

Onset of Activity of a Fixed Association of Fish Hydrolysate Proteins and Superoxide Dismutase in Dogs with Inappropriate Behaviours: An Open Pet-owners' Evaluation

Marlène Lacreusette^{1*}, Sarah Muller¹, Bruno Jahier² and Christelle Navarro¹

¹Marketing and Communication, MP Labo, Grasse, France

²Scientific Affairs, MP Labo, Grasse, France

*Corresponding Author: Marlène Lacreusette, Marketing and Communication, MP Labo, Boulevard Marcel Pagnol, Grasse, France.

Received: March 14, 2024; Published: April 11, 2024

Abstract

Complementary feeds are identified as an additional option to behavioural therapy and pharmacological treatments for the management of inappropriate behaviours in dogs. This study aimed to investigate the benefit of a combination of hydrolysed fish proteins and melon superoxide dismutase (Anxivet[®], MP Labo, France) formulated to reduce signs of fear in dogs. Fifty dogs were administered with the combination once daily for 28 days. Pet-owners completed a questionnaire before (D0) and after (D2, D7, D14 and D28) product administration. Results showed a significant improvement of 8 out of 24 behavioural signs ($0.008 < p < 0.0425$) and of the overall behavioural score ($p = 0.0150$) at D28 compared to baseline. The interaction with the surroundings and the inappropriate behaviours evolution were enhanced in more than 50% of the dogs as soon as D2 and continued to improve significantly over time ($p < 0.05$). Similarly, the owner satisfaction level was almost 6 on a scale of 1 to 10 at D2, and then significantly increased throughout the study period ($p < 0.0259$). In conclusion, this study suggests that the tested product demonstrates rapid beneficial effects on dogs with inappropriate behaviours, providing onset of activity to complete the previous data from a double-blinded, placebo-controlled study.

Keywords: Dog; Behaviour; Interaction; Nutritional Supplement; Owner Survey

Abbreviations

ROS: Reactive Oxygen Species; SOD: Super Oxide Dismutase

Introduction

Inappropriate behaviours are abnormal responses to adverse situations that have physiological and behavioural manifestations [1]. Increased heart rates, hypertension, hypersalivation, elimination, and secretion of hormones including epinephrine, norepinephrine, and cortisol, have been described in dogs exposed to excessive chronic stress [1-3]. In addition to physiological changes, stress can induce emotional states such as fear and anxiety. The latter is defined as the result of anticipation of prospective or imagined danger or uncertainty, whereas fear is caused by present or threatened danger [4]. Fear and anxiety-related problems can include separation anxiety, noise phobias, and fear-related aggression [5].

It was suggested that oxidative stress might be clinically translated as physical and emotional stress [6,7]. Oxidative stress is the result of impaired balance between pro-oxidant molecules including free radicals and antioxidant defenses [8,9]. These natural defense antioxidant enzymes such as superoxide dismutase (SOD) play a key role in diminishing oxidative stress [8-10]. Thus, the decrease of reactive oxygen species (ROS) resulting from nutritional antioxidant supplementation containing exogenous SOD, could be an effective fear and inappropriate behaviours management strategy in dogs [8,9].

Inappropriate behavioural reactions include freezing, hiding, pacing, withdrawal/escape attempts, submission, trembling/shaking, yawning, salivating, panting, paw lifting, barking/growling, piloerection, coprophagy, self-grooming, changes in locomotor activity, cowering/lowered body posture, nosing, digging, foot licking, and aggression [1,2,11,12]. These disorders are considered as an important issue in veterinary behavioural medicine [5,11,13]. Firstly, the prevalence of anxiety and fear-related behaviours in the canine species is high, with 29% of dogs experiencing anxiety and 17% to 49% showing an aversion to noise (e.g. thunderstorms, fireworks and gunshots) [1,4,12]. Secondly, it has been demonstrated that fear and anxiety have a negative impact on health and lifespan in dogs [5]. Thirdly, the dogs' interactions with their owners and animal counterparts can be profoundly affected by these problems [5]. Therefore, some authors underline the importance for the owners to be able to recognise behavioural signs of anxiety or fear in order to avoid stressful situations [14].

Basically, the management of fearful or anxious dogs relies on behavioural therapy [11]. In addition, psychotropic drugs can help speeding the rate of animals' recovery [11]. However, owners are often reluctant to administer psychotropic drugs to their dogs because of their potential side effects [11,13,15]. Natural compounds including pheromones and plant extracts are available on the market but very few have demonstrated evidence of efficacy compared to a placebo [4,15,16]. Recently, diet supplements have been increasingly considered as a natural and safe alternative to manage behavioural disorders in humans and dogs [2,17] and have been developed over the last few years [2,17]. The protective effect of melon SOD extract against oxidative stress has been demonstrated in several studies in humans and in mice [8,18]. It has also been shown to improve stress-related behavioural signs and quality of life in healthy people in a double-blind, placebo-controlled clinical trial [7]. Fish protein hydrolysates demonstrated calming-type properties in mice and rats [13,17], but also in dogs [4,15] and humans [13]. Furthermore, the effectiveness of a new complementary feed combining fish hydrolysate and melon SOD was shown to be effective in reducing mild fear and uncomfortable reactions in dogs in a double-blind, placebo-controlled, randomized study [1]. This previous study lasted 30 days and was focused on efficacy at the end of the treatment period, meaning that the duration of the onset of action has not been evaluated.

Goal of the Study

The present survey had 3 goals: i) to evaluate if the owners are able to recognize the signs of fear and inappropriate behaviours in their dogs, ii) to identify the most relevant behaviours for the owners to recognize fear and inappropriate behaviours in their pets, iii) to assess the onset of effectiveness of a combination of melon SOD extract and fish protein hydrolysate for managing fear and inappropriate behavioural signs in dogs.

Materials and Methods

Animals

Fifty client-owned dogs were recruited in a database prepared by an independent company specialising in customer surveys. The enrolled dogs had moderate to severe inappropriate behaviours to environmental stimuli for at least 3 months according to their owner. They were not under current medications.

Survey design

The owners were asked to test a complementary feed for 28 days. During the test period, they had to complete a multiple-choice questionnaire online and to score their animal's behaviour. Assessments were performed 5 times: at inclusion on D0 (basal assessment before initiation of product administration), on D2, D7, D14 and D28. Twenty-four behavioural signs were evaluated by the owners on D0 and D28. They are listed in table 1. They were scored on a scale of 1 to 10 with 1: no signs and 10: extremely pronounced signs.

Behavioural sign	
1.	Seeking for attention
2.	Decreased closeness to preferred associates
3.	Scanning the environment
4.	Ritualized/Repeated activities
5.	Excessive barking
6.	Hyperactivity
7.	Whining
8.	Excessive shaking
9.	Trying to escape
10.	Excessive grooming
11.	Decreased grooming
12.	Inappropriate urination
13.	Lowering of the head
14.	Hiding or trying to hide
15.	Inappropriate defecation
16.	Panting
17.	Immobility
18.	Nose licking
19.	Inability to meet a direct gaze
20.	Mydriasis even in the light
21.	Smacking jaws together
22.	Hypersalivation
23.	Anal sac expression
24.	Excessive scratching

Table 1: Behavioural signs assessed by the dogs' owners at D0 and D28.

The animals' interaction with their environment and behaviour evolution were assessed by the owners on D2, D7, D14. Both parameters were classified as "Strongly deteriorated", "Slightly deteriorated", "No change", "Slightly improved", or "Strongly improved". A 0-to-10 rating scale was used to evaluate the owners' satisfaction at D2, D7, D14, D28. The efficacy score of the test product on inappropriate behaviours and its ease of administration were noted on D28.

Test product

The product tested in this survey was Anxivet® capsules (MP Labo, Grasse, France) containing each 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase (SOD). The dogs were administered by their owner one capsule per 5 kg body weight once daily for 28 days from D0 to D28. The capsule was swallowed whole or opened and its content mixed with the animal's food.

Data analysis

Statistical analyses were performed using the Statgraphics® Centurion XVI software. An overall behavioural score was calculated by adding the 24 behavioural subscores together. All comparisons were performed on animals with a complete data set. The non-parametric sign test was used to compare the mean behavioural scores between D0 and D28. A Friedman test was performed to compare the owners' satisfaction level over time, completed by pairwise Sign tests as post-hoc comparisons in case of statistical significance. Interaction with the environment and behavioural evolution were compared between time points by using a McNemar test. The significant threshold was set at 5%.

Results

Animals

At the end of the study period, 9 dogs had incomplete data on at least one behavioural score on D0 and 3 had incomplete data on all the behavioural scores on D28. The enrolled dogs were of various breeds including small, medium, and large breeds, with ages varying from 1.5 to 12 years (mean ± SD was 5.9 ± 2.6 years). At enrolment, 48% of the dogs were considered to present consistent severe inappropriate behaviours by their owners and 52% have been described as having moderate inappropriate behaviours.

Behavioural scores

Except for decreased grooming, mydriasis even in the light, and smacking jaws together, all behavioural signs improved between D0 and D28 with significant differences for 8 of them (Figure 1). The subscores seeking for attention ($p = 0.0214$), ritualized/repeated activities ($p = 0.0425$), whining ($p = 0.0259$), and immobility ($p = 0.0371$) were significantly decreased at D28 compared to D0. Excessive scratching ($p = 0.0148$), excessive barking ($p = 0.0119$), hyperactivity ($p = 0.0008$), and excessive grooming ($p = 0.0093$) showed the most remarkable changes after 28-day administration of the product.

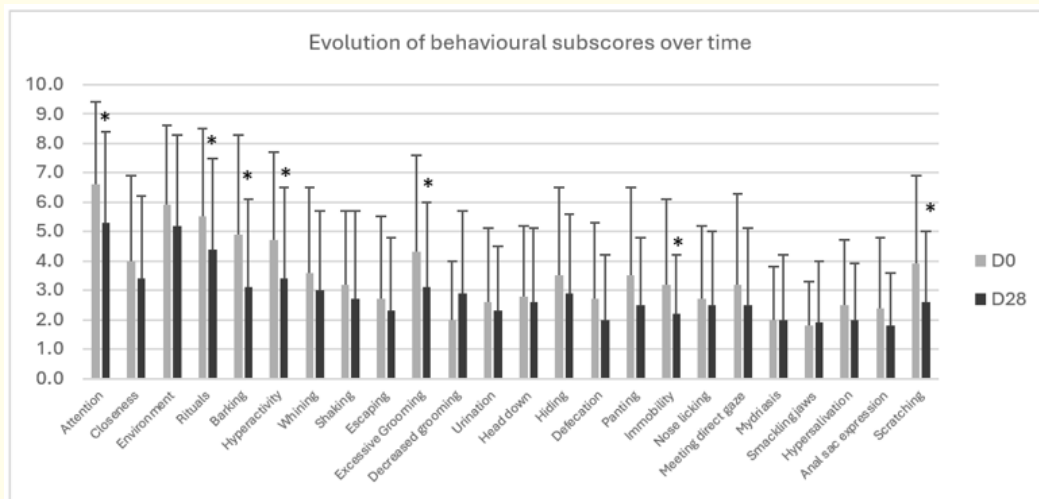


Figure 1: Evolution over time of the behavioural subscores (mean ± SD) in dogs after oral administration of 1 capsule of Anxivet® containing 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase, per 5 kg body weight once daily for 28 days from D0 to D28. *Denotes significant difference from baseline value ($p < 0.05$).

When considering the overall behavioural score, the decrease observed at D28 was significant compared to baseline ($p = 0.0150$), with a percentage difference of 19% (Figure 2).

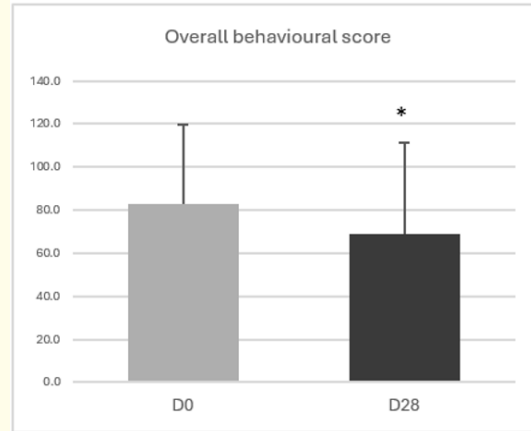


Figure 2: Evolution over time of the overall behavioural score (mean \pm SD) in dogs after oral administration of 1 capsule of Anxivet[®] containing 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase, per 5 kg body weight once daily for 28 days from D0 to D28. *Denotes significant difference from baseline value ($p < 0.05$).

Interaction with the environment

Interaction with the surroundings was improved in most of the dogs at D2, D7, and D14 vs D0 with a steady increase in the number of dogs showing a better interaction with their environment over time (Figure 3). It was assessed as slightly or strongly improved by 52% of the owners on D2, 72% on D7, and 78% on D14. Furthermore, the evolution was significant between D2 and D7 ($p < 0.05$) and between D2 and D14 ($p < 0.01$). No animals experienced a deterioration of this parameter from D0 to D14.

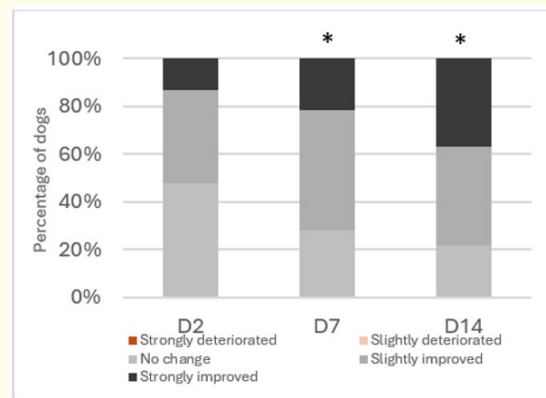


Figure 3: Evolution over time of the dogs' interaction with their environment, after oral administration of 1 capsule of Anxivet[®] containing 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase, per 5 kg body weight once daily for 28 days, from D0 to D14. *Denotes significant difference with D2 ($p < 0.05$).

Inappropriate behaviours evolution

Emotional tension was less noticeable by the owners on D2, D7, and D14 compared to baseline with a continuous improvement from D0 to D14 (Figure 4). It was reported as slightly or strongly reduced by 56% of the owners on D2, 72% on D7, and 84% on D14. Moreover, the changes were significant between D2 and D14 ($p < 0.005$).

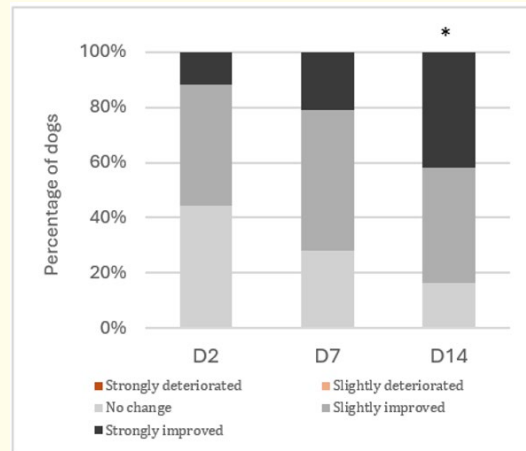


Figure 4: Evolution over time of the dogs' emotional tension, after oral administration of 1 capsule of Anxivet® containing 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase, per 5 kg body weight once daily for 28 days, from D0 to D14. *Denotes significant difference with D2 ($p < 0.05$).

Owners' satisfaction scores

At D28, the mean efficacy score of the test product reached 7.3 (± 2.5) on a scale of 1 - 10 and 81% of the owners described a reduction of inappropriate behaviours in their animal compared to D0 with return to normal behavior in 62% of the dogs. Improvement was observed by the owners within 2 days in 5 dogs (14%), within 7 days in 58% of the dogs and within 14 days in 92% of the dogs.

As soon as D2, the mean score of the owners' satisfaction was almost 6 on a scale of 1 to 10 and reached 7.4 on D28. Owners' satisfaction significantly increased on D7, D14, and D28 compared to D2 ($p = 0.0259$, $p = 0.0008$, and $p = 0.00003$, respectively) with percentage increases of 12% at D7, 22% on D14, and 28% on D28 (Figure 5). The owners' satisfaction score was even significantly higher on D14 and D28 compared to D7 ($p = 0.0176$ and $p = 0.0005$, respectively).

On D28, 87% of the owners reported being satisfied with the results obtained after four weeks of administration of the product. Lastly, the mean score of product's ease of administration was 7.4/10 (± 2.7) and the mean score of product's acceptance by the dog was 8.7 (± 1.6). The product was easy and very easy to give for 85% of owners.

Discussion

The administration of the tested combination over a four-week period resulted in significant enhancement of the overall behavioural score and of 8 individuals behavioural subscores, interaction with the surroundings and inappropriate behaviours level were improved

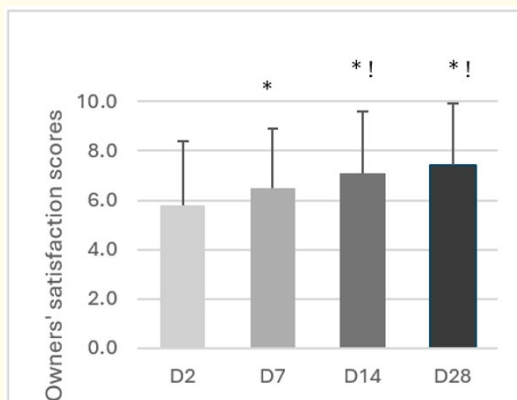


Figure 5: Evolution over time of the owners' satisfaction score (mean \pm SD) following oral administration of 1 capsule of Anxivet[®] containing 250 mg of hydrolysed fish proteins and 28 IU of melon superoxide dismutase, per 5 kg body weight once daily for 28 days from D0 to D28. *Denotes significant difference with D2 ($p < 0.05$). ! denotes significant difference with D7 ($p < 0.05$).

as soon as D2 in more than half of the dogs and continued the same trend over time in most dogs with significant differences compared to D2. These observations were corroborated by the high mean efficacy and owners' satisfaction scores on D28.

Previous double-blind, randomised, placebo-controlled clinical trials have shown the value of supplementation with melon juice concentrate alone [7], fish hydrolysate alone [4], and the combination of both ingredients [1] in emotional regulation in humans and dogs. The results of the present survey are comparable to those achieved by Titeux, *et al.* after administration of the same product to 39 dogs over a 30-day period [1]. In that study, the tested combination was effective in reducing fear and emotional behaviours in dogs. These data were the first tangible evidence of the interest of the product in dogs with inappropriate behaviours. The aim of the present survey was to assess the owners' perception of the effectiveness of the same combination of active ingredients with a focus on its onset of activity. Inappropriate behaviours can be highly variable in dogs. Fifty-four signs were identified in a questionnaire study administered to owners of dogs suffering from inappropriate behaviours, and more generally, the literature shows a great diversity of the signs related to stress [1,12,19]. Moreover, depending on the studies, different test procedures [1] and scales including the EDED (Emotional Disorders Evaluation in Dogs) scale [11] and the C-BARQ (Canine Behavioral Assessment and Research Questionnaire) [2], have been used to assess behavioural reactions to stressors. The questionnaire used in our survey was adapted from the AAHA 2015 guidelines as described by Chala, *et al.* [20]. This variability of assessment tools, added to the fact that the inappropriate behavioural level may vary from one subject to the other independently of the test procedures and perceived stress scale used, makes it difficult to compare the results of the different studies. Still, most results may be underestimated since they are based in most studies on a non-exhaustive list of inappropriate behaviours signs. Therefore, if significant results are shown in a trial, it can be hypothesized that somehow the tested product may have a value in inappropriate behaviours management.

It was reported that owners may be able to easily recognize trembling, shaking, and high-pitched vocalization as signs of inappropriate behaviours, but may not recognize less overt signs [19]. Our results showed that 8 subscores (Seeking for attention, Ritualized/Repeated activities, Whining, Immobility, Excessive scratching, Excessive barking, Hyperactivity, and Excessive grooming) were significantly decreased at the end of the study. These observations were not linked to the intensity level of the behavioural scores reported at D0

since some subscores above 5/10 at baseline did not significantly decrease at D28, and in the contrary some subscores below 5/10 (e.g. whining and immobility) improved significantly. This implies either that some behavioural signs were more impacted by the product supplementation than others or that the owners were more sensitive or more able to notice some behaviours. The last hypothesis appears to be the most reasonable since the mechanisms explaining a difference of efficacy of the active substances contained in the product according to the signs seem difficult to explain. These observations need to be confirmed by further studies, but it is interesting to note that while 16 out of the 24 behavioural subscores did not show a significant decrease, interaction with the environment and inappropriate behaviours evolution were reported as significantly improved by the owners and were associated with a good owners' satisfaction level.

Dogs may be exposed to stressors in everyday life, and it may be difficult to avoid difficult situations by eliminating stressors, especially when dogs mirror the stress levels of their owners as shown by Sundman, *et al.* [21]. Furthermore, inappropriate behaviours may have a negative impact on the human-pet bond and on dogs' health and lifespan [5]. Therefore, long-term behaviour management will be required in most cases. The safety profile of feed supplements is particularly interesting in this context and offer an additional option to anxiolytic drugs and their potential side effects [11]. Furthermore, a quick onset of action is an undeniable asset for the success of the therapy and leads to prolonged administration of the product by pet owners. Our survey showed that visible results were obtained as soon as D2 after initiation and then continued to improve until the end of the 28-day follow-up period. Finally, the ease of administration of the product will be a positive factor for long-term administration.

The survey limitations include the subjectivity of the assessments made by the owners without veterinary control examination or measurement of blood cortisol levels. Furthermore, since our survey did not include a control group, it can be assumed that the owners' assessments may have been biased by the fact that they knew that their animal was receiving an active supplement to manage their inappropriate behaviours. The aim of this study was mostly to evaluate the owner's perception of the effects and onset of perceived activity of the tested product on the behavioural unhappiness of their dog in real conditions rather than the efficacy by itself as this had already been done by Titeux, *et al.* [1].

Conclusion

This survey shows that, according to the owners, the combination of hydrolysed fish proteins and melon SOD has positive effects in dogs with inappropriate behaviours. The product appears to be a safe and effective solution for long-term behaviour management in dogs exposed to stressors in their daily lives.

Acknowledgments

The authors thank Dr Katherine Baldwin for the English proof-reading of the manuscript.

Conflicts of Interest

The authors were employees of MP Labo when the study was performed or published.

Funding Support

This research was funded by MP Labo, the manufacturer of Anxivet®.

Bibliography

1. Titeux E., *et al.* "Effects of a new dietary supplement on behavioural responses of dogs exposed to mild stressors". *Veterinary Medicine and Science* 7.5 (2021): 1469-1482.

2. Kato M., *et al.* "Effects of prescription diet on dealing with stressful situations and performance of anxiety-related behaviors in privately owned anxious dogs". *Journal of Veterinary Behavior: Clinical Applications and Research* 7.1 (2012): 21-26.
3. Grigg EK., *et al.* "Stress-related behaviors in companion dogs exposed to common household noises, and owners' interpretations of their dogs' behaviors". *Frontiers in Veterinary Science* 8 (2021): 760845.
4. Landsberg GM., *et al.* "Assessment of noise-induced fear and anxiety in dogs: Modification by a novel fish hydrolysate supplemented diet". *Journal of Veterinary Behavior* 10.5 (2015): 391-398.
5. Dreschel NA. "The effects of fear and anxiety on health and lifespan in pet dogs". *Applied Animal Behaviour Science* 125.3-4 (2010): 157-162.
6. Epel ES., *et al.* "Accelerated telomere shortening in response to life stress". *Proceedings of the National Academy of Sciences of the United States of America* 101.49 (2004): 17312-17315.
7. Milesi MA., *et al.* "Effect of an oral supplementation with a proprietary melon juice concentrate (Extramel®) on stress and fatigue in healthy people: A pilot, double-blind, placebo-controlled clinical trial". *Nutrition Journal* 8 (2009): 40.
8. Vouldoukis I., *et al.* "Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress". *Phytotherapy Research* 18.12 (2004): 957-962.
9. Carillon J., *et al.* "Superoxide dismutase administration, a potential therapy against oxidative stress related diseases: Several routes of supplementation and proposal of an original mechanism of action". *Pharmaceutical Research* 30.11 (2013): 2718-2728.
10. Ighodaro OM and Akinloye OA. "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid". *Alexandria Journal of Medicine* 54.4 (2018): 287-293.
11. Beata C., *et al.* "Effects of alpha-casozepine (Zylkene) versus selegiline hydrochloride (Selgian, Anipryl) on anxiety disorders in dogs". *Journal of Veterinary Behavior: Clinical Applications and Research* 2.5 (2007): 175-183.
12. Grigg EK., *et al.* "Influence of dog appeasing pheromone (DAP) on dogs housed in a long-term kennelling facility". *Veterinary Record Open* 2.1 (2015): e000098.
13. Messaoudi M., *et al.* "Anxiolytic and antidepressant-like effects of garum armoricum® (GA), a blue ling fish protein autolysate in male wistar rats". *Current Topics in Nutraceutical Research* 6.3 (2008): 115-124.
14. Mariti C., *et al.* "Perception of dogs' stress by their owners". *Journal of Veterinary Behavior: Clinical Applications and Research* 7.4 (2012): 213-219.
15. Landsberg GM., *et al.* "Dog-appeasing pheromone collars reduce sound-induced fear and anxiety in beagle dogs: A placebo-controlled study". *Veterinary Record* 177.10 (2015): 260.
16. Frank D., *et al.* "Systematic review of the use of pheromones for treatment of undesirable behavior in cats and dogs". *Journal of the American Veterinary Medical Association* 236.12 (2010): 1308-1316.
17. Freret T., *et al.* "Fast anxiolytic-like effect observed in the rat conditioned defensive burying test, after a single oral dose of natural protein extract products". *Nutrients* 13.7 (2021): 2445.
18. Carillon J., *et al.* "Endogenous antioxidant defense induction by melon superoxide dismutase reduces cardiac hypertrophy in spontaneously hypertensive rats". *International Journal of Food Sciences and Nutrition* 65.5 (2014): 602-609.

19. Hammerle M., *et al.* "AAHA canine and feline behavior management guidelines". *Journal of the American Animal Hospital Association* 51.4 (2015): 205-221.
20. Chala V., *et al.* "Pet owner evaluation of 2 feed supplements dedicated to stress management in privately owned cats and dogs: a controlled study". Proceeding of SEVC, Barcelona, Spain (2017).
21. Sundman AS., *et al.* "Long-term stress levels are synchronized in dogs and their owners". *Scientific Reports* 9 (2019): 7391.

Volume 9 Issue 1 January 2024

©All rights reserved by Marlène Lacreusette., *et al.*