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Research article

Assessing the effectiveness of cannabidiol additive supplementation on canine behavior and cortisol levels

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A B S T R A C T

In veterinary medicine, Cannabis has been used to treat pain conditions, inflammation, and seizures. However, little is known about its effect on dogs' behavior. This preliminary research aims to address this knowledge gap by evaluating the effectiveness of cannabidiol (CBD) oil in canine behavioral therapy. Twenty dogs, diagnosed with behavioral disorders and housed in a municipal shelter, participated in a double-blind trial. Ten dogs received CBD oil treatment, while the other ten received a control oil without CBD. Before (T0) and after (T1) the treatment, all the dogs underwent a temperament test to assess their behavior in the presence of four different stimuli: a human stranger, a novel object, a child-like doll, and a conspecific (another dog). Each stimulus was presented individually, and the dogs' behaviors were recorded on video and analyzed. Additionally, hair samples were collected using a shave-reshave technique for cortisol determination through Radio-Immuno-Assay. No behavioral differences were found between the two groups at both T0 and T1. There were no significant differences in the behavioral responses of either group when comparing T0 and T1. However, individual responses to the CBD oil treatment appeared to vary among subjects. A significant increase in hair cortisol levels (p-value <0.05) was observed in the group treated with CBD oil [T0 = 1.60 (1.44–1.93) pg/mg, T1 = 4.81(2.57–6.01) pg/mg]. These findings highlight the importance of individualized treatment when using Cannabis and encourage further research on the use of CBD oil in animal behavioral medicine.

1. Introduction

Animal welfare is a topic that garners substantial attention from consumers and is increasingly regarded as a crucial factor in society's evaluation of the acceptability and sustainability of animal care and utilization practices [1,2]. The public's perception of animal welfare is continually evolving and exerts a direct influence on legislation [3,4] as well as consumer purchasing decisions [5,6]. While discussions about animal experimentation [7,8] and livestock production [9–11] have received substantial attention, there has historically been relatively limited consideration given to the welfare of companion animals [12]. However, in recent years, there has been a noticeable shift, with an increasing focus on the well-being of companion animals, marking it as a central theme in contemporary discussions [13]. Based on the estimation provided by FEDIAF (14), the European pet population is approximately 309 million, with cats (112 million) and dogs (92 million) comprising the largest proportion. In modern times, these animals have become an integral part of the human family unit [14], and their well-being is deemed highly significant by their owners [15,16]. As per the most recent EU report [17] on public attitudes towards animal welfare in Europe, a majority of EU citizens (74 %), including Italians (80 %), express the belief that the welfare of companion animals should receive enhanced protection. For this reason, the use of feed additives (particularly plant extracts) to enhance the well-being and health of domestic animals, including companion animals, is becoming

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increasingly widespread [18–20].

Cannabis, also known as hemp, has a long history of cultivation spanning thousands of years for various purposes, such as rope, food, and oils [21]. Moreover, it has also been recognized for its medical properties to treat different symptoms and diseases [22]. Different Cannabis varieties differ in plant structure and cannabinoids content [23]. The most well-known species are *Cannabis sativa* and *Cannabis indica*, along with their hybrid combinations [24]. Cannabis consists of over 750 different chemical compounds, including terpenes, flavonoids, hydrocarbons, and phytocannabinoids, which collectively produce the so-called "entourage effect" [25,26]. Among these compounds, the two most recognized cannabinoids are tetrahydrocannabinol (THC) and cannabidiol (CBD). The THC has psychotropic effects and higher toxicity compared to CBD, which is a non-psychotropic compound with a higher safety threshold. Consequently, CBD has gained increasing usage in veterinary medicine in recent years [22]. In veterinary medicine, CBD has been used to address various conditions including pain, inflammation, seizures, and anxiety [22]. In this context, the endocannabinoid system (ECS) plays a pivotal role in facilitating the interaction between CBD and the organism. The ECS encompasses two primary receptor types, CB1 and CB2, which interact with both endogenous cannabinoids [27] and exogenous compounds derived primarily from Cannabis, such as CBD and THC [28]. This system is essential for maintaining organismic homeostasis and regulates various physiological and pathological processes, such as the modulation of the nervous and immune systems, or the transmission and modulation of pain signals and inflammation [29]. Gamble et al. [30], for example, found that a CBD-based treatment leads to a decrease in pain and a rise in activity in dogs with osteoarthritis.

Considering animal behavior, there have only been a limited number of studies examining the effects of CBD-based treatment on dogs' behavior. The CBD has been successfully used to control anxiety and stress conditions in animals [23,32]. Stress is a generalized response of the body to different factors that can disrupt individual homeostasis [31] and can lead to anxiety, fear, aggressiveness, and the development of displacement behaviors and stereotypies [32,33]. Stereotypies and displacement behaviors are considered indicators of poor welfare conditions, which can even lead to self-injury or self-mutilation, further compromising the animal's quality of life [33,34]. Dog shelters can be a stressful environment due to a lack of social interactions and stimuli, resource competition, and overcrowding [35,36], potentially leading to chronic stress conditions, and a rise in cortisol levels. Prolonged high cortisol levels can result in increased adrenal and thymus size, weight loss, reduced activation of lymphocytes, and increased susceptibility to infections and tumors [31].

Given the limited availability of literature about the effect of CBD on dog behavior, our objective was to assess the effects of CBD oil supplementation on dogs hosted in a shelter and affected by behavioral disorders. We hypothesize that this molecule will have an impact on dogs' temperament, and we predict an improvement in the behavior of treated dogs, with a decrease in behaviors caused by a state of anxiety (e.g., aggressive or discomfort behaviors) and an increase in positive behaviors (e.g., sociability and curiosity) response to novel stimuli. Additionally, we investigated the chronic response of the hypothalamic-pituitary-adrenal (HPA) axis by measuring cortisol levels in hair, and we predicted a decrease in cortisol levels in treated dogs. This decrease is expected to be a result of reduced anxiety, which can cause hyperactivity, avoidance, and compulsive behaviors, and an improvement in their overall welfare state. This condition would also be a beneficial effect due to an improvement in the management of the animals and a rise in their possibilities to get adopted.

2. Materials and methods

The study involved non-invasive procedures, and the administration of cannabinoids was supervised by the veterinarian, who would have administered them for therapeutic purposes regardless of this research. The experimental procedures comply with the ethical standards on animal experimentation following EU Directive 2010/63/EU [37] for animal experiments and were submitted to the Ethics Committee of the University of Bologna (Protocol n. 4567).

2.1. Animals and housing

The participants in this study were 21 domestic dogs diagnosed with various behavioral disorders by a veterinary behaviorist at the dog shelter where they resided. The main diagnosis included Hypersensitivity-Hyperactivity Syndrome (HSHA), Sensory Deprivation Syndrome (SDS), and Leadership Disorder (LD). These dogs exhibited avoidance, hyper-reactivity, and a lack of self-control. Some of them also displayed severe behavioral disorders such as compulsive wandering, compulsive limb licking resulting in skin lesions, and compulsive spinning. While some dogs had additional concurrent pathologies, none of them were undergoing pharmacological treatment during the study.

The research was conducted at a dog shelter in Italy, with a total capacity to accommodate 105 dogs. However, at the time of the study, there were 50 dogs present in the shelter. The shelter has 30 kennels, with a capacity to accommodate three dogs each. Each kennel consists of both an outdoor and an indoor area measuring 26.10 m^2, which was not visible from the outside. Additionally, there are three outdoor walking areas (measuring 357.0 m^2, 305.00 m^2, and 662.0 m^2), where the dogs were taken once a day. Finally, there are 10 kennels for initial reception, 3 kennels for unsociable dogs, and 2 kennels for isolation. These kennels are 9.0 m^2 and can host one dog. The dogs are fed twice a day by volunteers, who provide them with commercial food tailored to their dietary needs. Moreover, the veterinary behaviorist closely monitors the dogs to improve their behavior and increase their chances of adoption.

2.2. Treatment protocol

The veterinary behaviorist randomly divided the dogs into two different groups. As a double-blind study, the groups remained

unknown to the researchers until the end of the study. The first group (CTR) consisted of 10 dogs that received a control oil without CBD, while the second group (C) comprised 11 individuals who were supplemented with CBD oil (Table 1).

The control consists of MCT (Medium Chain Triglycerides) oil derived from coconut oil, supplemented with vitamin E. The CBD-based oil ("CanaCaress" by Canapa House) is composed of MCT oil combined with 5 % CBD, 2.5 % CBG (Cannabigerol), and 0.18 % THC. Both the oils were inserted into small lard bites and administered to the dogs twice a day, 1 h before mealtime and the treatment lasted 50 days. The dosage of CBD oil and the control was calculated by the shelter's veterinary behaviorist based on the animal's weight (Table 1).

2.3. Temperament test

The temperament tests were adapted from those proposed by Barnard et al. [38] for sheltered dogs. In this study, to ensure the safety of the researchers and dogs, the subjects were assessed within their kennels and were unable to have direct contact with the stimuli, but only interacted with test stimuli through the fence. Additionally, we replaced the plastic dog used in the reference study with three real conspecifics. One of the dogs of the C group was never visible during the tests, so was not considered for the behavioral evaluation.

The test sessions were video-recorded using a SONY® Handycam HDR CX-405, and the researchers were blinded to which group the dogs belonged to. The test consisted of four different phases.

a) Phase 1 - Human stranger: a female stranger, wearing sunglasses to avoid direct eye contact with the dogs, approached the animal in a friendly manner and then stood 1 m from the fence, engaging in a 90-s conversation with the dog.
b) Phase 2 - The doll: a 90 cm tall doll, manipulated by the same observer, was brought by hand to the fence, resembling a three-year-old child. The observer stood in front of the fence with the doll for 40 s.
c) Phase 3 - The ambiguous object: a garbage bag filled with paper and shaped like an "eight" was placed near the fence for 40 s.
d) Phase 4 - Dog stranger: the dogs were approached by a leashed unfamiliar dog, which walked along the fence for 40 s and then sat 1 m from the fence for 30 s.

Each dog underwent the test twice: the first test was conducted seven days before the beginning of the treatment (T0), and the second test was performed on the 50th and final day of the treatment protocol (T1).

The videos were analyzed using the BORIS (Behavioral Observation Research Interactive Software) software v.7.13.7 [39]. The observed behaviors were assessed based on an ethogram that comprised six different categories: aggression, fear, discomfort, curiosity, sociability, and maintenance [38]. For each dog, the relative duration of each behavioral category in every test phase was calculated.

Table 1
Characteristics of the dogs belonging to the Control and CBD oil groups. HSHA=Hypersensitivity-Hyperactivity Syndrome, SDS = Sensory Deprivation Syndrome, and LD = Leadership Disorder. M = Male, N=Neutered male, S=Spayed female. Furthermore, there are specified the CBD oil and control oil dosage calculated considering the dog's weight. BID = *bis in die (twice daily)*.

Name	Group	Breed	Age (Yr)	Weight (Kg)	Sex	Behavioral Problem	Co-Morbidities	Hair Sample	Drops/ BID	CBD mg/Kg	CBG mg/Kg
Baghera	Control	Mixed breed	4	30	M	HSHA	/	yes	7	/	/
Byron	Control	Mixed breed	4	26	N	HSHA	Food intollerance	/	7	/	/
Carola	Control	Mixed breed	1	15	S	SDS	/	/	4	/	/
Coffe	Control	Mixed breed	6	5.5	M	HSHA	/	/	4	/	/
Cortina	Control	Mixed breed	5	30	S	SDS	/	yes	5	/	/
Ebby	Control	Belgian sheperd	12	20	M	SDS	/	/	5	/	/
Jhonny	Control	Mixed breed	12	18	M	HSHA	Cardiomyopathy	yes	5	/	/
Mary	Control	Pitbull	5	30	S	HSHA	/	yes	7	/	/
Winston	Control	Maremma sheepdog	5	35	M	HSHA	/	/	7	/	/
Zeus	Control	Maremma sheepdog	6	48	M	SDS and HSHA	/	/	8	/	/
Aron	CBD *oil*	Mixed breed	5	35	M	SDS	/	yes	7	0.5	0.25
Billy	CBD *oil*	Border collie	5	15	N	HSHA	/	yes	5	0.83	0.415
Brando	CBD *oil*	Mixed breed	6	18	N	SDS	/	/	6	0.83	0.415
Creed	CBD *oil*	Amstaff	3	33	M	HSHA	/	/	7	0.5	0.25
Elvis	CBD *oil*	Mixed breed	7	38	M	SDS	/	yes	7	0.45	0.225
Iena	CBD *oil*	Mixed breed	5,5	30	M	HSHA	/	/	7	0.58	0.29
Igor	CBD *oil*	Amstaff	5	35	N	HSHA	Rupture of the right cruciate ligament	yes	7	0.5	0.25
Jason	CBD *oil*	Pit bull	10	22	M	HSHA	Arthrosis	yes	6	0.68	0.34
Toby	CBD *oil*	Mixed breed	5	40	M	SDS	/	yes	8	0.5	0.25
Togo	CBD *oil*	Mixed breed	5	23	N	LD	Chronic otitis	/	6	0.65	0.325
Corina	CBD *oil*	Mixed breed	4	30	S	SDS	/	yes	7	0.58	0.29

3. Cortisol

To avoid the sedation of animals, only dogs who well tolerated the sampling procedure (7 dogs of the C group and 4 dogs of the CTR groups) underwent three different hair collections in the same area near the cervical region. We utilized a shave-reshaving technique and collected the hair using an electric shaver. The first sample collection was conducted 53 days before the second collection, and the third collection took place 64 days after the second. The second sample was taken 8 days before the initiation of the trial. After the collection, all samples were identified with the name of the dog and the date, each hair sample was placed in plastic containers and stocked at room temperature until the analysis.

Cortisol concentrations were determined by Radio Immuno Assays (RIA). Cortisol was extracted from hair by putting 60 mg of trimmed hairs (1–3 mm) in a glass vial with 5 ml methanol [40]. All samples were dried under an air-stream suction hood at 37 °C and the dry-residue was dissolved into phosphate-buffered saline (PBS) 0.05 M, pH 7.5. The cortisol RIA was performed using an antiserum to cortisol-21-hemisuccinate-BSA (anti-rabbit), at a working dilution of 1:20000 and 3H-cortisol (30 pg/tube vial) as a tracer. Validation parameters of analysis were: sensitivity 0.19 pg/mg, intra-assay variability 5.9 %, and inter-assay variability 8.7 %. Radioactivity was determined using a liquid scintillation βcounter and a linear standard curve, ad hoc designed by a software program.

3.1. Statistical analysis

All the data were collected in Microsoft Excel (2019). The MIXED procedure of JMP (version 16.1 Pro, Statistical Analysis Systems Institute Inc., Cary, NC) was used for all the analyses. The normal distribution of the behavioral data was checked using Shapiro-Wilk's test before the analysis [41], and Box-Cox transformation was applied to normalize the variables, as they resulted in a non-normal distribution [42].

Different mixed models [43] were implemented in order to study the effects of treatment, phase and time, as followed reported. The first model tested the absence of differences between groups (CTR and C) at T0, with T0 included as a covariate in subsequent analyses. The fixed effects of the model were treatment (CTR and C), test phases (1, 2, 3, 4), and their interaction. The second analysis evaluated the evolution of analyzed behaviors at T0 and T1 in both groups. The fixed effects of this model were time (T0 and T1), phases (1, 2, 3, 4), and their interaction. The random effect of the model was the experimental unit (the dog), nested with age, sex, and behavioral disorder.

Due to the little number of cortisol data, we cannot assume the normality of the distribution. Thus, to compare the hair cortisol (HC) levels between the two groups (CTR and C) at T0 and T1, a Mann-Whitney *U* test was employed [42]. Additionally, to investigate the difference in HC levels within the same group between the beginning and end of the treatment, a Wilcoxon signed-rank test was performed.

A p-value ≤ 0.05 was considered statistically significant, while a p-value ≤ 0.01 was considered highly significant. The results were expressed as the median, 25th, and 75th percentiles.

4. Results

4.1. Comparison between groups

During the study, no dogs exhibited any of the possible side effects reported in the package leaflet (i.e., increased hunger, drowsiness, and, rarely, loose stools) related to the administration of the CBD oil.

Table 2
Influence of group, test phase, and their interaction on the behavioral response to the temperament test during the pre-treatment period. DF = degree of freedom. In bold the significant results, p-value<0.05

	Fixed Factor	Nparm	Num DF	Den DF	Rate F	Prob > F
Aggressive behavior	GROUP	1	1	60.7	0.000532	0.9817
	TEST PHASE	3	3	54	8.831548	**<0.0001**
	GROUP*TEST PHASE	3	3	54	1.476846	0.2311
Fear	GROUP	1	1	47.2	0.284939	0.596
	TEST PHASE	3	3	54	1.617198	0.1961
	GROUP*TEST PHASE	3	3	54	0.231802	0.8738
Discomfort	GROUP	1	1	44	0.342848	0.5612
	TEST PHASE	3	3	54	1.9996	0.125
	GROUP*TEST PHASE	3	3	54	0.41913	0.74
Curiosity	GROUP	1	1	61.1	0.511924	0.477
	TEST PHASE	3	3	54	1.345674	0.2693
	GROUP*TEST PHASE	3	3	54	1.247717	0.3016
Social behavior	GROUP	1	1	59.5	0.204179	0.653
	TEST PHASE	3	3	54	2.687172	0.0555
	GROUP*TEST PHASE	3	3	54	0.091112	0.9646
Maintenance	GROUP	1	1	67.2	0.298776	0.5865
	TEST PHASE	3	3	54	28.73028	**<0.0001**
	GROUP*TEST PHASE	3	3	54	0.199593	0.8962

At the pre-treatment period (T0), the statistical analysis of the behavioral response to different stimuli did not reveal any significant difference between the CTR and C groups. Additionally, no significant relationship was found between the interaction of the test phase and group and the percentage duration of the behavioral categories exhibited by the dogs. However, the test phase appeared to have a significant effect ($p < 0.05$) on the percentage duration of aggressiveness and maintenance behaviors (Table 2).

Similarly, during the post-treatment period (T1), the statistical analysis did not indicate any significant influence of the groups or the interaction between the test phase and group on the percentage duration of the behavioral categories observed. Nevertheless, once again, the test phase seemed to significantly ($p < 0.05$) impact the percentage duration of aggressiveness and maintenance behaviors (Table 3).

4.2. Comparison between pre and post-treatment

Considering the CTR group, no statistical differences were found between T0 and T1 regarding the percentage duration of the behavioral categories in the temperament test overall (Fig. 1) and during the individual phases (interaction between treatment periods and test phases). However, the statistical model revealed a significant influence ($p < 0.05$) of the test phase on the percentage duration of aggressiveness, sociability, and maintenance exhibited by the dogs in this group (Table 4).

Similarly, C dogs did not exhibit any significant changes in their behavioral response during T1 compared to T0, both in the overall test and the individual test phases. However, in this group as well, the test phase appeared to significantly ($p < 0.05$) influence the percentage duration of aggressiveness, fear, and maintenance behaviors (Table 5).

4.3. Hair cortisol

The HC concentration resulted statistically higher ($W = 0$, $r = 0.798$, 95%C.I. = 0.66–8.19, p-value = 0.01) in the CTR group (median = 3.33 (2.79–5.25) pg/mg) compared to group C (median = 1.60 (1.44–1.93) pg/mg) at T0, but not at T1 (P = 2.13 (1.28–2.84) pg/mg, C = 4.81(2.57–6.01) pg/mg). Considering only group C, the HC concentration was statistically higher at T1 compared to T0 ($V = 2$, $r = 0.767$, 95%CI = −5.92 to −0.22, p-value = 0.047), while this difference was not found in the group P (Fig. 1).

5. Discussion

Our research aims to assess the effects of CBD oil supplementation on dogs hosted in a shelter and affected by behavioral disorders. None of the dogs involved in the study showed any symptoms of intolerance to MCT and CBD oils, indicating that the treatment was well tolerated by all dogs. After the first temperament test (T0), both the CTR and C groups did not exhibit any significant difference in the relative duration of the different behavioral categories observed during the test phases. This result is desirable as it indicates that there were no significant differences between the two groups before the treatment began, establishing their homogeneity.

After the 50-day treatment, the temperament test was conducted again, and similar to the pre-treatment test, the statistical analysis found the two groups to be homogeneous. The results of the two temperament tests were compared, and no significant differences were found between T0 and T1 in both groups. This finding is consistent with existing literature, which also reports no significant differences in aggressiveness, fear, or daily management of dogs after treatment with CBD [44–46]. However, it is important to note that this study has several limitations, including the inability, due to the shelter environment and organization, to constantly monitor each dog and evaluate individual responses to the treatment. As a result of these constraints, standard microdoses of CBD and CBG (<0.5 mg/kg BID)

Table 3
Influence of group, test phase, and their interaction on the behavioral response to the temperament test during the post-treatment period. DFA = degree of freedom. In bold the significant results, p-value<0.05.

	Fixed Factor	Nparm	Num DF	Den DF	Rate F	Prob > F
Aggressive behavior	GROUP	1	1	40.1	0.637219	0.4294
	TEST PHASE	3	3	44	3.713198	**0.0182**
	GROUP*TEST PHASE	3	3	43.3	0.447927	0.72
Fear	GROUP	1	1	62	0.325178	0.5706
	TEST PHASE	3	3	44.2	0.884251	0.4566
	GROUP*TEST PHASE	3	3	43.2	1.117335	0.3525
Discomfort	GROUP	1	1	27.5	0.025899	0.8733
	TEST PHASE	3	3	39.2	1.344095	0.274
	GROUP*TEST PHASE	3	3	38.8	0.953356	0.4244
Curiosity	GROUP	1	1	46.9	0.368651	0.5467
	TEST PHASE	3	3	44.1	0.687714	0.5644
	GROUP*TEST PHASE	3	3	44.7	0.42276	0.7376
Social behavior	GROUP	1	1	52.7	0.002407	0.9611
	TEST PHASE	3	3	48.2	2.441818	0.0755
	GROUP*TEST PHASE	3	3	47.4	0.221519	0.881
Maintenance	GROUP	1	1	62	8.09E-05	0.9929
	TEST PHASE	3	3	51.1	4.888734	**0.0046**
	GROUP*TEST PHASE	3	3	47.6	1.266744	0.2965

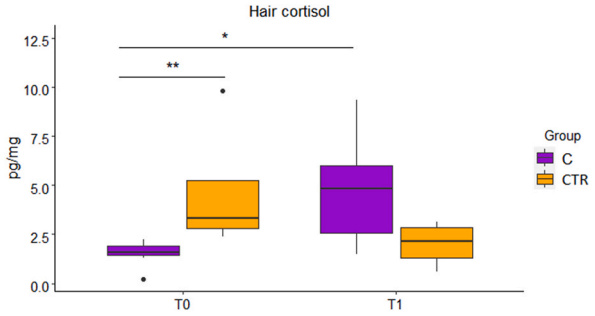

Fig. 1. Hair cortisol levels (pg/mg) of control (CTR) and CBD oil (C) groups at T0 and T1 The bar within the box represents the median, the borders of the box are upper quartiles, the bottom and top whiskers signify the lowest and highest cases within 1.5 times interquartile range (IQR), and outliers are shown through black full circles. * significant results ($p < 0.05$), ** highly significant results ($p < 0.001$).

Table 4
Influence of N.test (T0 or T1), test phase, and their interaction on the behavioral response to the temperament test of the Control group. DFA = degree of freedom. In bold the significant results, p-value<0.05.

	Fixed Factor	Nparm	Num DF	Den DF	Rate F	Prob > F
Aggressive behavior	N.TEST	1	1	58.1	0.060282	0.8069
	TEST PHASE	3	3	58.2	6.520202	**0.0007**
	N.TEST*TEST PHASE	3	3	58.2	0.48206	0.696
Fear	N.TEST	1	1	57.5	0.063448	0.802
	TEST PHASE	3	3	57.7	0.908947	0.4424
	N.TEST*TEST PHASE	3	3	57.7	0.923604	0.4352
Discomfort	N.TEST	1	1	58.5	0.913513	0.3431
	TEST PHASE	3	3	59	0.635269	0.5952
	N.TEST*TEST PHASE	3	3	59	0.701016	0.5552
Curiosity	N.TEST	1	1	58.1	0.142968	0.7067
	TEST PHASE	3	3	58.2	2.173269	0.1009
	N.TEST*TEST PHASE	3	3	58.2	1.410374	0.2489
Social behavior	N.TEST	1	1	58.2	0.409476	0.5247
	TEST PHASE	3	3	58.5	3.721825	**0.0162**
	N.TEST*TEST PHASE	3	3	58.5	0.354386	0.7861
Maintenance	N.TEST	1	1	58.3	1.625166	0.2074
	TEST PHASE	3	3	58.6	27.96893	**<0.0001**
	N.TEST*TEST PHASE	3	3	58.6	0.423367	0.7369

Table 5
Influence of N.test (T0 or T1), test phase, and their interaction on the behavioral response to the temperament test of the CBD oil group. DFA = degree of freedom. In bold the significant results, p-value<0.05.

	Fixed Factor	Nparm	Num DF	Den DF	Rate F	Prob > F
Aggressive behavior	N.TEST	1	1	59.6	0.69	0.4089
	TEST PHASE	3	3	59.5	13.7	**<0.0001**
	N.TEST *TEST PHASE	3	3	59.5	0.18	0.9105
Fear	N.TEST	1	1	59.2	0.21	0.6449
	TEST PHASE	3	3	59.2	2.86	**0.0443**
	N.TEST *TEST PHASE	3	3	59.2	0.2	0.8929
Discomfort	N.TEST	1	1	59.3	1.68	0.2002
	TEST PHASE	3	3	59.3	0.67	0.5721
	N.TEST *TEST PHASE	3	3	59.3	1.37	0.2611
Curiosity	N.TEST	1	1	60.5	1.07	0.3041
	TEST PHASE	3	3	60.3	0.15	0.9292
	N.TEST *TEST PHASE	3	3	60.3	0.11	0.9519
Social behavior	N.TEST	1	1	59.8	0.53	0.4694
	TEST PHASE	3	3	59.6	2.16	0.1018
	N.TEST *TEST PHASE	3	3	59.6	0.11	0.9551
Maintenance	N.TEST	1	1	59.5	1.85	0.179
	TEST PHASE	3	3	59.4	22	**<0.0001**
	N.TEST *TEST PHASE	3	3	59.4	0.56	0.6415

were employed to minimize side effects, and the dosage remained unchanged throughout the study period. Nonetheless, it would be beneficial to assess the treatment's effectiveness and adjust the doses according to individual responses [47,48].

However, when considering the results of the temperament test for individual subjects treated with CBD oil, it appears that the response to the treatment varies among individuals. This finding aligns with the observations made by Corsetti et al. [46], who reported a reduction in aggression towards humans in only some dogs following the administration of a CBD-based oil. It is also consistent with the studies conducted by Hartsel [48] and Vaughn [47], which demonstrated individual sensitivity to cannabinoids in terms of side effects, plasma concentration, and half-life of the molecules. The dogs that appeared to be most responsive to the treatment were those that had comorbidities resulting in chronic pain and those that exhibited stereotypies. In some of these subjects, the results of the temperament tests revealed a decrease in aggressiveness towards humans and other dogs, as well as a reduction in discomfort behaviors such as stereotypies. Additionally, an increase in curiosity and social behaviors towards both humans and other dogs was observed. These positive outcomes could be attributed to the anti-inflammatory and analgesic properties of CBD and CBG. Research by Camps et al. [49] demonstrated that pain can lead to an increase in aggressive behaviors and impulsivity in dogs. Dogs who did not exhibit aggressive behaviors prior to experiencing pain were found to become more impulsive and display aggression and defensive postures more frequently when approached or manipulated [49]. Other studies have reported the beneficial effects of cannabinoids, specifically CBD and CBG, in managing chronic pain in dogs due to their anti-inflammatory and analgesic properties [50,51]. Furthermore, research by Gamble et al. [52] showed that CBD oil administration can reduce pain associated with arthrosis. Moreover, CBD has been shown to have anxiolytic and anti-compulsive properties, as well as the ability to decrease unconditional fear, anxiety, and stress [53,54]. This could explain the observed reduction in anxiety-related behaviors and stereotypies in some subjects from the treated group.

Considering the concentration of HC in the two groups before and after the 50-day administration, we observed that the CTR group exhibited higher HC levels than the C group at T0. However, at the end of the treatment period, the only C group demonstrated a statistically significant increase in HC levels. Although these findings are only preliminary due to the limited number of dogs that were sampled, they partially align with various studies conducted on humans [55,56] and mice [57–60]. These studies reported that acute administration of THC (delta-9-tetrahydrocannabinol) in non-habitual users resulted in a significant increase in cortisol, corticosterone, and ACTH (adrenocorticotropic hormone). THC can bind to cannabinoid receptors and activate the HPA axis, and chronic exposure to THC can lead to dysregulation of the HPA axis, chronically elevated basal cortisol and ACTH levels, and reduced activation of the axis during acute stress. This condition ultimately results in a decreased ability to cope with stress [61]. In addition, we cannot exclude the potential influence of chronic pain or stress on the hair cortisol levels of the three animals affected by chronic diseases, as demonstrated in human studies [62]. However, a study did not find a difference in hair cortisol concentration between dogs affected by various chronic diseases and healthy dogs [63]. Therefore, further research is necessary to investigate the correlation between chronic pathologies and hair cortisol concentration.

6. Conclusion

In conclusion, this study did not identify statistically significant changes in the behavior of dogs treated with CBD oil twice a day. However, it did reveal certain behavioral modifications in specific subjects that received cannabinoids, including a reduction in aggressiveness and discomfort, as well as an increase in curiosity and social behaviors. These preliminary findings highlight the importance of individualized treatment when employing cannabis-based supplementations and emphasize the need for further research to assess the use of cannabinoids in veterinary behavioral medicine.

Availability of data and materials

Data are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Giovanna Marliani: Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Conceptualization. **Lucrezia Vaccari:** Writing – original draft, Methodology, Investigation, Formal analysis. **Damiano Cavallini:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Carmen Silvia Montesano:** Methodology, Investigation, Formal analysis. **Giovanni Buonaiuto:** Writing – review & editing, Writing – original draft, Visualization, Supervision. **Pier Attilio Accorsi:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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