bouvret E., Pallet V., Layè S., Joffre C. Supplementation with low molecular weight peptides from fish protein hydrolysate reduces acute mild stress-induced corticosterone secretion and modulates stress responsive gene expression in mice. *J. Funct. Foods.* 76: 104292; 2021.
- Dramard V., Kern L., Hofmans J., Reme C.A., Nicolas C.S., Chala V., Navarro C. Effect of l-theanine tablets in reducing stress-related emotional signs in cats: an open-label field study. *Ir Vet J.* 2018; 71:21.
- Galley J.D., Nelson M.C., Yu Z., Dowd S.E., Walter J., Kumar P.S., Lyte M., Bailey M.T. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol.* 2014; 14:189.
- Gevaert B., Veryser L., Verbeke F., Wynendaele E., De Spiegeleer B. Fish Hydrolysates: A Regulatory Perspective of Bioactive Peptides. *Protein Pept Lett.* 23:1052-1060; 2016.
- Giussani S., Colangeli R., Fassola F., Merola I., Possenti M., 2013. *Medicina comportamentale del cane, del gatto e di nuovi animali da compagnia.* Poletto Editore. Milan.
- González M.A., Santamarina P.G., Diéguez Casalta F.J., Suárez Rey M.L., De la Cruz P.L.F. Risk factors associated with behavioral problems in dogs. *J. Vet. Behav.* 6(4):225–231; 2011.
- Howell T.J., King T., Bennett P.C. Puppy parties and beyond: the role of early age socialization practices on adult dog behavior. *Vet. Med. (Auckl).* 6:143-153; 2015.
- Kim Y.M., Lee J.K., Abd el-aty A.M., Hwang S.H., Lee J.H., Lee S.M. Efficacy of dog-appeasing pheromone (DAP) for ameliorating separation-related behavioral signs in hospitalized dogs. *Can. Vet. J.* 51:380-384; 2012.
- Küçük A., Gölgeli A., Saraymen R., Koç N. Effects of age and anxiety on learning and memory. *Behav. Brain Res.* 195(1):147-52; 2008.
- Landsberg G. Therapeutic agents for the treatment of cognitive dysfunction syndrome in senior dogs. *Prog. Neuropsychopharmacol Biol. Psychiatry.* 29:471-479; 2005.
- Landsberg G., Radosta L., Ackerman L., 2023. *Behavior Problems of the Dog and Cat.* 4th Edition - Elsevier.
- Landsberg G., Mougeot I., Kelly S., Milgram N.W. Assessment of noise-induced fear and anxiety in dogs: Modification by a novel fish hydrolysate supplemented diet. *J. Vet. Behav.* 2015; 10.
- Le Faouder J., Arnaud B., Lavigne R., Lucas C., Com E., Bouvret E., Dinel A.L., Pineau C. Fish Hydrolysate Supplementation Prevents Stress-Induced Dysregulation of Hippocampal Proteins Relative to Mitochondrial Metabolism and the Neuronal Network in Mice. *Foods.* 11(11):1591; 2022.
- Maehata H., Kobayashi Y., Mitsuyama E., Kawase T., Kuhara T., Xiao J.Z., Tsukahara T., Toyoda A. Heat-killed *Lactobacillus helveticus* strain MCC1848 confers resilience to anxiety or depression-like symptoms caused by subchronic social defeat stress in mice. *Biosci. Biotechnol. Biochem.* 83:1239-1247; 2019.
- Marin I.A., Goertz J.E., Ren T., Rich S.S., Onengut-Gumuscu S., Farber E., Wu M., Overall C.C., Kipnis J., Gaultier A. Microbiota alteration is associated with the development of stress-induced despair behavior. *Sci. Rep.* 7:7:43859; 2017.
- Mills D., Cracknell N. Management of fear of fireworks in dogs. *Vet. Rec.* 157(18):.564; 2005.
- Modina S.C., Andreis M.E., Moioli M., Di Giancamillo M. Age assessment in puppies: Coming to terms with forensic requests. *Forensic Sci. Int.* 297:8-15; 2019.
- Mondo E., Marliani G., Accorsi P.A., Cocchi M., Di Leone A. Role of gut microbiota in dog and cat's health and diseases. *Open Vet. J.* 9:253-258; 2019.
- Mota-Rojas D., Mariti C., Zdeinert A, Riggio G., Mora-Medina P., Del Mar Reyes A., Gazzano A., Domínguez-Oliva A., Lezama-García K., José-Pérez N., Hernández-Ávalos I. Anthropomorphism and Its Adverse Effects on the Distress and Welfare of Companion Animals. *Animals (Basel).* 11(11):3263; 2021.

- Nathan P.J., Lu K., Gray M., Oliver C. The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent. *J. Herb. Pharmacother.* 6:21-30; 2006.
- Negar D., Mahdi P.B., Reza A., Bahman M. Problematic behaviors in companion dogs: A survey of their prevalence and associated factors. *J. Vet. Behav.* 39: 6-13; 2020.
- Notari L., Kirton R., Mills D.S. Psycho-Behavioral Changes in Dogs Treated with Corticosteroids: A Clinical Behavior Perspective. *Animals (Basel).* 12(5):592; 2022.
- Overgaauw P.A.M., Vinke C.M., Hagen M., Lipman L.J.A. A One Health Perspective on the Human-Companion Animal Relationship with Emphasis on Zoonotic Aspects. *Int. J. Environ. Res. Public Health.* 17(11):3789; 2020.
- Pageat P., 1998. *Pathologie du comportement du chien.* Editions du point Vétérinaire, Maison-Alfort.
- Palestrini C., Mineroa M., Cannasa S., Bertesellia G., Scagliaia E., Barbieria S., Cavalloneb E., Puricellic M, Servida F., Dall'Ara P. Efficacy of a diet containing caseinate hydrolysate on signs of stress in dogs. *J. Vet. Behav.* 5:309-317; 2010.
- Pike A.L., Horwitz D.F., Lobprise H. An open-label prospective study of the use of l-theanine (Anxitane) in storm-sensitive client-owned dogs. *J. Vet. Behav.* 10:324-331; 2015.
- Piotti P., Pierantoni L., Albertini M., Pirrone F. Inflammation and Behavior Changes in Dogs and Cats. *Vet. Clin. North Am. Small Anim. Pract.* 54(1):1-16; 2024.
- Riggio G. A case of Sensory Deprivation Syndrome in a mongrel dog. *Dog Behavior.* 1:31-36; 2017.
- Robinson E., Templeman J.R., Thornton E., Croney C.C., Niel L., Shoveller A.K. Investigating the effects of incremental conditioning and supplemental dietary tryptophan on the voluntary activity and behavior of mid-distance training sled dogs. *PLoS One.* 15:e0232643; 2020.
- Sacchettino L., Gatta C., Chirico A., Avallone L., Napolitano F., d'Angelo D. Puppies Raised during the COVID-19 Lockdown Showed Fearful and Aggressive Behaviors in Adulthood: An Italian Survey. *Vet. Sci.* 10(3):198; 2013.
- Sacchettino L., Gatta C., Giuliano V.O., Bellini F., Liverini A., Ciani F., Avallone L., d'Angelo D., Napolitano F. Description of Twenty-Nine Animal Hoarding Cases in Italy: The Impact on Animal Welfare. *Animals.* 13(18):2968; 2023.
- Sacchettino L., Gatta C., Maruccio L., Boncompagni C., Napolitano F., Avallone L., d'Angelo D. Combining cannabis and melatonin treatment with a rehabilitation program improved symptoms in a dog with compulsive disorder: A case report. *Res. Vet. Sci.* 160:26-29; 2023.
- Sargisson R.J. Canine separation anxiety: strategies for treatment and management. *Vet. Med. (Auckl).* 5:143-151; 2014.
- Sherman B.L. Separation anxiety in dogs. *Compend. Contin. Educ. Vet.* 30:27-42; 2008;.
- Siracusa C., Provoost L., Reisner I.R. Dog- and owner-related risk factors for consideration of euthanasia or rehoming before a referral behavioral consultation and for euthanizing or rehoming the dog after the consultation. *J. Vet. Behav.* 22:46-56; 2017.
- Susan L. J., Adrian D.J. Behavioral Effects of Cytokines. *Brain Behav. Immun.* 15 (4): 371-387; 2001.
- Templeman J.R., Davenport G.M., Cant J.P., Osborne V.R., Shoveller A.K. The effect of graded concentrations of dietary tryptophan on canine behavior in response to the approach of a familiar or unfamiliar individual. *Can. J. Vet. Res.* 82:294-305; 2018.
- Texeira A.R., Hall N.J. Effect of greeting and departure interactions on the development of increased separation-related behaviors in newly adopted adult dogs. *J. Ve.t Behav.* 41: 22-32;2021.
- Yeh Y.M., Lye X.Y., Lin H.Y., Wong J.Y., Wu C.C., Huang C.L., Tsaid Y.C., Wang Y.C. Effects of *Lactiplantibacillus plantarum* PS128 on alleviating canine aggression and separation anxiety *Appl. Anim. Behav. Sci.* 247:105569; 2022.
- Zamora-Sillero J., Gharsallaoui A., Prentice C. Peptides from Fish By-product Protein Hydrolysates and Its Functional Properties: an Overview. *Mar Biotechnol (NY).* 20:118-130; 2018.

Effetti benefici di un nuovo integratore nutraceutico, Calmina®, (buonapet)
sui disturbi comportamentali nei cani: 5 case report

Luigi Sacchettino¹, Viviana Orsola Giuliano¹, Anna Terracciano², Federica Manunta³, Luigi Avallone¹,
Danila d'Angelo¹, Francesco Napolitano^{1,4*}

¹ *Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli
Federico II, 80137 Napoli, Italia*

² *Veterinario Comportamentalista, 04020 Itri, Italia*

³ *Veterinario Comportamentalista e consulente presso la "Libera Accademia Cinofila Italiana Interdisciplinare"
(LAICI), 25126 Brescia, Italia*

⁴ *CEINGE-Biotecnologie Avanzate Franco Salvatore, 80145 Napoli, Italia*

Sintesi

I disturbi comportamentali negli animali domestici, tra cui l'aggressività, l'ansia da separazione o la fobia dei rumori, sono sempre più diffusi negli ultimi anni e raggiungono oltre l'80% in tutto il mondo, rappresentando così una delle principali preoccupazioni per il benessere degli animali e per il rapporto uomo-animale. Nonostante la disponibilità di terapie convenzionali, basate sull'uso di farmaci piuttosto efficaci, i caregivers degli animali domestici sono spesso riluttanti al loro utilizzo, preferendo piuttosto i composti naturali. Pertanto, lo scopo del presente studio è stato quello di descrivere gli effetti di un nuovo integratore alimentare, Calmina®, in cinque cani di famiglia, affetti da comportamenti disfunzionali. A seconda della gravità dei sintomi, gli animali sono stati trattati da uno a quattro mesi. Abbiamo osservato un miglioramento dei sintomi comportamentali e una maggiore facilità di gestione da parte dei proprietari. Nel complesso, i nostri dati preliminari suggeriscono che la somministrazione di Calmina® potrebbe essere utilizzata per ridurre i comportamenti legati all'ansia e facilitare il programma di riabilitazione. Sono necessari ulteriori studi per analizzare meglio i potenziali effetti in un campione più ampio di cani con disturbi comportamentali e nel trattamento a lungo termine, nonché la tolleranza e la sicurezza.