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Effects of a new dietary supplement on behavioural responses of dogs exposed to mild stressors

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Abstract

Background & Objectives: The effectiveness of a new dietary supplement (derived from fish hydrolysate and melon juice concentrate rich in superoxide dismutase) in reducing fear and stress-related behaviours in pet dogs was examined in a double-blind, placebo-controlled, randomized study.

Methods: 39 dogs were recruited after the owners had filled out a fear susceptibility index questionnaire. Over a 30-day period, one group of dogs received the supplement, and another group a placebo.

Twelve behavioural variables were recorded in a series of four subtests (ST1-ST4) on days 0, 15 and 30. Saliva cortisol levels were measured before and after each set of STs.

Results: The dogs rated as more fearful displayed significantly higher cortisol values before the day 0 test session, were less active, spent less time playing with the experimenter, and approached the unfamiliar object less frequently. The owners did not correctly guess whether their dog had received the supplement or not. Behaviours of dogs were significantly different across the three sessions, with significant increases of stress-related behaviours (time spent in the door zone, number of interactions with the door, of whining, and of lip-licking). Conversely time spent with the experimenter increased, interactions and curiosity for the novel object and play with the experimenter decreased, presumably due to a habituation process. This suggests that the design of the four subtests session was relevant to test for mild stressors situations. Moreover, supplemented and placebo dogs responded differently to the three test sessions, indicating a supplement effect on dogs' behaviours and their adaptation to mild stressors situations.

Conclusion: The trial results suggested that the supplement facilitates activity and curiosity in a familiar environment, promotes dog-human interactions with an increased human familiarity, and tends to reduce subtle stress behaviours. Our results suggest that the supplement was effective in the context of mild stressors and habituation.

1 | INTRODUCTION

Fear is a major issue in veterinary behavioural medicine (Martinez Pernas et al., 2011), and dog owners frequently consult for this problem in their pets (Levine, 2009; Overall et al., 2001). In a recent survey, 49% of owners stated that their dog was scared of loud noises (Blackwell et al., 2013). Fear is considered to be an emotional state of alarm and agitation caused by a present, threatening danger (Adolphs, 2013; Sherman & Mills, 2008; Stellato et al., 2017); it triggers adaptive, behavioural responses that will enable the animal to mitigate the threat (avoidance or defensiveness) or escape it (Adolphs, 2013; Sherman & Mills, 2008). An individual facing a threat can display the 'four Fs' behavioural responses, that is, fight, flight, freeze or flirt (Marks, 1987). Thus, fear is potentially one of the most common reasons for aggressive behaviour in dogs, even though the owner may not necessarily link this aggression to fear *per se* (Tiira & Lohii, 2014).

When a stimulus is perceived as a threat, the fear emotion also triggers a stress-related physiological response. Stress is a normal response to adverse (i.e., stressful) situations and has behavioural, physiological and immune manifestations (Beerda et al., 1997). Fearful dogs show physiological responses like tachycardia, hypersalivation and elimination (Sherman & Mills, 2008). Some dogs produce a stress response to many day-to-day stimuli or also anticipate potential threats: this leads to a state of chronic physiological stress (Dreschel, 2010) that could be related to an anxious state, associated with a shorter lifespan (Dreschel, 2010) and poor welfare (Beerda et al., 1998).

Various behavioural tests have been developed to measure acute fear or stress responses in dogs (Carlone et al., 2018; Landsberg et al., 2015). These consist of standardised experimental situations in which stimuli serve to elicit behaviours in the observed individuals (Araujo et al., 2013). The stressors can correspond to everyday scenarios (Stellato et al., 2017) or be stronger (e.g., stimuli mimicking thunderstorms; Dreschel & Granger, 2005; Landsberg et al., 2015). The main behaviours involved are freezing (i.e., staying attentively and tonically immobile), withdrawal/escape attempts, submission (i.e., low posture) and defensive aggression (Stellato et al., 2017; Walker et al., 1997). Other more subtle behaviours have been also described, such as acute responses (trembling/shaking, yawning, salivating, panting, paw-lifting, barking/growling and piloerection) or chronic ones (coprophagy, self-grooming, repetitive behaviours (pacing), changes in locomotor activity, nosing and digging; Beerda et al., 1998, 1999, 2000; Stellato et al., 2017). Furthermore, stress activates the hypothalamic-pituitary-adrenal (HPA) axis, and therefore the cortisol level is commonly used to assess the physiological level of stress of dogs submitted to fearful and/or stressful situations (Beerda et al., 1997; Landsberg et al., 2015).

Hence, there is a need for treatment options that decrease fear and stress reactions in dogs. Clomipramine is the only psychoactive drug currently licensed for the treatment of anxiety and has also been tested successfully in a setting of noise phobia (Crowell-Davis et al., 2003; Seksel & Lindeman, 2001; Sherman & Mills, 2008). However, clomipramine's onset of action is slow, and side effects have been reported. Recently, an oromucosal gel formulation of dexmedetomidine (an alpha-2 adrenergic receptor agonist) was developed as a new treatment for dogs suffering from noise aversion (Korpivaara et al., 2017). The gel's short duration of action (with a half-life ranging from 0.5 to 3 h), the associated contraindications and adverse reactions and the need for prescription by a veterinarian make it difficult to use in practice (Summary of Product Characteristics for Sileo, 2015). Accordingly, several dietary supplements have been tested for their ability to reduce fear and stress: these include L-theanine (Araujo et al., 2010), a combination of tryptophan and alpha-casozepine (Kato et al., 2012) and fish hydrolysate. The latter was evaluated at two different dose levels and showed some effectiveness in reducing (i) a hyperactivity response to thunder and (ii) the associated cortisol response (Landsberg et al., 2015). A preliminary study of rats treated with fish hydrolysate evidenced an increase in basal levels of gamma-aminobutyric acid in both the hippocampus and the hypothalamus. The researchers suggested that the hydrolysate had a diazepam-like effect on the stress responsiveness of the rat pituitary-adrenal axis and sympathetic adrenal activity (Bernet et al., 2000). In contrast to diazepam, this anxiolytic-like effect might not be associated with impaired learning (Messaoudi et al, 2008a, 2008b) and had a mild antidepressant effect (Dorman et al., 1995, Messaouidi et al., 2008a, 2008b). Last, oral supplementation with a melon juice concentrate rich in superoxide dismutase (SOD) was associated with an anti-stress effect on healthy people in a double-blind, placebo-controlled clinical trial (Milesi et al., 2009).

In view of the above, the primary objective of the present double-blind, placebo-controlled study was to evaluate the effectiveness of a new dietary supplement (a fish hydrolysate combined with a melon juice concentrate) on fear and stress-related behaviours in dogs. The study's behavioural tests were based on literature procedures used to assess behavioural reactions in dogs faced with a variety of stimuli similar to everyday situations (Carlone et al., 2018; Hoummady et al., 2016; King et al., 2003; Landsberg et al., 2015). The participating dogs were randomised to receive supplement or placebo daily for 30 days. We hypothesised that dogs who received the supplement would show a decrease over time in saliva cortisol levels and fear- and stress-related behaviours in response to mild stressors when compared with dogs who received a placebo.

1. When your dog hears loud noises (cars, fireworks, thunder, etc.), does he/she start to shiver or bark or try to run away?

☐ No, never (0 pt) ☐ Yes, sometimes (1 pt) ☐ Yes, frequently (2 pts) ☐ Yes, always (3 pts)

2. When an unfamiliar person comes close to your dog at home, does your dog start to shiver or bark or try to run away?

☐ No, never (0 pt) ☐ Yes, sometimes (1 pt) ☐ Yes, frequently (2 pts) ☐ Yes, always (3 pts)

3. When an unfamiliar person comes closer to your dog during a walk, does your dog start to shiver or barks or try to run away?

☐ No, never (0 pt) ☐ Yes, sometimes (1 pt) ☐ Yes, frequently (2 pts) ☐ Yes, always (3 pts)

4. When your dog is left alone at home, does he/she start barking or destroying furniture or other objects?

☐ No, never (0 pt) ☐ Yes, sometimes (1 pt) ☐ Yes, frequently (2 pts) ☐ Yes, always (3 pts)

FIGURE 1 Fear susceptibility index adapted from the C-BARQ questionnaire (Hsu & Serpell, 2003) and the inclusion criterion

2 | MATERIALS AND METHODS

The study took place in a 15 mš experimental room at the National Alfort Veterinary School (Maisons-Alfort, France). The tests were performed between April and July 2016. All procedures were performed in full compliance with the European Union's Directive 2010/63/EU on the protection of animals used for scientific purposes. All dog owners gave their informed consent prior to the study, which was approved by the local Animal Care and Use Committee (Comité Ethique en recherche Clinique d'Alfort [COMERC], National Alfort Veterinary School, Maisons-Alfort, France; reference: 2016-01-15).

2.1 | Animals

Thirty-nine pet dogs of various breeds (Australian Shepherd, Border Collie, German Shepherd, Pyrenean Shepherd, Cursinu, Bernese Mountain Dog, Labrador Retriever, Golden Retriever, Beagle, Poodle, Boston Terrier, Jack Russel Terrier, Yorkshire Terrier, Brazilian Terrier, Whippet, Bichon, West Highland White Terrier, Akita Inu, Shiba Inu, Chihuahua and crossbreeds) were recruited by advertising on social networks or in the vaccination waiting room of the Centre Hospitalier Universitaire Vétérinaire d'Alfort (University Hospital, National Alfort Veterinary School). Twelve females and six males were neutered.

2.1.1 | Inclusion based on a fear susceptibility index

Prior to a dog's inclusion in the study, the owner answered four questions related to fear and stress-related reactions from the dog-specific Canine Behavioural Assessment and Research Questionnaire (C-BARQ)–a questionnaire with proven reliability and validity (Hsu & Serpell, 2003; Figure 1). The scores for the four questions (from 0 to 3 points) were added together, so the total possible score ranged from 0 (not at all fearful) to 12 (very fearful). In our study, 9 was the highest score obtained, and 1 was the lowest.

### 2.1.2 | Other inclusion and exclusion criteria

Eligible dogs underwent a general health check before inclusion in the study. Aggressive dogs and dogs that were difficult to handle were excluded (one dog was excluded). Good hearing was checked with a multiclicker (Clix®); the dog had to turn its head towards the clicker. Pregnant or lactating bitches and dogs being treated with corticoids or psychotropic medications were not included.

A total of 39 dogs (26 females and 13 males) aged from 1 to 6 years (mean ± standard deviation (SD) age: 4.0 ± 1.7 years) participated in the study.

2.2 | The experimental protocol

2.2.1 | Protocol

The trial lasted 31 days: on Days 0, 15 (mean ± SD time point: 15.8 ± 2.5 days) and 30 (30.4 ± 2.1 days), the dogs underwent four standardised behavioural tests. The dietary supplement or placebo capsule was given to the dog daily in the morning for a 30-day period, starting on Day 1. The supplement contained 500 mg of fish hydrolysate (GABOLYSAT PTP 55) and 11 mg of SOD B Primo-antioxidant® M (5 IU/mg) per capsule. Dogs weighing more than 10 kg received two capsules a day (either supplement or placebo), and dogs weighing less than 10 kg received one capsule a day. After randomisation, 18 dogs were assigned to the supplement group, and 21 dogs were assigned to the placebo group.
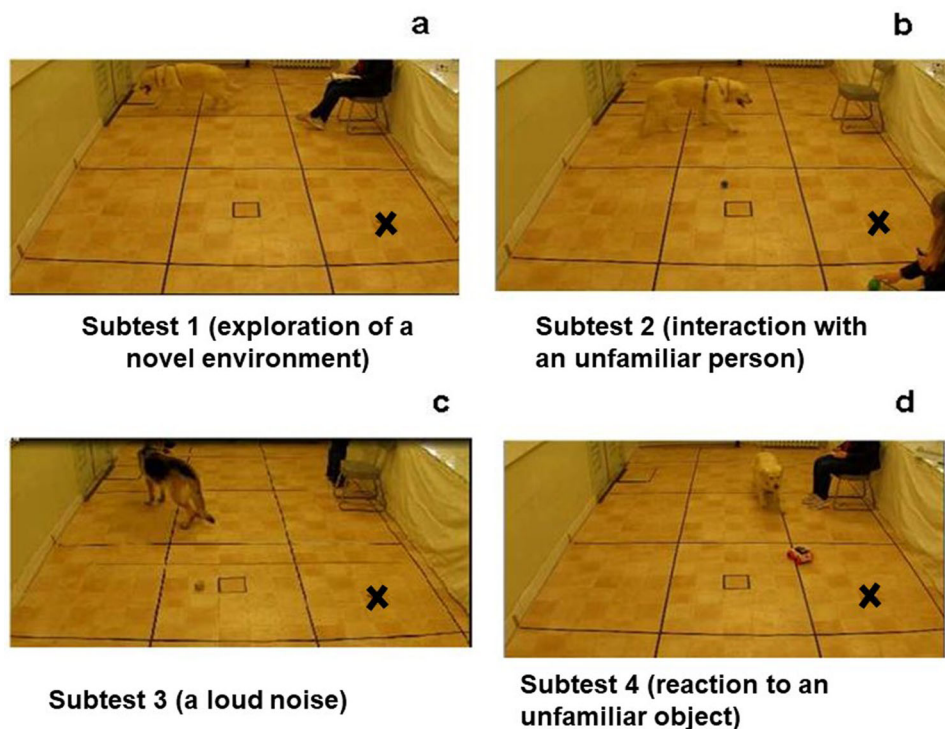
FIGURE 2 Behavioural tests designed in the study, with four subtests (ST1: novel environment, ST2: interaction with an unfamiliar person, ST3: loud noise, ST4: novel object)

2.2.2 | Behavioural tests

The behavioural tests took place in a 15 mš experimental room in which the floor was divided into 1 mš squares, with zones marked out for behavioural tests (Figure 2) and a chair placed in one square. Prior to testing, owners were welcomed by the experimenter, and for dogs that were driven to the Veterinary School, they were walked on average for half an hour on the campus to recover from the transport and were all relaxed before entering the room. The room temperature was not controlled and ranged from 20 to 25°C during the tests.

Each behaviour test session began when the test dog entered the room, off the leash, in the absence of his/her owner and the door was closed. Each test session lasted for 6 min and 50 s and was divided into four subtests (STs). All tests were recorded with a video camera (EOS 700D, Canon France), and the videos were rated offline using Boris software v6.3.4 (Friard & Gamba, 2016). Three experimenters (woman, for Day 0, man for Day 15 and woman for Day 30) participated in the study.

ST1. Exploration of a novel environment (duration: 3 min). The experimenter sat quietly in a chair in the experimenter zone and avoided physical and visual interaction with the dog (Figure 2a). The dog was free to explore the room during this time.

ST2. Interaction with an unfamiliar person (duration: 2 min). The experimenter stood up from the chair, held two balls and a rope and squatted in the play zone. He/she called the dog and invited him/her to play three times (Figure 2b). To avoid habituation, a different experimenter participated in the sessions on Days 0, 15 and 30.

ST3. Loud noise (duration: 1 min and 20 s). A sudden, 85 dB noise (Clix ® CD), which corresponds to the noise of a vacuum cleaner was played for 20 s (Figure 2c) with a standard CD player calibrated in a sound field with a precision sound pressure level meter (Type 2235 with Microphone Type 2235 +1/3−1/1 Octave Filter Set Type 1626, Brüel &Kjær Sound & Vibration Measurement A/S). After the noise had been played, the experimenter sat in the room for 1 min. The same vacuum cleaner sound was played three times in all so that the dog could habituate to it.

ST4. Response to a novel (unfamiliar) object (duration: 30 s). From his/her chair, the experimenter operated a remote control car and placed it in zone X. The dog was observed during this time (Figure 2d). The same novel object (the remote control car) was used three times so that the dog could habituate to it.

Once the four STs were over, the door was opened and the dog invited to leave the room. To assess the behaviour of each dog in ST1–4, 12 behavioural variables (Table 1) were quantified and recorded offline by two experimenters, whose level of inter-rater agreement had been previously confirmed with Kendall's tau test (mean $\pm$ SD: 0.983 ± 0.012 for 11 videos).

2.2.3 | Saliva cortisol sampling

On Days 0, 15 and 30, a saliva sample was collected with an oral swab (Salimetrics Kit®, Salimetrics LLC). The tip of the swab was placed in both cheek pouches and under the tongue for up to approximately

TABLE 1 Behavioural variables recorded during the test

Behavioural variable	Unit	Description	Sub-test (ST)
Door zone	Seconds	Time spent in front of the door, with at least half of the body inside the door zone (immobile for 2 s)	ST1, ST2, ST3, ST4
Experimenter zone	Seconds	Time spent within 1 m of the experimenter	ST1, ST3, ST4
Interaction with the door	Number	Biting, scratching or touching the door or jumping towards it for least 2 s (one interaction lasts for 2 s)	ST1, ST2, ST3, ST4
Zone crossing	Number	Number of 1 mš zones crossed with at least half of the body	ST1
Whining	Number	Each distinct pitch during a whining vocalisation	ST1, ST2, ST3, ST4
Yawning	Number	Opening the mouth with the tongue extended	ST1, ST2, ST3, ST4
Lip-licking	Number	Fast extension of the tongue passing by the lip/nose	ST1, ST2, ST3, ST4
Play	Seconds	Time spent playing with the toys and/or the experimenter	ST2
Turning the head towards noise	Number	The dog turns its head towards the source of the noise	ST3
Approaching an unfamiliar object	Number	Approach movement towards the unfamiliar object (a remote control car)	ST4
Turning the head towards an unfamiliar object)	Number	The dog turns the head towards the unfamiliar object and remains immobile	ST4
Touching the unfamiliar object	Number	The dog touches the unfamiliar object (one contact lasts for 2 s)	ST4

2 min. The swab was then folded into the swab storage tube. Sampling was made just before the dog entered the testing room and just after it left (approx. 10 min. interval). The tubes containing saliva were stored at 4°C, taken to the laboratory to be centrifuged, frozen at −20°C and subsequently analysed at the Unité de Recherche Vétérinaire Intégrée (URVI) lab (Namur University).

The minimum volume of saliva required for analysis was 50 μl. Although all of the 39 dogs were sampled, only 32 gave more than 50 μl of saliva (Dreschel & Granger, 2005). At day 0, 15 samples for supplemented dogs were analysed, 13 for placebo dogs, at day 15, respectively 16 and 15, and at day 30 respectively 14 and 13. The saliva cortisol levels were assayed by a specialist laboratory (URVI, Namur). The samples were defrosted and centrifuged (1000 relative centrifugal force, 5 min, 4°C) and assayed in duplicate aliquots using 25 μl of the sample with a high-sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics kit®, LLC). The kit's lower limit of sensitivity was 0.007 μg/dl. Average intra- and inter-assay coefficients of variation were below 5.3% and 13.2%, respectively. Salivary cortisol samples were collected between 10:00 AM and 5:00 PM. Based on the evidence documenting no significant diurnal variation of cortisol in dogs (Castillo et al., 2009; Johnston & Mather, 1978; Koyama et al., 2003), the time of the salivary cortisol sampling was not strictly controlled.

2.2.4 | Feedback from owners: questionnaire

A feedback questionnaire was asked to be filled out by the dogs' owners after the 30 days of the study, after the behavioural tests were realised (Figure 3).

2.3 | Statistical analyses

Dogs with a fear susceptibility index of between 1 and 5 were classified as 'less fearful' ($n = 21$), whereas those with an index of 6 to 9 were classified as 'more fearful' ($n = 17$). Mann–Whitney non-parametric tests were used to compare less fearful vs. more fearful dogs with regard to behavioural variables and saliva cortisol levels (before and after the test session).

Cortisol concentrations before and after the tests (on Days 0, 15 and 30) were compared using a non-parametric Wilcoxon test. Mann–Whitney non-parametric tests were used to assess supplement vs. placebo differences in behavioural variables and pre-test and post-test cortisol differences (i.e., delta post-pre test) on Days 0, 15 and 30.

Generalised linear mixed models (GLMMs) were used to analyse the effect of treatment (placebo vs. supplement), time (Days 0, 15 and 30) as fixed effects and with individuals as a random effect on the 12 behavioural variables and the deltas of post-pre test cortisol concentrations. The binary category of 'more' or 'less' fearful was considered as a random effect for zone crossing only. Models were fitted with a Poisson distribution and final GLMMs were selected based on Akaike Information Criterion for removal of non-significant effects and interactions.

Fisher's exact tests were used to compare the distribution of the owners' answers as to whether they thought their dog had received the supplement or placebo with the real distribution.

The threshold for statistical significance was set to $p < 0.05$ for behavioural variables that were not repeated along the four STs (Perneger, 1998). Considering 'door zone', 'interaction with the door',

First of all, thank you for participating in our study. To obtain feedback on our project, would you be kind enough to complete the following questionnaire?

Q1/ I think that my dog received:

☐ the placebo   ☐ the supplement   ☐ I don't know

Q2/ I have the feeling that my dog is now:

☐ less anxious, less fearful and less stressed   ☐ more stressed and more anxious

☐ the same as before the study (no change)   ☐ I don't know

If you think you know whether your dog received the supplement or the placebo:

Q3/ If you think your dog received the supplement, are you ready to continue giving it? If you think that your dog received the placebo, would you be willing to give the supplement?

☐ Yes   ☐ No   ☐ I don't know

Q4/ Are you happy to have participated in this study ?

☐ Yes   ☐ No

Do not hesitate to give us any other feedback:

FIGURE 3 Feedback questionnaire from the owners

'whining', 'yawning' and 'lip-licking', following Bonferroni's correction, significance was set at 0.0125 and considering 'experimenter zone', significance was set at 0.017. Quantitative data were expressed as the mean $\pm$ SD or the median (interquartile range (IQR)). Statistical analyses were performed with SigmaPlot software (version 12, Systat Software Inc.) and with R statistical software (R Development Core Team, version 3.5.1; RStudio Inc., version 1.1.456).

3 | RESULTS

3.1 | Behavioural and physiological characterisation of dogs according to the fear susceptibility index

On the basis of the study questionnaire, there were 21 dogs in the 'less fearful' class and 17 in the "more fearful" classes. The mean $\pm$ SD fear susceptibility index was 4.9 $\pm$ 2.9.

The 'less fearful' and 'more fearful' classes differed significantly with regard to six behavioural variables (Table 2): zone crossing during ST1 ($p = 0.007$; U = 239; df = 38), time spent playing during ST2 ($p = 0.046$; U = 264; df = 38) and approaching to the novel object during ST4 ($p = 0.023$; U = 259; df = 38). Hence, 'less fearful' dogs moved more than 'more fearful' dogs, played more and approached the novel object more frequently.

In addition, the mean pre-test cortisol value on Day 0 was significantly higher in 'more fearful' dogs ($n = 14$, 6.64 $\pm$ 3.66 nmol/L) than in 'less fearful' dogs ($n = 17$, 5.23 $\pm$ 3.24 nmol/L; $p = 0.013$;

U = 56; df = 30), whereas the mean post-test cortisol value was similar ('more fearful' $n = 13$, 7.04 $\pm$ 4.17 nmol/L; 'less fearful' $n = 15$, 5.48 $\pm$ 3.49 nmol/L; U = 215, $p = 0.231$).

Once the study had been unblinded, we calculated the median [IQR] fear susceptibility scores: 5.0 [2.0; 8.0] for the placebo group and 3.5 [2.75; 7.25] for the supplement group. Hence, there was no significant difference between the two groups in terms of fear susceptibility (U = 176, $p = 0.722$, df = 38). The 'more fearful' and 'less fearful' dogs had therefore been allocated evenly to the placebo and supplement groups.

3.2 | Placebo vs. supplement comparisons of stress reactions during the tests

Cortisol values measured after the behavioural tests including Days 0, 15 and 30 ($n = 86$; 6.41 $\pm$ 5.62 nmol/L) were significantly higher than the values measured before the tests ($n = 91$; 5.76 $\pm$ 5.97 nmol/L; W = 925, $p = 0.047$).

Differences in the median [IQR] cortisol level between post and pre-tests were not significant when comparing placebo vs. supplement on Day 0 (supplement: 0.3 [-0.8–1.4] $n = 15$; placebo: 1 [-0.3–3] $n = 13$; U = 222; $p = 0.128$; df = 27), Day 15 (supplement: 0.06 [-0.6–1.9] $n = 16$; placebo: 0.8 [-1.5–3.5] $n = 15$; U = 250; $p = 0.707$; df = 30) or Day 30 (supplement: 0.04 [-0.4–1.9] $n = 14$; placebo: 0.0 [-0.6–1.4] $n = 13$; U = 169; $p = 0.544$; df = 26). Similarly, no significant treatment effect was found following GLMM analysis of differences in cortisol between post and pre-tests.

TABLE 2 Behavioural variables in 'less fearful' dogs (simplified C-BARQ score on Day 0: 1–5) vs. 'more fearful' dogs (simplified C-BARQ score on Day 0: 6–9)

Behavioural variable	"Less fearful" dogs n=21		"More fearful" dogs n=17			
	Median	Interquartile range (IQR)	Median	IQR	U	p-value
ST1 Door zone	65	26–117	75	47–122	355.5	0.490
ST1 Experimenter zone	4.5	0–26	0	0–36	315	0.606
ST1 Interaction with door	6	3.5–13.5	8	6–12.5	349.5	0.606
ST1 Zone crossing	**34**	**26.5–43.5**	**17**	**5–30**	**239**	**0.007**
ST1 Whining	8	0.5–23.5	0	0–39	290	0.217
ST1 Yawning	0	0–0	0	0–0	235	0.764
ST1 Lip-licking	1	0–4	0	0–1	267	0.042
ST2 Door zone	0	0–45	33	4–82	409	0.019
ST2 Play	**68**	**0–117**	**7**	**0–27**	**264**	**0.046**
ST2 Interaction with door	0	0–2	1	0–6	379.5	0.139
ST2 Whining	0	0–9.5	0	0–7	307.5	0.415
ST2 Yawning	0	0–0	0	0–0	333.5	0.909
ST2 Lip-licking	0	0–3	0	0–1	301.5	0.325
ST3 Door zone	14	0–66	47	15–74	397.5	0.053
ST3 Experimenter zone	0	0–13	0	0–14	338.5	0.817
ST3 Interaction with door	1	0–3.5	1	0–3.5	328.5	0.939
ST3 Turning the head	1	1–2	2	1–3	381	0.125
ST3 Whining	2	0–4.5	0	0–12.5	302	0.375
ST3 Yawning	0	0–0	0	0–9	364	0.483
ST3 Lip-licking	1	0–2	0	0–0.5	262	0.025
ST4 Door zone	0	0–10	9	0–28	382	0.117
ST4 Experimenter zone	0	0–0	0	0–6	353	0.362
ST4 Interaction with door	0	0–0	0	0–0.5	336	0.869
ST4 Turning the head	2	1–4.5	2	0.5–4	310	0.528
ST4 Approaching object	**1**	**0.5–1**	**0**	**0–1**	**259**	**0.023**
ST4 Touching object	0	0–1	0	0–0.5	310.5	0.463
ST4 Whining	0	0–0.5	0	0–1	342.5	0.662
ST4 Yawning	0	0–0	0	0–0	314	0.211
ST4 Lip-licking	0	0–0.5	0	0–0	309	0.337

Note: Variables with a significant intergroup difference are shown in bold type. ST: subtest. One dog was excluded from this analysis because of missing data for ST3 and ST4 on Day 0.

3.3 | Treatment and time effects on behavioural variables

3.3.1 | Time spent in the door zone

Time spent in the door zone was influenced by the time of sessions (Table 3) and the interaction between time and treatment, except for ST3 with no significant interaction. Time spent in the door zone increased on Day 15 (for ST1, ST2, ST4), compared with Day 0, on Day 30, compared with Day 0 (ST2, ST3, ST4) and on Day 30, compared with Day 15 (ST2, ST3, ST4). However, the increase in time spent in the door zone differed between treatments. Considering ST1, the increase in time spent in the door zone was higher for dogs receiving the placebo

on Day 15, compared with Days 0 and 30 than for supplemented dogs (respectively, estimate ± SD = 0.17 ± 0.05, z = 3.27, p = 0.001; estimate ± SD = 0.18 ± 0.05, z = 3.55, p = 0.0004). Conversely, during ST2, the increase in time spent in the door zone was higher for supplemented dogs on Days 15 and 30 than for dogs receiving the placebo (respectively, estimate ± SD = 0.46 ± 0.006, z = 6.89, p < 0.0001; estimate ± SD = 0.67 ± 0.06, z = 10.85, p < 0.0001).

3.3.2 | Interactions with the door

The number of interactions dogs had with the door showed significant variations for ST2, ST3 but not for ST1 and ST4 (Table 3).

TABLE 3 Results of treatment and time effects (generalised linear mixed models analyses) on behavioural variables for the four ST

Behavioural variables	Fixed effects	Estimate ± standard deviation	z-value	p-value
ST1 Door zone	Days D0–D15	0.07 ± 0.03	2.72	0.007
ST2 Door zone	Days D0–D15	0.17 ± 0.04	4.14	<0.0001
	Days D15–D30	0.17 ± 0.04	4.72	<0.0001
	Days D0–D30	0.34 ± 0.04	8.85	<0.0001
ST3 Door zone	Days D0–D30	0.17 ± 0.04	4.67	<0.0001
	Days D15–D30	0.25 ± 0.04	6.72	<0.0001
ST4 Door zone	Days D0–D15	0.22 ± 0.07	3.10	0.002
	Days D15–D30	0.16 ± 0.06	2.55	0.01
	Days D0–D30	0.38 ± 0.07	5.62	<0.0001
ST1 Experimenter zone	Supplement vs. placebo	−1.17 ± 0.70	−1.66	0.098
	Days D0–D15	0.55 ± 0.05	10.79	<0.0001
	Days D0–D30	0.56 ± 0.05	11.06	<0.0001
ST3 Experimenter zone	Days D0–D30	0.21 ± 0.07	3.12	0.002
ST4 Experimenter zone	Days D0–D15	0.63 ± 0.13	4.85	<0.0001
	Days D0–D30	0.76 ± 0.13	6.03	<0.0001
ST2 Interaction with the door	Days D0–D15	0.24 ± 0.14	1.78	0.08
	Days D15–D30	0.32 ± 0.12	2.67	0.008
	Days D0–D30	0.56 ± 0.13	4.37	<0.0001
ST3 Interaction with the door	Days D0–D30	0.36 ± 0.13	2.79	0.005
	Days D15–D30	0.43 ± 0.13	3.31	0.0009
ST1 Zone crossing	Supplement vs. placebo	0.30 ± 0.18	1.65	0.098
	Days D0–D15	0.17 ± 0.05	3.85	0.0001
	Days D0–D30	0.10 ± 0.04	2.31	0.02
ST1 Whining	Days D0–D15	0.21 ± 0.05	4.72	<0.0001
	Days D15–D30	−0.16 ± 0.05	−3.56	0.0004
ST2 Whining	Days D0–D15	0.39 ± 0.08	4.89	<0.0001
	Days D15–D30	−0.23 ± 0.08	−3.07	0.002
ST4 Whining	Days D0–D30	0.59 ± 0.17	3.58	0.004
	Days D15–D30	0.42 ± 0.16	2.68	0.007
ST2 Yawning	Supplement vs. placebo	1.54 ± 0.84	1.84	0.07
ST2 Lip-licking	Days D0–D15	0.69 ± 0.19	3.73	0.002
	Days D15–D30	−0.89 ± 0.20	−4.49	<0.0001
ST2 Play	Days D0–D15	−0.10 ± 0.04,	−2.74	0.006
	Days D0–D30	−0.07 ± 0.04	−2.07	0.04
ST4 Turning the head	Days D0–D30	−0.29 ± 0.15	−2.00	0.046
ST4 Approaching object	Days D0–D30	−0.82 ± 0.32	2.53	0.01
	Days D15–D30	−0.64 ± 0.33	−1.93	0.054
ST4 Touching object	Days D0–D15	−0.95 ± 0.34	−2.80	0.005
	Days D0–D30	−0.96 ± 0.34	−2.82	0.005

During ST2 and ST3, the number of interactions dogs had with the door increased with time (Table 3). Moreover, during ST2, it increased on Day 30, compared with Days 0 and 15 for dogs receiving the placebo (respectively, estimate ± SD = 0.68 ± 0.15, z = 4.45, p < 0.0001; estimate ± SD = 0.51 ± 0.14, z = 3.50, p < 0.0001), whereas no variations were noted for supplemented dogs.

3.3.3 | Time spent in the experimenter zone

Time spent in the experimenter zone was influenced by time for ST1, ST3 and ST4 (Table 3, Figure 4): it increased on Days 15 and 30, compared with Day 0, for ST1, ST3 and ST4. Considering ST1, treatment tended to show a significant effect: Supplemented dogs stayed
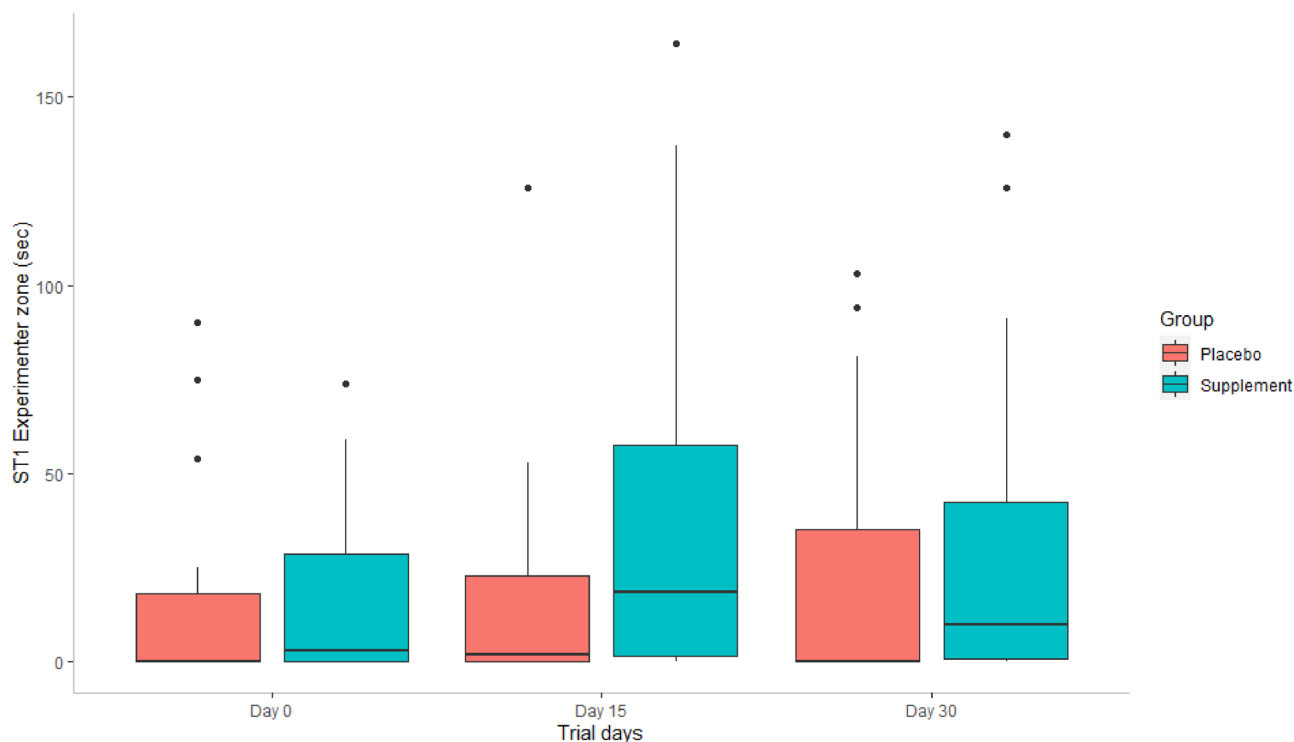
FIGURE 4 Time spent in the experimenter zone on Days 0, 15 and 30 for supplemented and placebo dogs

longer in the experimenter zone than dogs receiving the placebo (estimate $\pm$ SD = 1.17 $\pm$ 0.70, z = 1.66, p = 0.098). Interaction between treatment and time was significant for ST1: Time spent in the experimenter zone was significantly higher on Day 15 for supplemented dogs (estimate $\pm$ SD = 0.48 $\pm$ 0.12, z = 4.05, p < 0.0001; estimate $\pm$ SD = 0.27 $\pm$ 0.12, z = 2.26, p = 0.02). Hence, time spent in the experimenter zone tended to be significantly higher for supplemented dogs on Day 15 (estimate $\pm$ SD = 1.68 $\pm$ 0.7, z = 2.36, p = 0.02).

3.3.4 | Number of zone crossing

The number of zone crossing during ST1 increased with time (Table 3), tended to be higher for supplemented dogs (estimate $\pm$ SD = 0.30 $\pm$ 0.18, z = 1.65, p = 0.098; Table 3) and showed an interaction between time and treatment. On Day 0, the number of zone crossing was higher for placebo dogs (estimate $\pm$ SD = 0.53 $\pm$ 0.19, z = 2.85, p = 0.004) than for supplemented dogs, and this number decreased over time for placebo dogs (Days 15 vs. 0: estimate $\pm$ SD = −0.30 $\pm$ 0.06, z = −5.14, p < 0.0001; Days 30 vs. 0: estimate $\pm$ SD = −0.26 $\pm$ 0.06, z = −4.71, p < 0.0001). Conversely, the number of zone crossing increased for supplemented dogs on Day 30, compared with Day 0 (estimate $\pm$ SD = 0.14 $\pm$ 0.07, z = 2.08, p = 0.04).

3.3.5 | Number of whining

The number of whining was influenced by time for ST1, ST2 and ST4 (Table 3): it was higher on Day 15, compared with Days 0 and 30 for ST1

and ST2, and higher on Day 30, compared with Day 0 during ST4, and higher on Day 15, compared with Day 30 during ST4. In addition, significant effects of interaction between treatment and time were found. During ST1, for supplemented dogs, the number of whining was higher on Day 15 (D0–D15: estimate $\pm$ SD = 0.42 $\pm$ 0.07, z = 6.46, p < 0.0001; D15–D30: estimate $\pm$ SD = 0.24 $\pm$ 0.06, z = 3.87, p = 0.0001; D0–D30: estimate $\pm$ SD = 0.18 $\pm$ 0.07, z = 2.66, p = 0.008). Conversely, at ST1, the number of whining for dogs receiving the placebo did not change significantly. For ST4, similar variations were observed: The number of whining was higher for supplemented dogs on Day 30, compared with Day 0 (estimate $\pm$ SD = 0.86 $\pm$ 0.23, z = 3.80, p = 0.0001), whereas the number of whining for dogs receiving the placebo did not change with time. For ST2, the effect of time was significantly different for placebo and supplemented dogs (D0–D15: estimate $\pm$ SD = 0.41 $\pm$ 0.16, z = 2.56, p = 0.01; D15–D30: estimate $\pm$ SD = 0.60 $\pm$ 0.17, z = 3.60, p = 0.0003; D0–D30: estimate $\pm$ SD = 1.01 $\pm$ 0.18, z = 5.68, p < 0.0001): indeed, supplemented dogs whined less on Day 30, while placebo dogs whined more on Days 15 and 30. For ST3, results showed the same trend: a significant effect of time different for placebo and supplemented dogs (D0–D15: estimate $\pm$ SD = 0.50 $\pm$ 0.17, z = 2.90, p = 0.004; D0–D30: estimate $\pm$ SD = 0.58 $\pm$ 0.17, z = 3.40, p = 0.0007): supplemented dogs whined less on Day 30, while placebo dogs whined more on Days 15 and 30.

3.3.6 | Number of yawning

For ST1, ST2 and ST3, no effect of time was shown (Table 3). The number of yawning tended to be higher for placebo dogs than for

TABLE 4 Behavioural changes in dogs, as assessed by the owners

Owners whose dog <u>really</u> received:	Less anxious (positive behavioural changes)	More anxious (negative behavioural changes)	No behavioural changes
the placebo (*n* = 21)	3 out of 20 (15%)	2 out of 20 (10%)	15 out of 20 (75%)
the supplement (*n* = 18)	5 out of 18 (28%)	0 out of 18 (0%)	13 out of 18 (72%)
Owners who *thought* their dog received:	Less anxious (positive behavioural changes)	More anxious (negative behavioural changes)	No behavioural changes
the placebo (*n* = 24)	0 out of 23 (0%)	2 out of 23 (8.7%)	21 out of 23 (91.3%)
the supplement (*n* = 13)	8 out of 13 (61.5%)	0 out of 13 (0%)	5 out of 13 (38.5%)

supplemented dogs at ST2 (estimate $\pm$ SD = 1.54 $\pm$ 0.84, z = 1.84, p = 0.07), though not significantly.

3.3.7 | Number of lip-licking

For ST1, ST3 and ST4, no significant effects were found (Table 3). For ST2, lip-licking was higher on Day 15, compared with Days 0 and 30 (Table 3). The increase on Day 15 was different between supplemented and placebo dogs: For supplemented dogs, lip-licking was higher on Day 15, compared with Days 0 and 30 (respectively, estimate $\pm$ SD = 0.86 $\pm$ 0.23, z = 3.15, p = 0.002; estimate $\pm$ SD = 1.61 $\pm$ 0.37, z = 4.41, p < 0.0001), whereas for placebo dogs, lip-licking was not significantly higher on Days 15 and 30, compared with Day 0 (respectively, estimate $\pm$ SD = 0.53 $\pm$ 0.25, z = 2.11, p = 0.04; estimate $\pm$ SD = 0.46 $\pm$ 0.25, z = 1.85, p = 0.065).

3.3.8 | Turning the head towards the object, approaching the object, touching the object

Results of these three variables showed a time effect, with a significant decrease on Day 30, compared with Day 0, considering turning the head towards the object, approaching and touching the object; a decrease on Day 15, compared with Day 0, considering touching the object and a decrease on Day 30, compared with Day 15, for approaching object (Table 3).

3.3.9 | Play with the experimenter, turning the head towards the noise

A time effect was shown considering play with the experimenter, with a decrease on Days 15 and 30, compared with Day 0 (Table 3). For supplemented dogs, time playing with the experimenter was lower on Day 15 than on Day 0 (estimate $\pm$ SD = −0.18 $\pm$ 0.05, z = −3.54, p = 0.0004), while no significant change was found for placebo dogs. A significant effect of time was revealed between groups (D0–D15: estimate $\pm$ SD = 0.17 $\pm$ 0.07, z = 2.27, p = 0.02; D15–D30: estimate $\pm$ SD = 0.15 $\pm$ 0.08, z = 1.96, p = 0.049).

No effects of time or treatment were found for the variable 'turning the head towards the noise'.

3.3.10 | Results of the questionnaire assessing the owners' perception

The owners' perception of positive, negative or no behavioural changes in their dogs and of whether they thought that their dog had received the supplement or the placebo was assessed in a questionnaire (Table 4).

The owners' perception of the dog's anxiety (less anxious, more anxious, or no change) was similar in the placebo and supplement groups (p = 0.461 in Fisher's test). However, the difference in perception was significant when comparing owners who thought their dog had received the placebo with owners who thought their dogs had received the supplement; the proportions of owners reporting less anxiety were 0% and 62%, respectively, (p < 0.0001 in Fisher's test).

4 | DISCUSSION

4.1 | The fear susceptibility index and its relationship with behavioural tests

It is known that individuals' responses to stimuli vary with their temperament or their coping style (Koolhaas et al., 2010). By calculating a fear susceptibility score based on four C-BARQ questions related to fear and stress (Hsu & Serpell, 2003; Serpell & Hsu, 2001), we screened for dogs who were described by their owner as displaying fear and stress reactions. However, this type of questionnaire is necessarily subjective because it depends on the perception of the person answering (Diederich & Giffroy, 2006; Ley et al., 2009a, 2009b). In fact, quantitative objective behavioural tests are currently recommended as a means of avoiding bias due to observer subjectivity (Groothuis & Carere, 2005). In the present study, we linked questionnaires and behavioural tests and compared cortisol concentrations and behavioural variables in 'less fearful' dogs vs. 'more fearful' dogs. We hypothesised that dogs described as 'fearful' by their owners would show more frequent stress-related behaviours and have a higher post-test cortisol level in our experimental paradigm. These results

would also underpin the behavioural tests' construct validity (Taylor & Mills, 2006): Mild stressors would be more appropriate because they would stimulate more behavioural and physiological reactions from 'fearful' dogs. Even though several researchers have insisted on the need to evaluate behavioural tests (and temperament tests in particular) with regard to reliability and validity (Diederich & Giffroy, 2006; Jones & Gosling, 2005; Taylor & Mills, 2006), the latter criteria have rarely been examined.

In line with these predictions, our data showed that dogs classified as 'more fearful' (on the basis of their owner's perception) displayed significantly higher cortisol values just before but not after Day 0 session, were less active, spent less time playing with the experimenter and approached the unfamiliar object less frequently. All these behaviours have been described as fear or stress-related behaviours (Beerda et al., 1998, 1999, 2000; Stellato et al., 2017; Walker et al., 1997). These results confirm the degree of agreement between the fear susceptibility index (adapted from simplified C-BARQ scores) and the stress-related behaviours displayed during the tests. We, therefore, suggest that the fear susceptibility index is a relevant tool, despite its subjectivity and reliance on the owner's answers. Van den Berg et al.'s (2010) statistical analysis also showed that the C-BARQ questionnaire can be a reliable tool for measuring stranger-directed aggression.

4.2 | Between-test and intergroup differences in saliva cortisol concentrations

In all dogs (for the three sessions Days 0, 15 and 30), the saliva cortisol concentration was higher after the tests than before the tests. We hence suggest that the four behavioural STs (novel environment, interaction with an unfamiliar person, loud noise and novel object) were relevant as a mild stressor situation.

There were, however, no intergroup differences in the saliva cortisol concentrations when comparing post-pre-test variations. Salivary cortisol is considered to be an important biomarker in stress research. However, given the highly complex psychoneurobiological mechanisms underlying the activation of the HPA axis, the cortisol level may only be moderately related to psychological stress (Hellhammer et al., 2009). Activation of the HPA axis depends on the stressor, and is subject to a time lag (Beerda et al., 1998); hence, the sample must be collected at the cortisol peak associated with the stressor, which is difficult to predict in most studies. However, several studies showed no significant diurnal variation of cortisol in dogs (Castillo et al., 2009, Johnston et al., 1978, Koyama et al., 2003). In the present study, we could not determine whether the sudden noise, the unfamiliar person or the novel object had an impact on cortisol activation since differences appeared for several behavioural variables during the four STs.

4.3 | Time and supplement vs. placebo variations in stress-related behaviours

Once the study had been unblinded, we found that the placebo and supplement groups of dogs had similar fear susceptibility indices on Day 0. In terms of fear and stress responsiveness, the dogs were evenly attributed to the placebo and supplement groups; hence, any discrepancies in results could not be related to a difference in fearfulness between the dogs in each group.

Time spent in the door zone, the number of interactions dogs had with the door and the number of zone crossing significantly increased with time (when comparing Days 0, 15 and 30). In addition, time spent with the experimenter increased, similarly to the number of whining and the number of lip-licking (during interaction with an unfamiliar person only for lip-licking). This suggests that the design of the four STs session was relevant to test for mild stressors situations, with the increase of stress-related behaviours. Conversely, interactions and curiosity for the novel object decreased, similarly to play with the experimenter. This could have been due to a normal habituation process. The fact that the experimenter changed for the three sessions did not seem to impact the dogs' behaviours since changes in time were observed for both Days 15 and 30, compared to Day 0.

Moreover, our results showed that supplemented and placebo dogs responded differently to the three test sessions, indicating a supplement effect on dogs' behaviours and their adaptation to mild stressors situations. Time spent in the door zone and interaction with the door was significantly different between supplemented and placebo dogs: during ST1 (ST novel environment), time spent near the door was lower for supplemented dogs but higher during ST2 (ST interaction with an unfamiliar person). Conversely, interactions with the door were lower at ST2 for supplemented dogs. This could be linked to the fact that supplemented dogs stayed longer in the experimenter zone during ST1, suggesting that dogs would be more prone to interact and communicate (i.e., indicating the door) with an unfamiliar person. In the same way, lip-licking increased during ST4 for supplemented dogs, possibly linked with fear-related behaviours in a social context (Beerda et al., 1998). Play with the experimenter decreased for supplemented dogs on Days 15 and 30 but not for dogs who received the placebo. This increase in approaching the experimenter, presumably communicating with him/her, and the decrease in play behaviours may be associated with an improvement of human familiarity due to the supplement. An alleviation of fear and stress for supplemented dogs when faced with an unfamiliar person may have prompted them to spend more time near the experimenter (who changed from one test day to the next). We suggest that the supplement may enhance human familiarity, possibly through an anxiolytic effect (Dorman et al., 1995; Messaouidi et al., 2008a, 2008b; Milesi et al., 2009). The number of whining increased for supplemented dogs at ST1 (novel environment) and ST4 (novel object) with time, while it did not change for placebo dogs. Conversely, the number of whining decreased during ST2 (interaction with experimenter) and ST3 (loud noise) on Day 30 for supplemented dogs, while it increased for placebo dogs on Days 15 and 30. According to a previous study (Stellato et al., 2017), subtle stress-related behaviours such as whining occur relatively rarely and could explain the variations we observed for the different STs.

The number of zone crossing increased with time, but placebo dogs were less active (showing a decrease in zone crossing) than supplemented dogs (showing an increase in zone crossing) along the three

sessions. We can hypothesise that the dogs in the supplement group showed more curiosity and thus were more active, while dogs in the placebo group became less curious and more habituated to the testing room over the three sessions. On the contrary, pacing and enhanced activity have been described in previous studies and linked to stress-related behaviours. Since 'less fearful' dogs in our study showed lower activity, we expect that a decrease in activity was linked with increased curiosity. Hence, we suggest that the supplement may have enhanced activity and stimulated the learning processes in a familiar environment. This might be related to the mild anxiolytic effects of the fish hydrolysate and SOD already observed in rats and humans, respectively, and which might also be relevant in dogs (Dorman et al., 1995; Messaouidi et al., 2008a, 2008b; Milesi et al., 2009).

We, therefore, suggest that the supplement facilitates curiosity in a familiar environment, promotes dog-human interactions, and reduces subtle stress behaviours (i.e., yawning). According to a previous study (Stellato et al., 2017), subtle stress-related behaviours such as whining, lip-licking and yawning occur relatively rarely; this agrees with our present observations. Stellato et al. (2017) concluded that the rarity of subtle behaviours makes them of limited use for assessing mild fear responses to everyday scenarios. However, Stellato et al.'s tests took place in a fenced outdoor pen. In the present study, the tests were conducted indoors; this decreased the potential for escape-related behaviour and thus presumably enhanced fear and stress reactions. Moreover, our study featured three test sessions, which probably stimulated habituation and behavioural modifications linked to learning processes. We, therefore, consider that these subtle signs of stress were relevant in our standardised environment. This assumption is in line with the results of Beerda et al. (1998), who found a higher frequency of subtle behaviours than Stellato et al. (2017). In our study, some dogs frequently and repeatedly displayed these measured subtle stress (yawning, lip-licking and whining) during the tests. We did not measure panting (because panting could have been due to stress or high temperatures since the room temperature was not controlled) nor tail wagging (because a few dogs were docked). It is likely that the tests performed in the present study were stressful enough to elicit some fear and stress responses (as described in Beerda et al., 1997, 1998, 1999, 2000) but not stressful enough to observe them at a high frequency. Taken as a whole, these findings confirm that our test comprised mild stressors.

Compared with a previous study of a fish hydrolysate alone (Landsberg et al., 2015), our study of a supplement combining the fish hydrolysate and SOD revealed positive effects on dog familiarity, curiosity and learning processes influenced by mild stressors. In Landsberg et al.'s (2015) study, the fish hydrolysate supplement was associated with low levels of activity and saliva cortisol in dogs faced with an acute, short stressor. However, the dogs tested by Landsberg et al. (2015) were Beagles from a standardised animal research facility, whereas the participating dogs in our study dogs were pets. Along with the differences in the supplement's composition and the type of stressors used in the behavioural test, we believe that this difference in the study population also explains disparities in the results.

4.4 | Questionnaires based on the owners' perception

A satisfaction questionnaire is rarely implemented but enables the experimenter to explore the dog owner's perception and putative placebo effects. With regard to perception, we conclude that the owners were generally unable to tell whether their dog had received the supplement.

Owners who had effectively administered the supplement to their dog reported a reduction in fear-related behaviour. On the other hand, some of the owners who had administered the placebo to their dog reported an increase (or a smaller decrease) in fear-related behaviours. These results are in agreement with Korpivaara et al.'s (2017) placebo-controlled study of a dexmedetomidine oromucosal gel, in which effectiveness was assessed on a simple, owner-reported scale; 37% of the owners whose dogs had received the placebo reported a good or excellent effect, and 5% reported an unexpected poor effect.

In addition, owners who thought their dog received the supplement were more likely to report a reduction in fear-related behaviour than owners who thought their dog received the placebo. This finding reveals the importance of testing dogs in a controlled, blinded protocol and of not relying solely on questionnaires. It also suggests that any behavioural treatment may be associated with a large placebo effect as Munana et al. (2010) observed for epilepsy treatment. Our results suggest that the placebo effect operates when owners are asked to report changes in their dog's behaviour. We suggest that testing dogs in a standardised room as part of a double-blind behavioural protocol is essential for assessing the putative effects on canine behaviour exerted by a dietary supplement or other compounds.

5 | CONCLUSION

Our study shows that the supplement tested here might be useful on a short-term basis for reducing fear and stress reactions in the context of everyday life mild stressors. In particular, the supplement may lower stress reactions and modify behaviours related to habituation processes to unfamiliar environments and people. Supplemented dogs were more active, more familiar and more prone to communicate with a stranger. The supplement may facilitate curiosity in a familiar environment, promote dog-human interactions, and reduce subtle stress behaviours.

In view of the present study's double-blind design and our selection of 'more fearful' and 'less fearful' dogs using a fear susceptibility index, we suggest that the combination of fish hydrolysate and melon juice concentrate contributed to the observed behavioural improvements. The supplement could therefore be useful for reducing mild fear and stress reactions in the context of everyday life stressors, enhancing learning processes, promoting curiosity and familiarity with humans; these features may be particularly useful in boosting the effectiveness of behavioural therapies.

ETHICS STATEMENT

All procedures were performed in full compliance with the European Union's Directive 2010/63/EU on the protection of animals used for scientific purposes. All dog owners gave their informed consent prior to the study, which was approved by the local Animal Care and Use Committee (COMERC, National Alfort Veterinary School, Maisons-Alfort, France; reference: 2016-01-15).

CONFLICT OF INTEREST

Dr Titeux, Pr Gilbert and Dr Padilla provided the research for this study as contracted by MP Labo, which granted equipment and cortisol analyses.

AUTHOR CONTRIBUTIONS

The experiments were designed by Caroline Gilbert and Emmanuelle Titeux. The experiments were performed by Stephanie Padilla and Emmanuelle Titeux. The data were analysed by Stephanie Padilla, Emmanuelle Titeux and Caroline Gilbert. The article was written by Emmanuelle Titeux and Caroline Gilbert, reviewed by Bernard-Marie Paragon.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.560.

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