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RESEARCH ARTICLE

Twenty-Four Hour and One-Week Steady State Pharmacokinetics of Cannabinoids in Two Formulations of Cannabidiol and Cannabidiolic Acid Rich Hemp in Dogs

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ABSTRACT

The aim of this study was to compare the pharmacokinetics of a cannabidiol (CBD) and cannabidiolic acid-rich (CBDA) hemp extract in a sesame oil base and a soft gel capsule formulation. During acute twenty-four hour pharmacokinetic evaluation, maximum serum concentration (C_{max}) was higher for all measurable components in the serum (CBD, CBDA, Δ -9-tetrahydrocannabinol [THC] and tetrahydrocannabinolic acid [THCA]) following soft gel administration versus the oil formulation. Similar times of maximal serum concentration (T_{max}) were observed with both presentations. Based on the area under the curve, a significant increase in CBDA absorption was observed following soft gel dosing. Whilst comparable, the steady state pharmacokinetic data after one week of twice daily dosing shows an increased concentration of CBDA, with a slightly lower CBD concentration with soft gel administration compared to oil. Although THC and THCA concentrations remain comparably low from both presentations, THCA absorption was superior regardless of formulation. When examining acceptance, soft gels are associated with increased palatability and less rejection of oral dosing with oil. Dogs, and potentially even people, may show increasing tolerance to this soft gel formulation; as such consideration should be given to the ease of administration and superior CBDA absorption with the use of a soft gel formulation of a CBD/CBDA hemp blend.

Introduction:

As we continue to see an increase in the use of cannabidiol (CBD) rich supplements in people and their pets, our attention should move towards seeking definitive evidence of the efficacy of such products in providing positive outcomes in clinical medicine. Whilst there are some emerging publications demonstrating pharmacokinetics, safety and efficacy in the use of CBD products in dogs for various clinical conditions¹⁻⁵, ongoing research is warranted. It is prudent to explore the absorption and fate of specific presentations of products prior to release to the public.

Based on previous studies^{1, 2, 6} a dose of 2mg/kg of a CBD/CBDA rich hemp extract is considered an appropriate dose in canines. Further investigations at 2mg/kg dosing of a 50:50 mixture of CBD and CBDA showed superior absorption of a soft chew formulation compared to an oil base, and that administration with food may increase absorption of the active molecules^{1, 7}. Previous published studies of a similar blend of CBD/CBDA have proven safety and have shown pharmacokinetics of this specific blend as well as other blends of cannabinoids^{1, 2, 6, 8, 9, 10}.

It is postulated that CBDA undergoes biotransformation in the mammalian body into CBD¹¹. This same group found that administration of oral CBDA rather than CBD resulted in three times the serum concentration of CBD in human subjects. Importantly, this suggests that CBDA may be more bioavailable and may be converted *in-vivo* to CBD, aiding the absorption of CBD following lower dosing levels. Recent data in dogs and humans, however, refutes the assumption that there is bioconversion of CBDA to CBD^{6, 12}. Similarly, there is experimental data of the presence of THC in body systems following administration of CBD alone¹³, but converse evidence disputing that this occurs¹⁴.

It is increasingly accepted that maximal absorption of CBD is superior when administered with a fatty meal^{1, 15-17}. More specifically, the work of Deabold *et al.*¹ evaluated this in canine patients fed a soft chew food matrix product, suggesting improved absorption and higher maximal concentrations of CBD.

Nearly all full spectrum hemp products currently marketed, including those labelled as CBD isolates, will contain some level of THC and THCA. This level is usually declared as less than 0.3% content, and whilst some work has been undertaken evaluating absorption⁵, little is known regarding

the absorption of these cannabinoids in canines and their subsequent psychotropic and neuroprotective effects.

Wakshlag *et al.*⁶ demonstrated CBDA and THCA absorption and retention in dogs, and the effect of different vehicle mediums on delivery of these compounds orally. In their work, evidence is provided that THC uptake remains low in all presentations, which concurs with the work of Taylor *et al.*¹⁷. They further highlighted the synergism that CBDA and THCA may possess working alongside CBD, namely the “entourage effect”. This means that more benefit can be observed utilising whole hemp extract versus hemp isolates. Importantly, these authors clearly pointed out that absorption data will potentially vary considerably between products, due to variability between carrier oils and cannabinoid and terpene profiling. As such, it is imperative that companies research and provide individual data on their specific blend and presentation, as part of due diligence, before the release of hemp-based products to market.

The aim of this study was to evaluate pharmacokinetic (PK) data and explore potential administration protocols of a soft gel formulation of a CBD/CBDA rich hemp extract, compared to an original oil-based presentation of the same CBD/CBDA hemp extract. Evaluation of the presence and kinetics of absorption of various important cannabinoid compounds and *in-vivo* conversion into metabolites was studied. Observation of side effects of administration of either preparation was undertaken following an initial observation of the possibility of increased mild gastrointestinal events associated with soft gel administration.

Materials and Methods:

Eight purpose bred research Beagle dogs were used for this 28-day study, consisting of seven males and one female. Screening for suitability consisted of physical examination, complete blood count and serum biochemistry (Antech Diagnostics, Fountain Valley, CA). These dogs were between one and seven years of age and weighed between 11.47 and 12.3 kg at the start of the investigation. Standard measured feeding regime for the facility, consisting of dry food (Purina Dog Chow; Nestlé Purina Petcare, St Louis, Missouri) was administered for the duration of the study. One packet of wet food (Cesar Classic Loaf; Mars Petcare, McLean, Virginia) was offered following administration of product in the morning of Days 1 and 22.

The CBD/CBDA rich hemp oil utilized in the study was submitted for cannabinoid analysis by a certified ISO/IEC 17025 laboratory (Proverde Laboratories, Milford, MA). Each milliliter of the oil was determined to contain 32mg of CBD, 35mg of CBDA, 1.3mg of THC, 1.4mg of THCA, 0.9mg of cannabigerolic acid (CBGA), 1.3mg of cannabichromene (CBC) and 0.5mg of cannabigerol (CBG). Required volumes of this oil were then also formulated into soft gel capsules containing 6 mg total cannabinoid (small soft gels) or 19mg total cannabinoid (large soft gels); these strengths were calculated to facilitate accurate dosing of the experimental cohort.

In the morning of Day 1 of the study, all dogs were administered approximately 2mg/kg of a soft gel capsule containing a CBD/CBDA blend (ElleVet Sciences, South Portland, ME). Following dosing, blood was drawn at 0, 0.5, 1, 2, 4, 8 and 24 hour time intervals and stored at -80°C for later cannabinoid analysis. A physical examination was performed by the facility veterinarian at 1, 4 and 24 hours following dosing. Basic clinical parameters such as heart and respiratory rate, temperature, skin, hair, and body condition were recorded, including any behavioural changes and neurological assessment of gait for ataxia.

On Days 2 through 7, all dogs received an oral dose of the same concentration using a soft gel capsule formulation, administered with approximately 50 grams of wet food, twice daily with an 8-hour dosing interval (8 am and 4 pm daily). On Days 3 and 7, the same clinical examination and assessment as Day 1 was performed by the facility veterinarian, one hour following the morning dose. On Day 7, 6 hours after morning dosing, blood was drawn, and serum was separated and stored at -80°C for later pharmacokinetic analysis of cannabinoids.

Following a 2-week washout period, on the morning of Day 22 of the protocol, all dogs were administered 2mg/kg of hemp in sesame oil containing a CBD/CBDA rich blend (ElleVet Hemp CBD + CBDA Oil for Dogs; ElleVet Sciences, South Portland, ME). Blood was again drawn at 0, 0.5, 1, 2, 4, 8 and 24 hour time intervals and stored at -80°C for later analysis. A physical examination was performed by the facility veterinarian at 1, 4 and 24 hours following dosing. Basic clinical parameters were collected as in Phase one (during soft gel administration).

On Days 22 through 28, all dogs received an oral dose of the same hemp in sesame oil blend,

administered with approximately 50 g of wet food, twice daily with an 8-hour dosing interval (8 am and 4 pm). On Days 24 and 28, the same clinical examination and assessment as Day 22 was performed by the facility veterinarian, one and four hours following the morning dose. On Day 28 and 6 hours after dosing, blood was drawn with further serum separated and stored at -80°C for later pharmacokinetic analysis of cannabinoids.

Serum cannabinoid assessment was performed (Toxicology Research Laboratory, University of Illinois at Chicago, IL) as per Wakshlag et al. ⁶ using liquid tandem chromatography mass spectroscopy (LC-MS/MS). Pharmacokinetic analysis examining maximal serum concentration (C_{max}), time of maximal absorption (T_{max}), T_{max} half-life elimination (T_{1/2} elim), area under the serum concentration curve (AUC 0- ∞) and mean residence time (MRT), as well as calculated steady state mean serum concentration (C_{ss} Pred) were assessed using a pharmacokinetic software package (PK Solutions 2.0, Montrose, CA). Serum cannabinoid concentration, 6 hours after 6 days of twice daily administration of 2mg/kg (C Obs 6d) and predicted concentration after twice daily administration of 2 mg/kg (C_{ss} Pred) were also compared for each presentation. Statistical analysis was performed on measurable cannabinoids from the serum of all dogs which were above the lower limit of quantification from the LC-MS results across the population. These cannabinoids included CBD, CBDA, THC and THCA which were evaluated between the soft gel and oil administration pharmacokinetic data using a Student's T-test. In addition, data from C Obs 6d and C_{ss} Pred results using a Student's T-test was also assessed as all data fit the assumptions of normality following Shapiro-Wilks Test (Prism 6.0, Carlsbad CA). Significance was set at $p \leq 0.05$ for all testing.

Results:

Dog Physical Examination and Observation:

Overall food consumption for the duration of the study appeared unchanged. All but one dog increased bodyweight during the period, with an average weight gain of 2.8% during the entire trial, with normal eating behaviors.

Following normal initial physical examination on day one, no abnormalities in physical examination were noted in any dog after soft gel dosing. Slightly dilated pupils (mydriasis) with slowed return to normal size were seen on Day

3 after the morning dose on one occasion in one dog. Dilated pupils with normal light response were observed in one dog following morning dosing on day 24 and day 28 of oil administration. Behaviorally all dogs appeared to be normal in their disposition showing no signs of somnolence or lethargy with no overt clinical signs of ataxia during the entire dosing regimen.

Soft Gel and Oil Dosing Tolerance

One episode of vomiting of semi-digested food was observed immediately following evening dosing on Day 3 (soft gel administration) in one dog. Throughout the period of oil administration, licking was seen on 51 occasions and head shaking on 35 occasions. Grimacing was observed 29 times, with 2 episodes of chomping following administration and a single gagging event seen during the period of dosing.

Gastrointestinal events (loose stool [n=6]; vomiting digested food [n=2]) were recorded in six

dogs on a total of eight occasions during the period of administration of soft gel, but only one dog on one occasion (loose stool) during the period of administration of oil. Notably however, gastrointestinal signs were observed in three of these dogs on five occasions during the washout period (loose stool [n=4]; foamy vomit [n=1]).

24 hour and one-week steady state pharmacokinetics:

Although absorption of cannabinoids appears to follow a similar profile in both soft gel and oil, the magnitude of cannabinoid absorption is greater overall when administered via a soft gel (Table 1; Figures 1 and 2). CBG and CBGA concentrations could not be assessed for 24-hr pharmacokinetics as many dogs had below the lower limit of detection on most assessment times; therefore data was not analysed further.

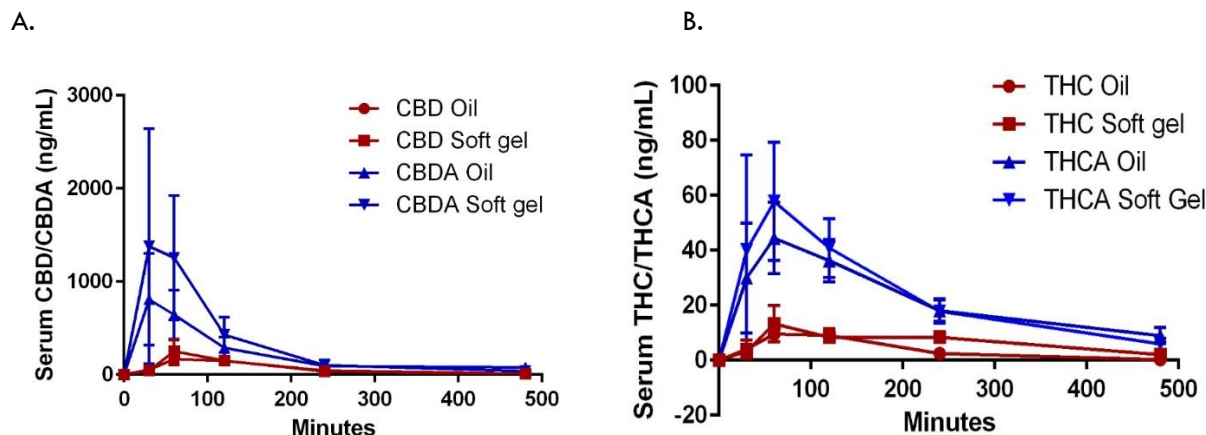
Table 1: Mean and standard deviations for 24 hr-pharmacokinetic parameters of CBD, CBDA, THC and THCA after dosing with either 2 mg/kg of Ellevet oil or soft gel formulations.

Superscript ^a indicates a significant difference ($p < 0.05$) between oil and soft gel administration. Superscript ^b indicates a significant difference ($p < 0.05$) between C Obs 6d and C_{ss} Pred serum concentrations.

Mean Values	C _{max} (ng/mL)	T _{max} (hrs)	T _{1/2} Elim (hrs)	AUC 0- ∞ (ng-h/mL)	MRT (hrs)	C Obs 6d (ng/mL)	C _{ss} Pred (ng/mL)
CBD (oil)	184.5 \pm 55.8 ^a	1.4 \pm 0.5	3.4 \pm 1.4	687.8 \pm 218.2	4.4 \pm 1.0	34.5 \pm 9.5 ^a	57.3 \pm 18.2 ^b
CBD (soft gel)	267.6 \pm 98.9	1.1 \pm 0.4	2.2 \pm 1.7	693.2 \pm 191.4	3.4 \pm 1.7	45.3 \pm 14.4	61.5 \pm 13.9 ^b
CBDA (oil)	923.1 \pm 427.1 ^a	0.7 \pm 0.3	3.2 \pm 0.8	2161 \pm 633 ^a	3.7 \pm 1.3 ^a	73.7 \pm 21.0	180.1 \pm 53.8 ^{ab}
CBDA (soft gel)	1826.0 \pm 1043.0	0.9 \pm 0.5	2.3 \pm 0.9	2786 \pm 910	2.7 \pm 0.8	50.2 \pm 17.6	232.2 \pm 75.9 ^b
THC (oil)	10.5 \pm 2.3	1.4 \pm 0.5	1.6 \pm 0.5 ^a	38.9 \pm 9.9	2.6 \pm 0.9	2.3 \pm 0.5	3.4 \pm 0.7 ^b
THC (soft gel)	14.1 \pm 5.8	1.3 \pm 0.5	1.1 \pm 0.3	47.7 \pm 11.2	2.0 \pm 0.3	2.7 \pm 0.9	4.0 \pm 0.9 ^b
THCA (oil)	46.4 \pm 11.3 ^a	1.0 \pm 0.5	4.0 \pm 0.6 ^a	259.0 \pm 43.2	5.4 \pm 1.0 ^a	10.2 \pm 2.0 ^a	21.6 \pm 3.6 ^b
THCA (soft gel)	62.9 \pm 22.1	0.9 \pm 0.5	3.3 \pm 0.5	255.7 \pm 60.8	4.2 \pm 0.5	8.6 \pm 2.3	21.3 \pm 5.1 ^b

C_{max} = Maximum serum concentration; T_{max} = time of maximum serum concentration; T_{1/2} Elim = half-life of elimination; AUC 0- ∞ = area under the serum concentration curve to infinity; MRT = mean residence time; C Obs 6d = serum concentration 6 hours after 6 days of BID administration of 2 mg/kg; C_{ss} Pred = calculated predicted mean steady state serum concentration.

Figure 1 – A: 24 hour mean and standard deviation of serum concentrations (ng/mL) of CBD and CBDA in 8 dogs. B: 24 hour mean and standard deviations of serum concentrations (ng/mL) of THC and THCA in 8 dogs.



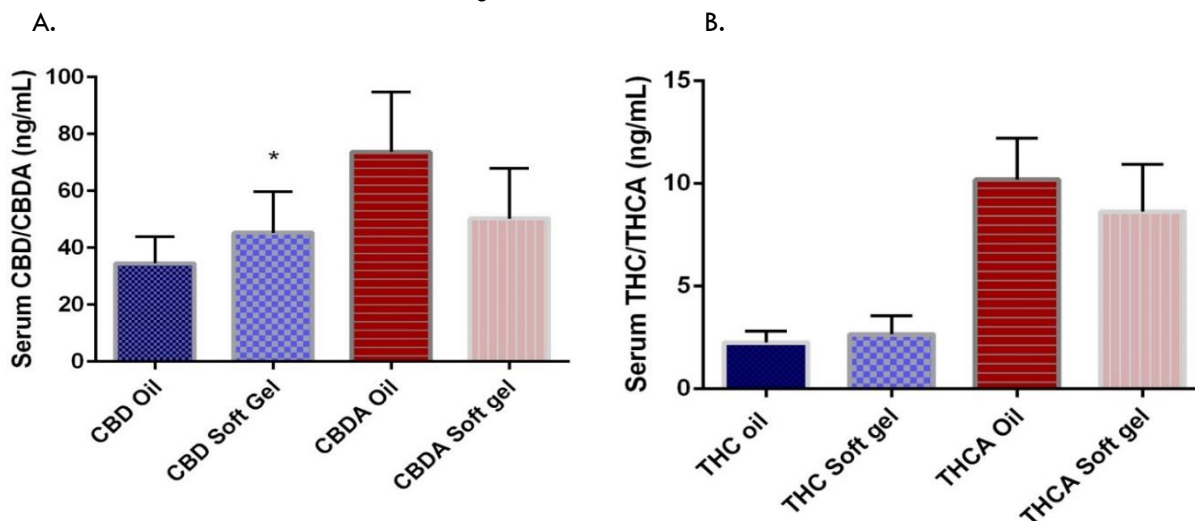
Maximal serum concentration of CBD was approximately 1 hour post administration and was significantly greater in the soft gel format ($P < 0.05$), but with no significant differences in the T_{max} . Serum concentrations of CBDA in the soft gel formulation showed a higher C_{max} , which was significantly different than the oil formulation ($P < 0.05$), with no significant differences in T_{max} between formulations. The half-life of elimination ($T_{1/2\text{ Elim}}$) for CBDA shows no difference between the two formulations; $AUC_{0-\infty}$ was significantly greater in the soft gel formulation, whilst MRT was significantly greater for CBDA in the oil formulation ($P < 0.05$). CBD concentrations show no differences between the formulations across $T_{1/2}$ life, $AUC_{0-\infty}$ or MRT .

Low levels of THC and THCA were detected in samples at the same time points post administration. Maximal serum concentration (C_{max}) of THC was detected 1 hour post administration, which was significantly greater in the soft gel format ($P < 0.05$), with no significant differences in T_{max} . Serum concentrations of THCA in the soft gel formulation showed a higher C_{max} , which was significantly different than the oil formulation ($P < 0.05$), with no significant

differences in T_{max} between formulations. Half-life of elimination ($T_{1/2\text{ Elim}}$) for THC and THCA showed a modest yet significant increase for the oil formulation ($P < 0.05$), while $AUC_{0-\infty}$ was no different for THC and THCA across the formulations. Levels of THC showed no differences between the formulations with regards to MRT , yet THCA showed a mildly increased MRT for the oil-based formulation ($P < 0.05$). Concentrations of CBG and CBGA were below the lower limit of quantitation for most of the dogs; thus, it appears to suggest that these components are also largely unavailable in this presentation, which correlates with the very low concentrations in the product. Interestingly, CBGA was detectable in the 24-hour pharmacokinetics study at times 0.5, 1 and 2 hours for most of the dogs but was below the quantitation limit following one week of dosing.

After one week of dosing, CBD, CBDA, THC, and THCA concentrations $C_{Obs\ 6}$ were significantly lower than the $C_{ss\ Pred}$ calculation (Table 1). The $C_{Obs\ 6d}$ concentrations were no different between formulations for CBDA, THC and THCA, while the soft gel format showed a mild yet significantly higher CBD concentration (Table 1; Figure 2 A and B).

Figure 2 - A: 6-day serum pharmacokinetic data (mean \pm SD) demonstrating concentration of CBD and CBDA following administration of hemp in a sesame oil-based formulation as an oil or soft gel formulation. B: 6-day serum pharmacokinetic data demonstrating concentrations of THC and THCA following administration of hemp in a sesame oil-based formulation as an oil or soft gel formulation. * indicates a significant difference between serum concentrations for CBD between soft gel and oil.



Discussion:

This study was initiated as part of due pharmacological diligence to better understand the tolerance and pharmacokinetics of a soft gel versus an oil formulation of a specific CBD/CBDA rich hemp product. The results of this study suggests that administration of a soft gel capsule containing a CBD/CBDA rich hemp blend was associated with fewer overall negative observations, compared to administration of a sesame oil containing a CBD/CBDA rich hemp blend. Taylor *et al.*¹⁷ report diarrhoea and nausea as one of the commonly reported side effects in their human cohort receiving purified cannabidiol. They found that most adverse events were mild or moderate in severity and did not result in discontinuation of the trial. Similarly, McGrath *et al.*¹⁸ reported that all dogs developed diarrhoea during the course of their study; whilst it was thought to be secondary to treatment with CBD or the vehicle oil used, other factors such as stress and dietary indiscretions could not be ruled out.

The ready acceptance of soft gel, opposed to the negative events observed with oil, would be beneficial for future choices regarding method of administration in people or in companion animals. The gastrointestinal events observed were mild and appeared to resolve with continued administration of soft gels; the ease of administration and comparable pharmacokinetics may outweigh the adverse effects initially observed. As diarrhoea

and vomiting were observed at a similar frequency during the washout phase, attributing these episodes to the formulation may not be valid.

Found in *Cannabis sativa*, CBDA is the native acid form, which is converted to CBD during a heat extraction process¹¹. Chemical extraction techniques preserve CBDA in the preparation utilized, facilitating absorption from the gastrointestinal tract following oral administration^{9, 19}. CBDA is known to possess anti-inflammatory and anti-hyperalgesic properties, reducing pain behaviours in a rodent model, which may be superior to those of CBD²⁰. The work of Takeda *et al.*²¹ suggests that CBDA demonstrates possible and selective COX-2 inhibition in an in-vitro setting, adding further to the argument for selection of CBDA as part of cannabinoid therapy, pending adequate gastrointestinal absorption, which is apparent in this study and others^{6, 12}.

Comparing both formulations, serum levels after a week of treatment appear similar. However, acute dosing 24-hour pharmacokinetic data shows superior maximal serum concentrations (C_{max}) of both CBD, CBDA, THC and THCA within 2 hours following administration of soft gels. This translates into a significantly greater CBDA concentration after soft gel dosing compared to oil, which may have utility in acute situational dosing of cannabinoid rich hemp.

Administration of soft gel gives rise to increased initial absorption of CBDA, yet levels appear to be relatively similar to CBD after 1 week of treatment. This may be due to differences in the metabolism of the two molecules which has yet to be studied, particularly in dogs, who may have different metabolic pathways than humans^{11,22}. In humans and rodents, there is prominent cytochrome p450 hydroxylation and eventual carboxylation to form 7-carboxy-cannabidiol (7-COOH-CBD) which is the primary metabolite found in humans and rodents^{23,24}. In pharmacokinetic assessments in dogs the formation of 7-hydroxy-cannabidiol (7-OH CBD) and 7-COOH-CBD is very low, suggesting differential metabolism, whereby in-vitro liver microsome work has suggested that the major metabolite of CBD in dogs may be 6-COOH-CBD²². Currently, the pathways for CBDA metabolism have not been defined in any species; however the slightly more rapid elimination than CBD and the one week steady state being lower than expected, does suggest that there may be an upregulation of metabolism of CBDA during chronic dosing, which has also been observed in a prior study in dogs⁶. The steady state data (C Obs 6d) shows good retention of CBD, CBDA, THC and THCA post soft gel administration compared to oil administration, although observed CBDA steady state concentration was slightly less with soft gel versus oil administration. The C_{ss} Pred suggests that the C Obs 6d are less than predicted. It is postulated that this is due to hepatic cytochrome P450 enzyme induction and upregulation, which is further evidenced in the work of Anderson and Chan²⁵, or that the twice daily dosing interval may not be appropriate to maintain serum concentrations as the predicted state and that more frequent dosing may be necessary.

More interestingly, across both formulations of the supplement, it is universally noted that there is a 3-6 fold increase in CBDA C_{max} and AUC compared to CBD when examining the 24-hour pharmacokinetics. Although CBDA research is in its infancy, other studies in dogs and people suggest that CBDA absorption is superior to CBD during acute dosing^{6,12}. Anderson and colleagues also noted that CBDA absorption when provided as an isolate, was actually quite poor, but when provided as a whole hemp extract, CBDA absorption increased over 10 fold¹². Their findings suggest that some of the minor cannabinoids and potentially lower levels of terpenes found in whole hemp extract may cause inhibition of efflux transporters in enterocytes, allowing for better absorption of

acidic forms of cannabinoids¹². This may be part of the well-known “entourage effect” that is often observed when using whole hemp extracts, compared to cannabinoid isolates^{26,27}. These differences in hemp cultivars highlights the need to understand cannabinoid pharmacokinetics of individual cultivars being marketed, as they may all demonstrate nuances regarding pharmacokinetics and indications for treatment.

The slightly better absorption of cannabinoids in general during initial dosing and differential absorption across dogs with the soft gel formulation may correlate to the mild adverse events noted, which appear to diminish to some extent after chronic dosing. Overall, across the two different products after administration for six days, soft gel dosing appears to be comparably efficacious to oil dosing, but with fewer aversive behaviours towards administration of the product. Dogs appear to build tolerance to soft gel administration and any associated adverse events with continued dosing. As such, the inference is to continue administration of the product to achieve a steady state, in the face of these mild adverse events.

Much like CBD and CBDA in this particular product, there are equal amounts of low levels of THC and THCA. The absorption of THCA is superior to THC, as evidenced in our data; THCA appears to be retained at a 3-4 fold increase after week long twice daily dosing, when compared to THC. The low levels of THC observed in serum and lack of psychotropic effects observed, suggest that this is a safe dose for dogs. There is little known about THCA's effects; rodent models suggest neuroprotective effects with no psychotropic effects from THCA^{28,29}. Moreover, based on our data, THCA appears to be absorbed intact much like CBDA. The implications of THCA for therapeutic means with proper dosing remains to be determined. However, the absorption kinetics make THCA a highly attractive molecule, due to its superior absorption, warranting further research on metabolism and interventions using THCA in cannabinoid formulations in both people and companion animals.

Conclusions:

Although soft gel dosing is associated with less negative events surrounding oral administration compared to dosing with oil, the incidence of gastrointestinal adverse events associated with soft gel administration may be slightly higher than with

oil alone. However, these adverse events are self limiting and appear to resolve with continued dosing with soft gel administration. Dosing in the form of a soft gel is associated with modestly improved absorption data, which may have implications for acute dosing. Chronic dosing shows little difference between products, suggesting either formulation may be appropriate depending on tolerance, although soft gels may be easier to administer. Interestingly, when delivering THC and THCA in equal quantities the absorption of THCA, regardless of the product, is superior to THC. Concentrations of CBDA are modestly higher than CBD after chronic dosing, suggesting that native acids in the plant may have superior absorption to the decarboxylated forms of cannabinoids.

Conflicts of Interest:

DJT, WSS and JJW are paid consultants of ElleVet Sciences.

No other conflicts of interest are reported. All authors contributed to the conception, design, analysis, and interpretation of data for this article and approved the final version.

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