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Twice-daily oral administration of a cannabidiol and cannabidiolic acid–rich hemp extract was well tolerated in orange-winged Amazon parrots (*Amazona amazonica*) and has a favorable pharmacokinetic profile

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OBJECTIVE
To determine the pharmacokinetics of 8 cannabinoids and 5 metabolites after oral administration of single and multiple doses of a cannabidiol (CBD)-cannabidiolic acid (CBDA)–rich hemp extract to orange-winged Amazon parrots (*Amazona amazonica*) as well as to evaluate the extract's adverse effects.

ANIMALS
12 birds.

PROCEDURES
Based on pilot studies, a single-dose study based on 30/32.5 mg/kg of cannabidiol/cannabidiolic acid of a hemp extract was administered orally to 8 fasted parrots, and 10 blood samples were collected over 24 hours after administration. After a 4-week washout period, the hemp extract was administered orally to 7 birds at the previous dose every 12 hours for 7 days, and blood samples were collected at the previous time points. Cannabidiol, Δ9-tetrahydrocannabinol, cannabinol, cannabichromene, cannabigerol, cannabidiolic acid, cannabigerolic acid, Δ9-tetrahydrocannabinolic acid, and 5 specific metabolites were measured by liquid chromatography-tandem/mass-spectrometry, and pharmacokinetic parameters were calculated. Adverse effects and changes in the plasma biochemistry and lipid panels were evaluated.

RESULTS
Pharmacokinetic parameters for cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, Δ9-tetrahydrocannabinolic acid, and the metabolite 11-hydroxy-9-tetrahydrocannabinol were established. For the multiple-dose study, cannabidiol/cannabidiolic acid mean C_{max} was 337.4/602.1 ng/mL with a t_{max} of 30 minutes and a terminal half-life of 8.6/6.29 hours, respectively. No adverse effects were detected during the multidose study. The predominant metabolite was 11-hydroxy-9-tetrahydrocannabinol.

CLINICAL RELEVANCE
Twice daily oral administration of the hemp extract based on 30 mg/kg/32.5 mg/kg of cannabidiol/cannabidiolic acid was well tolerated and maintained plasma concentrations considered to be therapeutic in dogs with osteoarthritis. Findings suggest different cannabinoid metabolism from mammals.

Introduction

There is a need in avian medicine to investigate additional drugs for chronic pain management. Currently, chronic painful conditions are treated with the long-term use of drugs such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), which can have potential adverse effects. Also, gabapentin and amantadine are used but there is little evidence yet regarding their efficacy in birds with chronic pain.

Phytocannabinoids, plant-derived cannabinoids (CBs), are chemicals that interact with the

endocannabinoid system (ECS) and are 1 of the active compounds of *Cannabis sativa* plant. There are over 150 CBs that have been identified from *C sativa*.[1] These compounds are analogs of Δ9-tetrahydrocannabinol (Δ-THC), cannabidiol (CBD), cannabichromene (CBC), cannabigerol (CBG), and cannabinol (CBN), among others. The main compound studied in veterinary medicine has been CBD, a nonintoxicating compound, which shows an affinity for CB_1 and CB_2 receptors.[2] There are different proposed mechanisms of action of cannabinoids. CBD is considered a partial agonist at CB_2 and an antagonist at CB_1 with low binding affinity to the CB_1 receptor.[3] CBD molecules also work on other receptor systems, such as transient receptor potential vanilloids (TRPVs), 5-HT1A (serotonin), and glycine receptors.[3,4] CBs have been recently studied for multiple therapeutic effects for their proposed antinociceptive, antipsychotic, antineoplastic, and anticonvulsant effects, which make them an attractive therapeutic alternative.[5]

The endocannabinoid system (ECS) has been identified in multiple animal species. The cannabinoid receptors CB_1 and CB_2 are involved in different physiological processes such as neuronal plasticity, pain, inflammation, and immune regulation among others.[2] In birds, CB_1 receptor has been identified in chickens[6] and budgerigars,[7] which supports the presence of a developed ECS in birds. A recent comparative genomic analysis[8] revealed that the CB_2 gene is evolutionarily absent in parrots, and based on these preliminary findings, it is possible that its absence may impact the regulation of neuroinflammation, making them more susceptible to it.

In 2018, in the United States, the Agriculture Improvement Act removed hemp from Schedule I of the Federal Controlled Substances Act. Hemp is a form of *C sativa* with low levels (≤ 0.3%) of THC.[9] Cannabidiol (CBD), a major phytocannabinoid, is found in many commercial products as the main component; however, there can be other phytocannabinoids present in significant quantities such as CBC, CBG, CBN, THC, and their acid precursors cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA). All these CBs may be responsible for some of the plant's many medicinal properties.[3] Hemp is defined in the 2018 Farm Bill as any part of the *C sativa* plant, including the seeds and all derivatives, extracts, cannabinoids, isomers, acids, salts, and other compounds with a THC concentration of not more than 0.3%.[10] A full-spectrum hemp extract includes cannabinoids (such as CBDA, CBD, CBG, CBC, and ≤ 0.3% of THC), flavonoids, terpenes, and other constituents within the cannabis plant.[9]

In mammals, CBD is metabolized by the liver to its active metabolite 7-hydroxy-cannabidiol (7-OH-CBD) and then further metabolized to its inactive metabolite 7-carboxy-cannabidiol(7-COOH-CBD).[11] 7-OH-CBD has anticonvulsant activity comparable to CBD.[12] There are other metabolites and the metabolic profiles of CBD are considerably different among mammal species.[13] These metabolite profiles have not been evaluated in avian species. Besides CBD, it is important to evaluate other CBs, their acid precursors, and their metabolites to have a better understanding of their absorption and metabolism in birds. One example is the acid precursor CBDA, which recently has gained research interest. CBDA loses its carboxyl group to form CBD when heated. CBDA has been shown to be more potent than CBD for the treatment of induced nausea and hyperalgesia reduction in rat models.[14,15] It also has been shown that it is a selective inhibitor for cyclooxygenase-2 (COX-2).[16] There is evidence that CBDA is readily absorbed in other species.[17] Other CBs could have different pharmacokinetic (PK) profiles, which support the importance of describing the PK profile for each of them when administered in combination.

In veterinary medicine, there has been a surge in CBs research. The pharmacokinetics of CBs have been investigated in multiple species of mammals including dogs,[17–21] cats,[22] cattle,[23] rabbits,[24] guinea pigs,[25] and horses.[26,27] Only limited studies have investigated the pharmacodynamics of cannabinoids in animals. For example, a recent study[18] investigated a 2-mg/kg dose of CBD administration that was associated with decreased pain scores and increased activity in dogs with osteoarthritis. The C_{max} at this dose was 102 ng/mL, which is the highest concentration measured associated with a pharmacodynamic effect. The therapeutic plasma concentrations of CBD are not well defined. Based on this information and considering the short half-life, it could be established that lower plasma concentrations are likely therapeutic. Conservatively, it could be speculated that a target plasma concentration above 50 ng/mL is likely therapeutic for OA-associated pain in dogs. In avian medicine, there is a recent pharmacokinetic study[28] of CB in Hispaniolan Amazon parrot (*Amazona ventralis*) evaluating the single-dose parameters of 2 different oral doses, 60 and 120 mg/kg of CBD, administered as a sole compound. This study was the first to describe cannabinoid pharmacokinetics in an avian species, though CBD metabolites, multiple-dose parameters, or adverse effects were not investigated. Reported adverse effects of hemp extract administration in dogs are similar to humans, including elevation of alkaline phosphatase (ALP) with long-term CBD administration.[18,19] Other adverse effects in humans are diarrhea, pyrexia, somnolence, decreased appetite, sedation, vomiting, ataxia, and elevated transaminases.[29,30]

There were 2 main objectives of this study. The first objective was to determine the plasma concentrations and pharmacokinetic profile of 8 CBs following oral administration of a full spectrum hemp-extract contatining cannabidiol (CBD), Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG); precursors cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and Δ9-tetrahydrocannabinolic acid (THCA); and the 5 metabolites 7-hydroxycannabidiol (7-OH-CBD), 7-nor-7-carboxycannabidiol (7-COOH-CBD), 11-nor-9-carboxy-Δ9-THC (COOH-THC), 11-hydroxy-Δ9-THC (11-OH-THC), and 11-nor-9-carboxy-Δ9-THC glucuronide (COOH-THC-Glu) in a single and a multiple-dose study in orange-winged

Amazon parrots (OWAP). The second objective was to evaluate safety parameters after multiple-dose administration including neurological (eg, abnormal ambulation, tremors, agitation, and sedation) and gastrointestinal adverse effects (eg, vomiting, diarrhea) and changes in the plasma biochemistry panel and lipid panel. It was hypothesized that a commercial hemp (*C sativa*) extract containing predominantly almost equal amounts of CBD and CBDA as well as smaller amounts of other cannabinoids, administered orally to OWAP at a dose based on 30/32.5 mg/kg of CBD/CBDA, would achieve and maintain above target plasma concentrations of CBD (50 ng/mL in plasma) and result in a pharmacokinetic profile comparable to other species studied in a single and a multiple-dose study without significant adverse effects. Other minor cannabinoids contained in the hemp extract would be absorbed and result in detectable plasma concentrations and similar pharmacokinetic trends as CBD/CBDA after oral administration, and common metabolites across species will also be found in OWAP.

Materials and Methods

Animals

The studies were approved by the Institutional Animal Care and Use Committee at the University of California-Davis (protocol No. 22582). Twelve healthy OWAP, 6 females and 6 male adults of ages ranging between 20 and 60 years old and maintained as a research colony at the University of California-Davis, were enrolled in the study. During the study period and beginning 14 days prior to the start of the study, birds were housed in a single room in individual stainless-steel cages (dimensions 91 X 71 X 165 cm) that contained 2 perches and hanging toys and allowed for visual and audible socialization with each other, with a 12-hour day-light cycle. The daily diet offered was a commercial pelleted diet (Roudybush low fat medium pellets; Roudybush Inc), and water ad libitum was provided via a sipper water line. Parrots were deemed healthy to participate in the study based on history, physical examination, and complete blood count. None of the birds received other medications or underwent any anesthetic procedures for at least 1 month prior to the start of the study.

Hemp extract

A more concentrated version of a commercially available hemp extract (Ellevet Sciences) from the same lot was used for all the studies, containing a total of 100 mg of CBs/mL in a sesame oil base. A third-party ISO 17025–certified laboratory for hemp testing (Proverde Laboratories, analytical testing services) performed the analysis. The sample was analyzed for plant-based cannabinoids by liquid chromatography (LC) to ensure known concentrations and ratios of the different cannabinoids. The analyzed extract contained CBD, 45.2; CBDA, 49; THC, 1.86; THCA, 0.59; CBG, 0.69; CBGA, 1.21; and CBC, 1.53 mg/mL. The dose of the single- and multiple-dose studies was based on CBD/CBDA (30/32.5 mg/kg, respectively) and corresponded to concentrations in the same volume of 1.23/0.39 mg/kg of THC/THCA, respectively. For the cannabinoids present in smaller quantities in the hemp extract the corresponding doses were 0.46 mg/kg CBG, 0.80 mg/kg CBGA, and 1.01 mg/kg CBC. The extract also contained 10 mg/mL of terpenes; these were not considered for dosing.

Pilot study

Three different doses were investigated in a pilot study to determine an appropriate and safe dose of the hemp extract as well as to define the appropriate time points for blood sample collection in the following single and multiple-dose studies. Six fasted birds (3 females and 3 males) were assigned to receive 1 of 3 doses based on 15/16.25 mg/kg (n = 2), 30/32.5 mg/kg (2), and 60/65 mg/kg (2) of CBD/CBDA each. Food was removed at the end of the light cycle to ensure a 12-hour fasting period. The hemp extract was administered to fasted birds into the crop via metal gavage tube. Blood samples were collected from the jugular veins at 0.5, 1, 2, 3, 4, 6, 12, and 24 hours postadministration for plasma concentration measurement of CBD/CBDA. The parrots were continuously monitored after oral administration for 18 hours for neurological (abnormal ambulation, tremors, agitation, or sedation) using an agitation-sedation score previously described for Hispaniolan Amazon parrots (*Amazona ventralis*)[31] prior to each sample collection and for gastrointestinal (vomiting, diarrhea) adverse effects.

Based on the plasma concentration results and adverse effects observed at the highest dose, it was elected to use a dose of 30/32.5 mg/kg of CBD/CBDA for the single- and multiple-dose studies. At the end of each study, each bird received supportive care that included the administration of 1 dose of 50 mL/kg of lactated Ringer solution SQ to compensate for intravascular volume loss during the study and meloxicam at 1 mg/kg, SQ once for pain management after multiple phlebotomies.

Single-dose study

Eight fasted birds, balanced for age and sex (4 males and 4 females), and randomly assigned to this group using statistical software (R, version 4.2.0, 2022; R Foundation For Statistical Consulting), ranging between the ages of 20 and 60 years old, participated in this study after a 4-week washout period from the pilot study. Food was removed at the end of the light cycle to ensure a 12-hour fasting period. Birds received a single dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA via a metal gavage tube, with an average volume of 0.26 mL of hemp extract (minimum, 0.24; maximum, 0.33). Blood samples were collected from the jugular veins at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postadministration for CBs and metabolites plasma concentration measurement.

The parrots were continuously monitored after oral administration for 12 hours for neurological (abnormal ambulation, tremors, agitation, or sedation) and gastrointestinal (vomiting or diarrhea)

adverse effects. An agitation-sedation scoring system previously described was used to record the level of sedation or agitation at each venipuncture time point. Birds were given access to food at 6 hours postadministration of the hemp extract.

Multiple-dose study

The same 8 birds used in the single-dose study were the treatment group in this phase and an additional 4 birds were assigned to the control group, also balanced for age and sex and randomly assigned as described above. After a 4-week washout period from the single-dose study, baseline blood samples for plasma biochemistry panels and lipid profiles were collected. The treatment group received a hemp extract dose based on 30/32.5 mg/kg of CBD/CBDA every 12 hours for 7 days via metal gavage tube and the control group received the same doses with equivalent volume of water. Prior to the administration of the final dose on day 8, blood samples were collected for plasma biochemistry panels and lipid profile at the 0 time point. Blood samples were then collected at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postadministration for CBs plasma concentration measurement.

For all time points, 0.3 mL of blood was collected from the jugular veins or ulnar veins using a 0.5-mL insulin syringe with a 28-gauge needle (BD Micro-Fine; BD Lo-Dose). The samples were transferred to lithium-heparin tubes (BD microtainer), placed in a cooler with ice packs, and centrifuged (3,500 X *g* for 8 minutes) within 30 minutes of collection. Plasma was extracted using a disposable transfer pipette, placed into labeled 2-mL polypropylene cryovials, and stored at −80 °C until analysis. For the plasma biochemistry and lipid panels, 0.6 mL of blood was processed as described above. In total, the amount of blood collected did not exceed more than 1% of their body weight.

The plasma biochemistry panel was performed at the William R. Pritchard Veterinary Medical Teaching Hospital, Clinical Diagnostic Laboratories, University of California-Davis, and the lipid panel was performed in the Department of Molecular Biosciences, School of Veterinary Medicine, University of California-Davis. For the lipid panel, 1-part plasma and 1-part LDL/very low-density lipoprotein precipitation buffer (abcam) were mixed together and incubated at room temperature for 10 minutes. The samples were then centrifuged for 10 minutes at 2,000 X *g* and 4 °C. After centrifugation, the supernatant high-density lipoprotein (HDL) fraction was removed. The original plasma sample and HDL fraction were assayed for total cholesterol (TC) and triglyceride (TG) with an enzymatic assay (Fisher Diagnostics). The HDL values were then subtracted from the total values to calculate the non-HDL values for each respective assay. Validation of the lipid profile was performed during a previous study[32] from the same University of California-Davis flock of parrots.

During the multiple-dose study birds were monitored for 8 hours on the first and last day of the hemp extract administration for sedation, behavior, and gastrointestinal effects as described previously and were monitored for 30 minutes after each dose for the rest of the days. To monitor for gastrointestinal adverse effects throughout the 7 days of dosing, birds were weighed once daily and urofeces, regurgitation, and evidence of eating (based on the presence of food particles on the paper) were evaluated using a white paper in the bottom on the cage that was changed daily during the morning treatments.

Cannabinoid and metabolite plasma concentration

Plasma concentration of the cannabinoids and metabolites analysis was performed using an exploratory (fit-for-purpose) method for fast measurement of 13 cannabinoids and their metabolites at the Toxicology Research Laboratory, University of Illinois at Chicago. The reference standards for CBD and CBDA were obtained from Restek Corporation; all other reference and internal standards were obtained from Cerilliant Corporation. The concentration in parrot plasma of cannabinoids (CBD, CBDA, THC, THCA, CBN, CBC, CBG, and CBGA) and their metabolites (11-OH-THC, 7-OH-CBD, 7-COOH-CBD, COOH-THC, and COOH-THC-Glu) was determined using high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Nexera X2 and LCMS 8050; Shimadzu Corp).

Parrot plasma (30 µL) was mixed with 15 µL of internal standards (100 ng/mL of CBD-d3, THC-d3, THCA-d3, 7-COOH-CBD-d3, 7-OH-CBD-d5, 11-OH-THC-d3, COOH-THC-d9, and COOH-THC-Glu-d3 in 50% methanol) in a 96-well plate. Proteins were precipitated and compounds were extracted by adding 75 µL of ice-cold acetonitrile to the samples and then vortexing for 1 to 2 minute and centrifuging at 4,000 rpm for 30 minutes at 4 °C. Supernatants (60 µL) were mixed with 60 µL of water in a different 96-well plate and centrifuged again (for 10 minutes). The processed samples (10 µL) were injected into Waters Atlantis T3 HPLC column (3 µm, 2.1 X 50 mm) with a guard cartridge (Waters VanGuard Atlantis T3) coupled to LC-MS/MS. The column was equilibrated with mobile phase A (0.1% formic acid in water) and mobile phase B (acetonitrile) at 50% B. The compounds were eluted by a linear gradient from 50% B to 95% B over 6 minutes and then held at 95% B for 1 minute. Subsequently, the column was reequilibrated at initial composition for 1 minute. Flow rate was 0.3 mL/min. The autosampler and column temperature were set a 4 and 30 °C, respectively. The compounds were detected in electrospray ionization positive and/or negative mode as described in **Supplemental Table S1**. Interface voltage was 4 kV and −3 kV, respectively. Interface, desolvation line, and heat block temperature were 300, 200, and 400 °C, respectively. Nebulizing, heating, and drying gas flow were 2.7, 5, and 5 L/min, respectively.

Concentrations of cannabinoids were calculated by LabSolutions software (Shimadzu Corp) using a quadratic calibration curve with $1/c^2$ weighing based on relative response (peak area of cannabinoids/peak area of internal standards). The

calibration curve range in parrot plasma is shown in Supplemental Table S1.

Pharmacokinetic analysis

Maximum plasma drug concentrations (C_{max}) and the time of maximum concentration (t_{max}) were obtained directly from a review of the concentration-time data. Calculated pharmacokinetic parameters were obtained using noncompartmental analysis with a commercially available software program (Phoenix Winnonlin v 8.3; Certara). Multiple dosing parameters were estimated at steady state. Pharmacokinetic parameters included the terminal-phase rate constant (λ_z), terminal-phase half-life (λ_z HL), the area under the curve from time 0 to infinity ($AUC_{0\rightarrow\infty}$), and the extrapolated percentage of the area under the curve (AUC %). The λ_z HL was calculated using the $t_{1/2} = 0.693/\lambda_z$ equation and AUC was calculated using the log-linear trapezoidal method. The accumulation index was calculated using the formula: $1/[1\text{-}e^{-(\lambda z \times tau)}]$, where tau represents the dosing interval.

Statistical analysis

Differences in plasma biochemical parameters between treatment and time were assessed using linear mixed models with treatment, time, and treatment X time interaction as fixed effects and individual birds as the random effect. Assumptions of linearity, homoscedasticity, and normality of the residuals as well as the presence of outliers were assessed on residual and quantile plots. When the interaction term was significant, post hoc comparisons were made with a Tukey adjustment. A statistical software (R, version 4.2.0, 2022, R Foundation for Statistical Computing) was used for statistical analysis using the R-package ggplot2 for statistical graphing.[33] An alpha of 0.05 was used for statistical significance.

Results

Pilot study

One adult bird in the lowest dose treatment group (15/16.25 mg/kg CBD/CBDA) experienced a seizure-like episode at 4 hours postadministration, which lasted approximately 2 minutes. This bird recovered without further complications within the following 30 minutes. In the treatment group receiving hemp extract based on 30/32.5 mg/kg CBD/CBDA, target plasma concentrations above 50 ng/mL for CBD and CBDA were achieved, and no adverse effects were appreciated. Both birds in the high-dose treatment group, receiving 60/65 mg/kg CBD/CBDA, experienced marked sedation. These birds were observed at the bottom of their cage, mildly sedated at 4 hours postadministration, and had a quiet mentation at 12 hours. These birds were unable to hold their head up, had wide stance, had wing drop, and were only responsive to loud auditive stimuli. One of them was in ventral recumbency. These 2 birds regained a normal posture at 18 hours postadministration and were deemed fully recovered at 24 hours postadministration.

Single-dose study

The 8 OWAP used for this study remained bright and alert throughout with no noted adverse effects over a 24-hour period. Of the 8 CBs measured, CBG, CBC, and CBN remained below quantitation levels at all time points and only low plasma concentrations of CBGA were detected in 3 parrots during the initial 4 hours postadministration. Therefore, pharmacokinetic parameters were not calculated for these CBs. For the metabolites, only 11-OH-THC had quantifiable plasma concentrations. 7-COOH-CBD, 7-OH-CBD, COOH-THC, and COOH-THC-Glu measurements were below the limits of quantitation. The plasma concentration-versus-time curves of CBD, CBDA, THC, THCA, and 11-OH-THC are shown in **Figure 1**, and their pharmacokinetic parameters are summarized in **Table 1**.

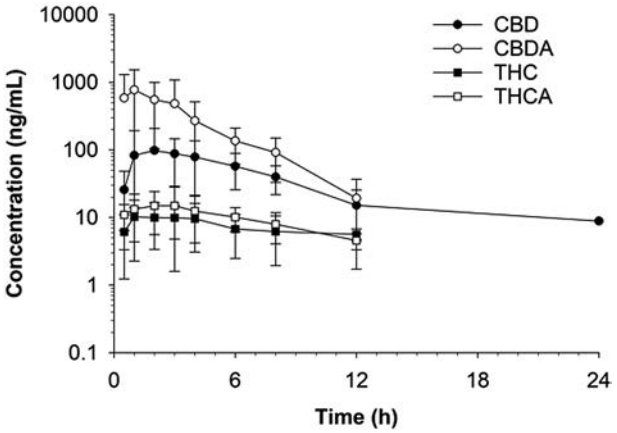

Figure 1—Mean ± SD plasma concentrations of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), and 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) in 8 orange-winged Amazon parrots (*Amazona amazonica*) after oral administration of a single-dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA. Time 0 was the time of hemp extract administration for the single-dose phase. The *y*-axis is logarithmically scaled.

Multiple-dose study

All birds maintained their body weight with less than 10% fluctuation, none of the birds had signs of appetite, sedation/agitation, or behavior changes, and none, except for 1 bird in the treatment group that developed intermittent diarrhea, had changes in urofeces as subjectively assessed.

Of the 8 birds in the treatment group, 2 were excluded from the pharmacokinetic analysis. One was due to a metacarpal injury on the fifth day of the study, and the other one was considered an outlier, based on disproportionally high plasma concentrations. On retrospective evaluation, this bird had a moderate amount of blood on the metal gavage tube at the time of the last drug administration.

The plasma concentration-versus-time curves of CBD, CBDA, THC THCA, and 11-OH-THC are shown in **Figure 2**, and their pharmacokinetic parameters are summarized in **Table 2**. The mean accumulation

Table 1—Mean ± SD values of pharmacokinetic parameters of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), and 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) in orange-winged Amazon parrots (*Amazona amazonica*) after oral administration of a single-dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA to 8 parrots.

Parameters	CBD	CBDA	THC	THCA	11-OH-THC
t_{max} (h) (range)	2.5 ± 1.6 (1–6)	1 ± 0.59 (0.5–2)	2.5 ± 0.93 (1–4)	3 ± 2.13 (1–8)	4.87 ± 2.64 (2–8)
C_{max} (ng/mL)	105 ± 103.8	602.1 ± 761.7	11.6 ± 6.33	15.8 ± 11.9	38.93 ± 17.83
$t_{1/2\lambda}$ (h)	2.92 ± 1.08	1.72 ± 41.5	4.58 ± 8.43	4.24 ± 3.25	3.04 ± 1.42
λ_z (1/h)	0.23 ± 0.07	0.18 ± 0.35	0.10 ± 0.13	0.14 ± 0.09	0.22 ± 0.11
$AUC_{0-\infty}$ (h·ng/mL)	601 ± 442.1	3,915.8 ± 1,1141.1	118.2 ± 47.0	154.2 ± 40.2	376.56 ± 142.47
AUC_{extrap} (%)	7.64 ± 3.34	4.73 ± 38.7	34.4 ± 28.0	19.5 ± 18.55	19.79 ± 12.06

Median and range are included for t_{max}. Hemp extract composition was 100 mg of total CBs/m, which corresponded to CBD, 45.2; CBDA, 49; THC, 1.86; THCA, 0.59; CBG, 0.69; CBGA, 1.21; and CBC, 1.53 mg/mL. The doses were CBD, 30; CBDA, 32.5; THC, 1.23; THCA, 0.39; CBG, 0.46; CBGA, 0.80; and CBC 1.01 mg/kg. All values were generated using noncompartmental analysis.

λ_z = Terminal slope. $AUC_{0-\infty}$ = Area under the plasma-concentration curve from time 0 to infinity. AUC_{extrap} = Percentage of area under the concentration-versus-time curve (AUC) extrapolated. C_{max} = Maximum concentration. $t_{1/2\lambda}$ = Terminal half-life. t_{max} = Time to maximum concentration.

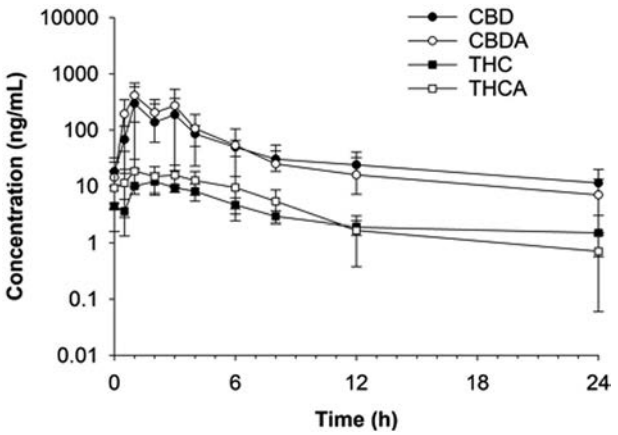

Figure 2—Mean ± SD plasma concentrations of cannabidiol (CBD), cannabidiolic acid (CBDA), Δ9-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), and 11-hydroxy- Δ9-tetrahydrocannabinol (11-OH-THC) in 6 orange-winged Amazon parrots (*Amazona amazonica*) after oral administration after 7 day twice daily oral administration of hemp extract based on 30/32.5 mg/kg of CBD/CBDA. Time 0 was the time of hemp extract administration on day 8 for the multiple-dose phase of the study. The *y*-axis is logarithmically scaled.

index values for all CBs and metabolite show evidence of weak accumulation after multiple-dose administration, given that accumulation index ≥ 1.2 but < 2.[34]

The results from the plasma biochemistry and lipid panels are summarized in **Table 3**. There was no significant effect of time or treatment on the lipid panel (TC, HDL-cholesterol [C], non-HDL-C, and total TG) and on chlorides, bicarbonates, phosphorus, total calcium, ALP, GLDH, and uric acid. Although not statistically significant 2 out of 6 birds in the treatment group had elevated ALP at 531 IU/L and 137 IU/L, respectively (reference interval, 17.5 to 119.6 IU/L).[35]

Plasma glucose, potassium, and total proteins (TP) were significantly higher after treatment by 10%, 80%, and 4%, respectively ($P = .008$, $P < .001$, and $P = .027$, respectively). AST was significantly higher after treatment (38%, $P < .001$) in the control group. None of these elevations were clinically significant. For sodium, there was a significant treatment-time interaction effect ($P = .0119$). Within the control treatment, there was a significant decrease in sodium by 2% ($P = .011$) while no decrease was seen in the CBD treatment over time ($P = .31$).

Table 2—Mean ± SD values of pharmacokinetic parameters of CBD, CBDA, THC, THCA, and 11-OH-THC in orange-winged Amazon parrots (*Amazona amazonica*) after 7 day twice daily oral administration of hemp extract based on 30/32.5 mg/kg of CBD/CBDA to 6 parrots.

Parameter	CBD	CBDA	THC	THCA	11-OH-THC
t_{max} (h) (range)	1 ± 2.63 (1-4)	2 ± 1.29(1-4)	1 ± 2.57 (1-3)	1 ± 1.86 (1-6)	3 ± 0.9 (2-4)
C_{max} (ng/mL)	216.3 ± 283.5	337.4 ± 281.1	13.6 ± 10.5	18.1 ± 9.9	44.9 ± 32.53
$t_{1/2\lambda}$ (h)	8.6 ± 2.3	6.29 ± 2.65	6.5 ± 4.2	2.61 ± 1.63	2.57 ± 1.68
λ_z (1/h)	0.081 ± 0.024	0.110 ± 0.055	0.106 ± 0.066	0.266 ± 0.110	0.270 ± 0.109
C_{min} (ng/mL)	18.7 ± 12.8	8.06 ± 6.02	1.55 ± 0.50	1.32 ± 1.29	6.48 ± 1.09
C_{avg} (ng/mL)	63.2 ± 48.9	83.3 ± 60.4	4.31 ± 2.14	7.72 ± 3.93	17.46 ± 9.08
AUC_{τ} (h·ng/mL)	758.4 ± 586.3	999.2 ± 724.3	51.7 ± 25.6	92.6 ± 47.1	209.6 ± 109.0
$AUC_{0-\infty}$ (h·ng/mL)	1,045.9 ± 866.1	1,202.3 ± 753.7	68.7 ± 35.2	100.3 ± 51.4	222.7 ± 113.6
AUC_{extrap} (%)	11.2 ± 4.6	4.2 ± 5.73	18.0 ± 10.3	3 ± 2.2	11.4 ± 10.7
Accumulation index	1.63 ± 0.25	1.40 ± 0.27	1.45 ± 0.46	1.08 ± 0.13	1.08 ± 0.13

AUC_{τ} = Area under the curve to the end of the dosing period. C_{avg} = Mean plasma concentration. C_{min} = Minimum blood plasma concentration.

See Table 1 for the remainder of the key.

Table 3—Mean ± SD serum biochemical analyses and lipid profile results pre- and postadministration of treatment group based on 30/32.5 mg/kg of CBD/CBDA, PO every 12 hours for 7 days and control group receiving equivalent volume of water.

		Pretreatment		Posttreatment	
Analyte	**Reference interval[35]**	**Treatment group (n = 8)**	**Control group (n = 8)**	**Treatment group (n = 6)**	**Control group (n = 4)**
Anion gap (mmol/L)	17.9–39.4	27 ± 6	31 ± 7	28 ± 2	27 ± 2
Sodium (mmol/L)	146.2–154.2	152 ± 3	155 ± 5	153 ± 2	152 ± 4
Potassium (mmol/L)	1.29–5.04	2 ± 0	2 ± 0	4 ± 1*	3 ± 1
Chloride (mmol/L)	105.4–114.2	112 ± 3	111 ± 3	114 ± 2	111 ± 2
Bicarbonate (mmol/L)	6.6–21.8	14 ± 3	14 ± 4	14 ± 1	18 ± 2
Phosphorus (mg/dL)	1.16–5.00	3 ± 1	4 ± 1	4 ± 1	4 ± 1
Calcium (mg/dL)	7.74–10.36	10 ± 1	10 ± 1	9 ± 1	10 ± 1
Bun (mg/dL)	0–2	1	1	2 ± 1	1
Glucose (mg/dL)	213–371	253 ± 21	253 ± 21	281 ± 15*	287 ± 28
TP (g/dL)	3.44–4.91	4	4 ± 0	4*	4
Albumin (g/dL)		2	2 ± 0	2	2
Globulin (g/dL)		2	2 ± 0	2	2
AST (U/L)	125–375	213 ± 32	234 ± 88	289 ± 45	369 ± 114*
Creatine kinase (U/L)	182–1459	310 ± 82	602 ± 368	706 ± 376	1431 ± 664
ALP (U/L)	17.5–119.6	79 ± 42	64 ± 31	136 ± 194	55 ± 16
Cholesterol (mg/dL)	110.4–363.0	249 ± 68	230 ± 60	275 ± 59	247 ± 42
GLDH (U/L)	0–3.6	1 ± 1	1 ± 1	1	1
Uric acid (mg/dL)	1.86–12.66	5 ± 2	6 ± 2	4 ± 2	5 ± 1
TC (mg/dL)	109–386	257 ± 73	233 ± 58	241 ± 62	244 ± 41
HDL-C (mg/dL)	53–219	184 ± 55	158 ± 46	171 ± 48	160 ± 40
Non-HDL-C (mg/dL)		72 ± 20	74 ± 24	69 ± 26	85 ± 25
TG (mg/dL)	69–234	62 ± 7	79 ± 9	60 ± 14	74 ± 14
HDL-TG (mg/dL)		49 ± 9	62 ± 9	40 ± 6	49 ± 7
Non-HDL-TG (mg/dL)		13 ± 7	17 ± 9	20 ± 12	24 ± 14

*Significance.
See Table 1 for the remainder of the key.

Discussion

One of the main goals of the present study was to evaluate the plasma concentrations and establish the pharmacokinetic parameters of different cannabinoids and their metabolites in OWAP. These parameters were established for CBD, CBDA, THC, THCA, and the metabolite 11-OH-THC in a single and in a multiple-dose studies, being the first studies to evaluate multiple cannabinoids and metabolites in a full spectrum hemp extract in birds. The pharmacokinetic parameters of CBG, CBC, CBN, and the metabolites 7-COOH-CBD, COOH-THC, COOH-THC-Glu, and 7-OH-CBD were not able to be determined because the plasma concentrations were below the limit of quantitation at most time points at the doses evaluated. As initially hypothesized, the findings suggest that twice daily administration of the evaluated hemp extract is well tolerated in healthy OWAP and results in plasma concentrations above 50 ng/mL at a dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA for at least 6 hours (252.1 and 78.3 ng/mL, respectively) following the administration, without significant adverse effects. In contrast to the initial hypothesis, minor cannabinoids CBC, CBG, and CBGA did not generate pharmacokinetic data due to concentrations below the level of quantitation at most time points. The dose was selected based on the pilot studies, in which both birds that received the highest dose of hemp extract based on 60/65 mg/kg of CBD/CBDA showed marked sedation during several hours postadministration, while a single dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA resulted in no adverse effects.

There were major differences in the plasma concentrations and pharmacokinetic parameters between CBs and their acid precursors. The higher concentrations of CBDA observed in the single-dose study suggest that it is better absorbed than CBD, demonstrated by its approximately 6 times greater C_{max} following administrations of almost equal doses. The $t_{1/2}$ of CBDA was shorter than CBD, 1.72 and 2.92 hours, respectively, indicating that CBDA is also metabolized or cleared faster. These differences in absorption and elimination of CBD and CBDA have been observed after single-dose pharmacokinetics in dogs[17] and rabbits.[24] At the 24-hour time point postadministration, from all the compounds measured, there were only detectable plasma concentrations of CBD. For the multiple-dose study, the difference in C_{max} between CBD and CBDA was not as marked as in the single-dose study; however, both $t_{1/2}$ were almost three times longer than those found in the single-dose study. This is also consistent with research performed in dogs evaluating three different forms of hemp extract after 2 weeks of twice daily administration where the serum concentrations of CBD and CBDA were nearly equal, suggesting absorption and retention.[17] A possible mechanism for this is that CBD is probably retained in tissues

as a lipophilic molecule and/or CBDA may inhibit CBD metabolism allowing for better retention.[36,37] Similarly, nonlinear pharmacokinetics of CBD and THC have been observed in dogs, attributed to saturation of P450 leading to increased bioavailability.[38] In a mice model that compared the pharmacokinetic parameters of different cannabinoids administered as a full-spectrum versus as a single compound, it was found that CBDA concentrations were 14 times higher when administered as a full-spectrum hemp extract than when administered as a single compound at an equal dose.[37] The conclusion of this study also suggested that CBDA might have more contribution to the pharmacological effects of the commercially available full-spectrum hemp extracts than previously assumed since the peak plasma concentrations of the other cannabinoids THC and THCA were considerably lower following administration of the full-spectrum extract to when administered as a single compound.[37]

Similar pharmacokinetic trends were observed with THC and THCA, where THCA reached higher plasma concentrations than THC but was metabolized or cleared faster; however, these 2 CBs were not administered at almost equal dosing, receiving 1.23/0.39 mg/kg of THC/THCA, respectively. THC and THCA had longer half-life than CBD and CBDA. This may be of relevance when evaluating the pharmacodynamic effects of CBs and could explain why during the pilot study, in the birds that experienced marked sedation, the effects lasted up to 18 hours postadministration. The accumulation index value for all CBs ranged between 0.95 and 1.88 after the multiple-dose study. An accumulation index of ≥ 1.2 but < 2 is considered weak,[34] suggesting minimal drug accumulation for all cannabinoids measured.

There were differences in the CBs metabolic pathways between OWAP and the species of mammals studied.[39,40] Of the 5 metabolites that were measured, the only metabolite that was consistently quantifiable was 11-OH-THC, which is considered the psychoactive metabolite of THC. Interestingly, in a recent study,[17] performed in dogs following administration of a similar hemp extract and at comparable doses of THC from the present study, 11-OH-THC was below the limit of quantitation at most time points precluding pharmacokinetic analysis. In a different canine study,[38] higher doses of THC of approximately 37 mg/kg resulted in 11-OH-THC maximum concentrations of 5.9 ± 2.7 ng/mL 2 hours postadministration. In the present study, a dose of THC of 1.22 mg/kg and THCA of 0.36 mg/kg resulted in a mean C_{max} of 44.9 ± 32.53 ng/mL of 11-OH-THC. This marked difference could suggest that birds have a different THC metabolism. In contrast, the CBD metabolite 7-COOH-CBD has been readily measured in dogs[17,21] at a dose of 2 mg/kg combined CBD/CBDA and horses[27] 2 mg/kg, PO and 0.1 mg/kg, IV of CBD. The pharmacokinetic profiles of 7-COOH-CBD have been generated for these species. In the present study the CBD metabolites 7-COOH-CBD and 7-OH-CBD, were below the limit of quantitation at most time points despite of similar-to-higher CBD concentrations when compared to canine[17, 21] and equine[27] studies, and pharmacokinetic analysis was not possible for these metabolites.

There were also marked differences between the results of the present study, and a recent study[28] published describing the pharmacokinetics of CBD alone in Hispaniolan Amazon parrots (*Amazona ventralis*) following administration of a single dose of 60 and 120 mg/kg orally. The C_{max} after a 60 mg/kg dose of CBD was 213 ng/mL, while in the present study a dose of hemp extract based on 30/32.5 mg/kg of CBD/CBDA resulted in a CBD mean C_{max} of 105 ng/mL in the single-dose study and 216 ng/mL in the multiple-dose study. The $t_{1/2}$ could not be determined at 60 mg/kg of CBD in the Hispaniolan Amazon parrots because the terminal portion of the curve did not have 3 points. However, at a dose of 120 mg/kg in the Hispaniolan Amazon parrots, the t_{max} of 0.5 hours was significantly shorter than in the one described in the OWAP of 2.5 hours following administration of the hemp extract based on 30/35 mg/kg of CBD/CBDA. In the Hispaniolan Amazon parrot study,[28] the plasma concentrations of different individuals were combined to obtain a plasma concentration versus time curve in an incomplete block study design. In the same study, the variability was not measured; however, interindividual pharmacokinetic differences were speculated. This variability could also be explained by the incomplete block study design used which did not account for all the birds in the study at each time point but also for the absorption and metabolism of CBD in the species studied. Also, the birds' crops were empty during administration without control over food consumption, which could have affected the absorption. Interindividual variability in the metabolism and pharmacokinetic parameters has been described in dogs and humans.[20,41] In the present study, despite having ensured a fasting period of 12 hours and being able to collect samples from all birds at all time points, there were also wide ranges in pharmacokinetic parameters.

Evidence regarding the association of a pharmacodynamic effect for pain management or others and plasma concentrations of CBD is limited and that evidence is even scarcer for the rest of the cannabinoids and their acid precursors. The therapeutic concentrations for pain management will differ based on the specific conditions being treated, endocannabinoid tone (overall functioning of the ECS), and metabolic status (P450, membrane transporters). In canine models, CBD administration was associated with decreased pain scores in dogs with osteoarthritis. A C_{max} of 102 ng/mL and 591 ng/mL following administration of 2 mg/kg and 8 mg/kg, respectively, resulted in plasma concentrations close to 102 ng/mL (eg, 50 ng/mL, which would be obtained after 1 half-life in that study)[18] target concentrations for OA-associated pain in dogs. In a different study,[19] looking at the anticonvulsive effects of CBD, a dose of 2.5 mg/kg twice a day showed a reduction in the frequency of seizures in dogs with idiopathic epilepsy; however there was a negative

association with the 450.1 ng/mL CBD plasma concentration in the dogs in the treatment group and seizure reduction. Although it is unknown if this is a therapeutic plasma concentration for birds for pain management, we elected to use a target plasma concentration of 50 ng/mL of CBD alone. Plasma concentrations of CBD and CBDA that were 500 and 1,711 ng/mL, respectively were associated with marked sedation in both birds at the highest dose evaluated in the pilot study. In contrast, CBD administered as a single compound in Hispaniolan Amazon parrots, a C_{max} of 562 ng/mL was not associated with sedation effects.[28] The sedative effects observed at the highest doses could be associated with the combined effect of CBD and CBDA and/or proportionally higher concentrations of other cannabinoids such as THC and THCA and their metabolite 11-OH-THC. Dose-dependent sedation has been reported as an adverse effect of CBD. In canine studies, at the doses evaluated, sedation has not been an adverse effect. Escalating doses as high as 62 mg/kg did not result in sedation in 1 study,[42] and in a different study[21] doses up to 12 mg/kg did not result in sedation after 28 days of administration. In the present study, there was no sedation observed following a single dose or multiple doses over 7 days.

The birds in this study received hemp extract without food administration. CBD and THC are highly lipophilic substances;[43] therefore, coadministration of a high-lipid meal could enhance their solubility and absorption.[41] Evidence in the rodent model suggests that the bioavailability of CBD and THC was 3 times higher when administered with a lipid vehicle than when administered with a lipid-free vehicle.[44] In contrast, a study[24] in rabbits that compared CBD/CBDA bioavailability with and without food administration showed that administration of a commercial hay-based food slurry immediately after the administration of the hemp oil decreased absorption of CBD/CBDA. This shows that the feeding status may affect absorption differently depending on the species and feeding strategies. It is of particular interest because differences in absorption could affect the therapeutic effect and increase the toxicity of these products. Therefore, further research investigating the absorption of a hemp extract with food administration is warranted. In a similar manner, caution also must be taken given when using different formulations for CBD or full hemp extracts in the market and each presentation and vehicle may affect the absorption and bioavailability. For example, in a canine pharmacokinetic study,[45] hemp was delivered in a powder form inside a gelatin capsule and resulted in no absorption in three of the dogs.

The reported adverse effects of long-term administration of hemp extract in mammals include elevation of liver enzymes, specifically ALP after 4 to 12 weeks of administration in dogs[18,19,21] and AST,[42] which have been associated with cytochrome p450 mediated oxidative hepatic metabolism.[46] A recent study[47] performed in dogs found concurrent elevation of ALP and ALP bone-derived isoform that is suspected a consequence of increased osteoblastic activity. Although in the study presented here there was no significant difference between ALP pre- and posttreatment, 2 out of 6 birds in the treatment group had elevated ALP posttreatment, with 1 having a marked elevation and 1 having a mild elevation. The elevations in ALP were observed after 4 to 12 weeks of hemp extract administration in canine studies, which could suggest that longer administration in the OWAP could have induced these elevations, but a different metabolism between avian species and mammals is also possible. Conversely, AST was significantly elevated in the control group at the end of the treatment, and while there is no clear explanation for this, in most cases AST was concurrently elevated with CK, which could be associated with nonspecific muscle activity during restraint. Also, the oil-based hemp extract used during this study did not induce dyslipidemia in OWAP after 7 days of administration, but it could be possible that longer term administration could induce it.

The limitations of the present study need to be considered. The parameters presented are specific to the hemp extract formulation used, and there are several commercial products available and different vehicles that may affect the absorption of the cannabinoids.[17] The parameters presented here are also specific for OWAP, and significant differences might occur with other species of birds. In addition, these parameters are specific for fasted birds, which needs to be taken into consideration when administered to a companion parrot since the plasma concentrations may change depending on the bird's fasting status. This also represents safety parameters for 7-day administration only, the safety beyond that period will need to be further assessed.

In conclusion, twice daily for 7 days administration of a full spectrum hemp extract with known CBs concentrations was well tolerated in healthy OWAP and resulted in target plasma concentrations in dogs without adverse effects at a dose based on 30/32.5 mg/kg of CBD/CBDA. Although there are similarities with mammal pharmacokinetic parameters, this study suggests that avian species could have differences in the cannabinoid metabolic pathway than mammals. Future studies in orange-winged Amazon parrots and other avian species evaluating the effect of feeding in the pharmacokinetics of CBs and pharmacodynamic studies to determine the association with plasma concentrations and effect in inflammatory mediators are warranted. As non-FDA products are available for use with CBD and CBDA, veterinarians should adhere to compounding regulations and be aware that pharmacokinetic properties may differ between compounded and FDA-approved products.

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References

1. Shahbazi F, Grandi V, Banerjee A, Trant JF. Cannabinoids and cannabinoid receptors: the story so far. *iScience.* 2020;23(7):1–22. doi:10.1016/j.isci.2020.101301
2. Chiurchiù V, van der Stelt M, Centonze D, Maccarrone M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: clues for other neuroinflammatory diseases. *Prog Neurobiol.* 2018;160:82–100. doi:10.1016/j.pneurobio.2017.10.007
3. Zhornitsky S, Potvin S. Cannabidiol in humans–the quest for therapeutic targets. *Pharmaceuticals.* 2012;5(5):529–552. doi:10.3390/ph5050529
4. Hazzah T, Andre C, Ritcher G, McGrath S. Cannabis in veterinary medicine: a critical review. *J Am Holist Vet Med Assoc.* 2020;61(Winter):17–41.
5. Hartsel JA, Boyar K, Pham A, Silver RJ, Makriyannis A. Cannabis in veterinary medicine: cannabinoid therapies for animals. In: *Nutraceuticals in Veterinary Medicine.* Springer International Publishing; 2019:121–155. doi:10.1007/978-3-030-04624-8_10
6. Stincic TL, Hyson RL. Localization of CB1 cannabinoid receptor mRNA in the brain of the chick (*Gallus domesticus*). *Brain Res.* 2008;1245:61–73. doi:10.1016/j.brainres.2008.09.037
7. Alonso-Ferrero ME, Paniagua MA, Mostany R, et al. Cannabinoid system in the budgerigar brain. *Brain Res.* 2006;1087(1):105–113. doi:10.1016/j.brainres.2006.02.119
8. Divín D, Goméz Samblas M, Kuttiyarthu Veetil N, et al. Cannabinoid receptor 2 evolutionary gene loss makes parrots more susceptible to neuroinflammation. *Proc Biol Sci.* 2022;289(1988):20221941. doi:10.1098/rspb.2022.1941
9. Kogan LR, Hellyer P, Downing R. The use of cannabidiol-rich hemp oil extract to treat canine osteoarthritis-related pain: a pilot study. *J Am Holist Vet Med Assoc.* 2020;58(Spring):35–45.
10. American Veterinary Association. Cannabis in Veterinary Medicine August 2020. Accessed January 9, 2023. https://www.avma.org/sites/default/files/2021-03/APH-CannabisResources-Report-20201207.pdf.
11. Jiang R, Yamaori S, Takeda S, Yamamoto I, Watanabe K. Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sci.* 2011;89(5–6):165–170. doi:10.1016/j.lfs.2011.05.018
12. Whalley BJ, Stott C, Gray RA. The human metabolite of cannabidiol, 7-hydroxy-cannabidiol, but not 7-carboxy-cannabidiol, is anticonvulsant in the maximal electroshock threshold test (MEST) in mouse [abstract no. 1.435]. 71st Annual Meeting of the American Epilepsy Society, Washington DC, December 1–5, 2017. Accessed April 11, 2020. https://www.aesnet.org/meetings_events/annual_meeting_abstracts/ view/381222.
13. Brown N, Harvey D. In vitro metabolism of cannabinoids in seven common lab animals. *Drug Metab Dispos.* 1990;18(6):1065–1070.
14. Rock EM, Parker LA. Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. *Br J Pharmacol.* 2013;169(3):685–692. doi:10.1111/bph.12162
15. Rock EM, Limebeer CL, Parker LA. Effect of cannabidiolic acid and Δ9-tetrahydrocannabinol on carrageenan-induced hyperalgesia and edema in a rodent model of inflammatory pain. *Psychopharmacology (Berl).* 2018;235(11):3259–3271. doi:10.1007/s00213-018-5034-1
16. Takeda S, Misawa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. *Drug Metab Dispos.* 2008;36(9):1917–1921. doi:10.1124/dmd.108.020909
17. Wakshlag JJ, Schwark WS, Deabold KA, et al. Pharmacokinetics of cannabidiol, cannabidiolic Acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. *Front Vet Sci.* 2020;7:505. doi:10.3389/fvets.2020.00505
18. Gamble LJ, Boesch JM, Frye CW, et al. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Front Vet Sci.* 2018;5:165. doi:10.3389/fvets.2018.00165
19. McGrath S, Bartner LR, Rao S, Packer RA, Gustafson DL. Randomized blinded controlled clinical trial to assess the effect of oral cannabidiol administration in addition to conventional antiepileptic treatment on seizure frequency in dogs with intractable idiopathic epilepsy. *J Am Vet Med Assoc.* 2019;254(11):1301–1308. doi:10.2460/javma.254.11.1301
20. Bartner LR, McGrath S, Rao S, Hyatt LK, Wittenburg L. Pharmacokinetics of cannabidiol administered by 3 delivery methods at 2 different dosages to healthy dogs. *Can J Vet Res.* 2018;82(3):178–183.
21. Vaughn DM, Paulionis LJ, Kulpa JE. Randomized, placebo-controlled, 28-day safety and pharmacokinetics evaluation of repeated oral cannabidiol administration in healthy dogs. *Am J Vet Res.* 2021;82(5):405–416. doi:10.2460/ajvr.82.5.405
22. Deabold KA, Schwark WS, Wolf L, Wakshlag JJ. Single-dose pharmacokinetics and preliminary safety assessment with use of CBD-rich hemp nutraceutical in healthy dogs and cats. *Animals.* 2019;9(10):832. doi:10.3390/ani9100832
23. Kleinhenz MD, Magnin G, Lin Z, et al. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (*Cannabis sativa*). *Sci Rep.* 2020(1);10:12753. doi:10.1038/s41598-020-69768-4
24. Rooney TA, Carpenter JW, KuKanich B, Gardhouse SM, Magnin GC, Tully TN. Feeding decreases the oral bioavailability of cannabidiol and cannabidiolic acid in hemp oil in New Zealand white rabbits (*Oryctolagus cuniculus*). *Am J Vet Res.* 2022;83(10):ajvr.22.01.0006. doi:10.2460/ajvr.22.01.0006
25. Spittler AP, Helbling JE, McGrath S, Gustafson DL, Santangelo KS, Sadar MJ. Plasma and joint tissue pharmacokinetics of two doses of oral cannabidiol oil in guinea pigs (*Cavia porcellus*). *J Vet Pharmacol Ther.* 2021;44(6):967–974. doi:10.1111/jvp.13026
26. Ryan D, McKemie DS, Kass PH, Puschner B, Knych HK. Pharmacokinetics and effects on arachidonic acid metabolism of low doses of cannabidiol following oral administration to horses. *Drug Test Anal.* 2021;13(7):1305–1317. doi:10.1002/dta.3028
27. Turner SE, Knych HK, Adams AA. Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses. *Am J Vet Res.* 2022;83(9):ajvr.22.02.0028. doi:10.2460/ajvr.22.02.0028
28. Carpenter JW, Tully TN, Rockwell K, KuKanich B. Pharmacokinetics of cannabidiol in the Hispaniolan Amazon parrot (*Amazona ventralis*). *J Avian Med Surg.* 2022;36(2):121–127. doi:10.1647/20-00076
29. Devinsky O, Patel AD, Thiele EA, et al. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. *Neurology.* 2018;90(14):e1204–e1211. doi:10.1212/WNL.0000000000005254
30. Thiele EA, Marsh ED, French JA, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet.* 2018;391(10125):1085–1096. doi:10.1016/S0140-6736(18)30136-3

31. Geelen S, Sanchez-Migallon Guzman D, Souza MJ, Cox S, Keuler NS, Paul-Murphy JR. Antinociceptive effects of tramadol hydrochloride after intravenous administration to Hispaniolan Amazon parrots (*Amazona ventralis*). *Am J Vet Res.* 2013;74(2):201–206.
32. Robertson JA, Sanchez-Migallon Guzman D, Graham JL, et al. Evaluation of orally administered atorvastatin on plasma lipid and biochemistry profiles in hypercholesterolemic hispaniolan Amazon parrots (*Amazona ventralis*). *J Avian Med Surg.* 2020;34(1):32–40. doi:10.1647/1082-6742-34.1.32
33. Wickham H. In: Wickham H, ed. *Ggplot2: Elegant Graphics for Data Analysis.* 2nd ed. Springer-Verlag; 2016.
34. Li L, Li X, Xu L, Sheng Y, Huang J, Zheng Q. Systematic evaluation of dose accumulation studies in clinical pharmacokinetics. *Curr Drug Metab.* 2013;14(5):605–615. doi:10.2174/13892002113149990002
35. Vergneau-Grosset C, Polley T, Holt DC, Vernau W, Paul-Murphy J. Hematologic, plasma biochemical, and lipid panel reference intervals in orange-winged amazon parrots (*Amazona amazonica*). *J Avian Med Surg.* 2016;30(4):335–344. doi:10.1647/2015-129
36. Eichler M, Spinedi L, Unfer-Grauwiler S, et al. Heat exposure of cannabis sativa extracts affects the pharmacokinetic and metabolic profile in healthy male subjects. *Planta Med.* 2012;78(7):686–691. doi:10.1055/s-0031-1298334
37. Anderson LL, Etchart MG, Bahceci D, Golembiewski TA, Arnold JC. Cannabis constituents interact at the drug efflux pump BCRP to markedly increase plasma cannabidiolic acid concentrations. *Sci Rep.* 2021;11(1):14948. doi:10.1038/s41598-021-94212-6
38. Chicoine A, Illing K, Vuong S, Pinto KR, Alcorn J, Cosford K. Pharmacokinetic and safety evaluation of various oral doses of a novel 1:20 THC:CBD cannabis herbal extract in dogs. *Front Vet Sci.* 2020;7:583404. doi:10.3389/fvets.2020.583404
39. Harvey DJ, Samara E, Mechoulam R, Samara E. Comparative metabolism of cannabidiol in dog, rat and man. *Pharmacol Biochem Behav.* 1991;40(3):523–532.
40. Whalley BJ, Lin H, Bell L, et al. Species-specific susceptibility to cannabis-induced convulsions. *Br J Pharmacol.* 2019;176(10):1506–1523. doi:10.1111/bph.14165
41. Stott CG, White L, Wright S, Wilbraham D, Guy GW. A phase I study to assess the effect of food on the single dose bioavailability of the THC/CBD oromucosal spray. *Eur J Clin Pharmacol.* 2013;69(4):825–834. doi:10.1007/s00228-012-1393-4
42. Vaughn D, Kulpa J, Paulionis L. Preliminary investigation of the safety of escalating cannabinoid doses in healthy dogs. *Front Vet Sci.* 2020;7:51. doi:10.3389/fvets.2020.00051
43. Millar SA, Stone NL, Yates AS, O'Sullivan SE. A systematic review on the pharmacokinetics of cannabidiol in humans. *Front Pharmacol.* 2018;9:1365. doi:10.3389/fphar.2018.01365
44. Zgair A, Wong JC, Lee JB, et al. Dietary fats and pharmaceutical lipid excipients increase systemic exposure to orally administered cannabis and cannabis-based medicines. *Am J Transl Res.* 2016;8(8):3448–3459.
45. Samara E, Bialer M, Mechoulam R. Pharmacokinetics of cannabidiol in dogs. *Drug Metab Dispos.* 1988;16(3):469–472.
46. Bornheim LM, Correia MA. Effect of cannabidiol on cytochrome P-450. *Biochemicd Pharmacology.* 1989;38(17):2789–2794. doi:10.1016/0006-2952(89)90432-2
47. Bradley S, Young S, Bakke AM, et al. Long-term daily feeding of cannabidiol is well-tolerated by healthy dogs. *Front Vet Sci.* 2022;9:977457. doi:10.3389/fvets.2022.977457

Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.