The endocannabinoid system (ECS) has been demonstrated to play an important role in analgesic and inflammatory pathways and is highly conserved across most species.1,2 Cannabidiol (CBD), cannabidiolic acid (CBDA), and tetrahydrocannabinol (THC) are all plant-derived cannabinoids (phytocannabinoids)3 that act on receptors, including transient receptor potential vanilloid 1, 5-hydroxytryptamine and those within the ECS.4 Notably, CBD and CBDA are nonpsychotropic,5 and CBD has been used in studies on canine osteoarthritis.6,7 In recent years, the increasing use of cannabinoids in veterinary medicine as over-the-counter therapeutics based on anecdotal information has highlighted the need for prospective clinical trials to characterize the pharmacokinetics, pharmacodynamics, and appropriate clinical application of these medications in animals.3,8

Dog and cat owners have indicated that they administer cannabinoids to their pets as treatment for a range of conditions, including chronic pain, seizures, neoplasia, and behavioral issues.5 Despite this varied use, prospective studies to assess the efficacy and potential risks of cannabinoids as a treatment have only been conducted pertaining to osteoarthritis and seizure activity.5 Further research to determine effectiveness, risks, and appropriate doses for individual conditions are needed.5 Recommended doses for CBD administration in domestic dogs range widely1,7–11; a recent study evaluating the safety and effectiveness of CBD and CBDA in a model system showed a difference in oral bioavailability with and without food.12

OBJECTIVE
To determine the pharmacokinetics of a solution containing cannabidiol (CBD) and cannabidiolic acid (CBDA), administered orally in 2 single-dose studies (with and without food), in the domestic rabbit (Oryctolagus cuniculus).

ANIMALS
6 healthy New Zealand White rabbits.

PROCEDURES
In phase 1, 6 rabbits were administered 15 mg/kg CBD with 16.4 mg/kg CBDA orally in hemp oil. In phase 2, 6 rabbits were administered the same dose orally in hemp oil followed by a food slurry. Blood samples were collected for 24 hours to determine the pharmacokinetics of CBD and CBDA. Quantification of plasma CBD and CBDA concentrations was determined using a validated liquid chromatography–mass spectrometry (LC-MS) assay. Pharmacokinetics were determined using noncompartmental analysis.

RESULTS
For CBD, the area under the curve extrapolated to infinity (AUC) was 179.8 and 102 hours X ng/mL, the maximum plasma concentration (Cmax) was 30.4 and 15 ng/mL, the time to Cmax (tmax) was 3.78 and 3.25 hours, and the terminal half-life (t1/2λ) was 7.12 and 3.8 hours in phase 1 and phase 2, respectively. For CBDA, the AUC was 12,286 and 6,176 hours X ng/mL, Cmax was 2,573 and 1,196 ng/mL, tmax was 1.07 and 1.12 hours, and t1/2λ was 3.26 and 3.49 hours in phase 1 and phase 2, respectively. Adverse effects were not observed in any rabbit.

CLINICAL RELEVANCE
CBD and CBDA reached a greater Cmax and had a longer t1/2λ in phase 1 (without food) compared with phase 2 (with food). CBDA reached a greater Cmax but had a shorter t1/2λ than CBD both in phase 1 and phase 2. These data may be useful in determining appropriate dosing of cannabinoids in the domestic rabbit.
pharmacokinetics of chronic CBD administration in dogs found that there was a dose-dependent plasma accumulation of CBD, and that oral doses of 1 to 12 mg/kg daily for 28 days were well tolerated. Only mild side effects have been reported to date and include gastrointestinal upset and elevated serum alkaline phosphatase.\textsuperscript{1,7,8,10,11} Pharmacokinetic studies on analgesics in rabbits have demonstrated that the doses needed to achieve therapeutically concentrations tend to be substantially greater in rabbits than other mammals\textsuperscript{13-15}; thus, a single dose of 15 mg/kg PO was selected for this study.

The objective of this study was to characterize the pharmacokinetics of CBD and CBDA administered orally in the domestic rabbit (\textit{Oryctolagus cuniculus}) in 2 single-dose studies, with and without a food slurry (phase 1 and phase 2, respectively). It was hypothesized that CBD and CBDA administered in food would extend the plasma concentration and half-life of CBD and CBDA in the rabbit. It was also hypothesized that a greater dose of cannabinoids relative to the currently published doses in dogs would provide and maintain greater and prolonged blood concentrations of cannabinoids in the domestic rabbit.

### Materials and Methods

#### Animals

Six clinically normal, specific pathogen (\textit{Pasteurella sp})-free New Zealand White rabbits (age, 8 months; sex, 3 males and 3 females), obtained from a commercial supplier were included in this study. The rabbits were housed individually in stainless steel cages for 12 hours/day and in indoor runs (7.8 X 0.9 X 1.8 m) for 12 hours/day at Kansas State University College of Veterinary Medicine. The runs and cages were both in a temperature- and humidity-controlled holding room. The rabbits were provided a photoperiod of 12 hours of light and 12 hours of dark. Rabbits were fed ¼ cup of a commercial pelleted diet per 5 pounds of body weight once daily (Oxbow Essentials Young Rabbit Food, Oxbow Enterprises Inc) and ad libitum timothy hay (Oxbow Western Timothy Hay, Oxbow Enterprises Inc). Water was available ad libitum via sipper bottles. Rabbits were allowed 7 days to acclimate to the study environment before the study began. All rabbits were evaluated by a veterinarian whose assessments included physical examinations and baseline blood work (PCV, total solids, and plasma chemistry panel) prior to each phase of the study. Physical examination findings and blood work results were within normal limits for all rabbits at each assessment. All animal husbandry and experimental procedures were performed under the approval of Kansas State University Institutional Animal Care and Use Committee (No. 4474) prior to initiation. Animals were adopted after completion of the study.

#### Cannabidiol, CBDA, and THC analyses in hemp oil

A confidence analysis was performed on the hemp oil oral suspension (22.5 mg/mL CBD, 24.6 mg/mL, CBDA; Canna Companion Services, LLC) prior to the study for CBD, CBDA, and THC content using high-performance liquid chromatography with ultraviolet detection (Confidence Analytics: Cannabis Analytics and Research Specialists). The oral suspension was also analyzed for heavy metal using inductively coupled plasma–mass spectrometry, for microbes using plate counting, for terpenes using gas chromatography with flame ionization detection, for solvents using headspace–gas chromatography–mass spectrometry (HS-GC-MS), for trace chemical residues using ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS), and for water activity using a hygrometer (Confidence Analytics: Cannabis Analytics and Research Specialists).

### Experimental design

#### Phase 1

Rabbits were not fasted prior to drug administration and their regular diet was available to them for the duration of the study. For drug administration, each rabbit was restrained manually and administered its dose of oral hemp oil containing CBD and CBDA via plastic oral syringes. Each rabbit was monitored to ensure it swallowed the entire dose. Dosing was based on 15 mg/kg CBD, which also provided a CBDA dose of 16.4 mg/kg; the total volume of hemp oil administered ranged from 1.33 to 1.67 mL.

Blood samples (0.75 mL) were obtained from the lateral saphenous and cephalic veins at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours. The blood was placed into 1-mL lithium heparin tubes without separators, which were placed in a cooler. Plasma was separated within 1 hour of collection via centrifugation for 10 minutes at 2,000 X g, and plasma supernatant was harvested and stored in cryogenic vials in an ultralow freezer (−79°C) at Kansas State University until analysis.

A 2-week washout period was provided before phase 2 of the study began.

#### Phase 2

For drug administration, each rabbit was restrained manually and administered its dose of oral hemp oil, followed immediately by 35 to 40 mL orally of a food slurry (Herbivore Critical Care Apple Banana, Oxbow Enterprises Inc) that was mixed according to the manufacturer’s instructions. The food slurry with CBD oil was administered via plastic oral syringes. Each rabbit was monitored to ensure it swallowed the entire dose. Venipuncture and sample processing followed the same methods as phase 1.

### Plasma drug and pharmacokinetic analysis

#### Extraction

All solvents used for the analysis were liquid chromatography–mass spectrometry grade. CBD, CBDA, and 9-THC were purchased as certified reference solutions at 1,000 μg/mL (Cerilliant Corp). Deuterated cannabinoid analogs were purchased in solution at 100 μg/mL and were used as internal standards (ISs). A stock solution of cannabinoids...
at 10 μg/mL in methanol was prepared and stored at -20°C. A stock solution of IS at 10 μg/mL was prepared in methanol and stored at -20°C. Fresh working standard solutions in acetonitrile with 0.1% formic acid were prepared daily at the following concentrations: 1, 2.5, 5, 10, 25, 50, and 100 ng/mL. The following quality control (QC) working solutions at 47.5, 475, and 950 ng/mL were prepared in acetonitrile with 0.1% formic acid, and 50 μL was added to 450 μL of negative control rabbit plasma to prepare the QCs at 4.75, 47.5, and 95 ng/mL (Sierra for Medical Science). Calibration standards were prepared by mixing 100 μL of the standards solutions, 100 μL of negative control rabbit plasma, and 100 μL of the IS mix at 100 ng/mL. QCs were prepared by mixing 100 μL of the spiked plasma, 100 μL of IS mix at 100 ng/mL, and 100 μL of acetonitrile 0.1% formic acid. To 100 μL of each unknown plasma sample was added 100 μL of IS mix at 100 ng/mL and 100 μL of acetonitrile 0.1% formic acid. Standards, QCs, and unknowns were vortexed for 30 seconds and spun down at 13,000 X g for 5 minutes. The supernatant was transferred to a clean microcentrifuge tube and 400 μL of water was added to each tube. Cleanup was performed by solid-phase extraction on reverse-phase cartridges. Standards, QCs, and samples were loaded on the cartridge and, after washing with water:methanol (75:25), each cartridge was allowed to dry. The cannabinoids were eluted with 2 X 25 μL of acetonitrile:methanol (90:10), and aqueous formic acid 0.2% was added to each well before analysis.

Analysis by UPLC-MS/MS
Cannabinoids were analyzed by UPLC-MS/MS coupled to a triple-quadrupole mass spectrometer. The chromatographic separation was performed with a UPLC column (column length 100 mm, column diameter 2.1 mm, with a 1.8 μm particle size), heated at 55°C. The flow rate was 0.5 mL/minute, the mobile phase consisted of a gradient of acetonitrile (B) and water containing 0.1% formic acid (A). The chromatographic separation was performed coupled to a triple-quadrupole mass spectrometer. The total run time was 12 minutes and the injection volume was 5 μL. Data acquisition was performed by electrospray ionization (ES) in positive (ES+) and negative (ES–) mode using multireaction monitoring. The precursor ion, the products ions, the qualifier ions, and the collision energy (CE), as well as the ionization mode (ES+ or ES–), are indicated below. The capillary voltage was set at 3.0 kV, the source temperature at 150°C, the desolvation temperature at 500°C, the desolvation nitrogen flow at 1,000 L/hour, and the cone nitrogen flow at 150 L/hour.

The ionization mode, the precursor ions, product ions, quantifier and qualifier ions, CV, and CE are detailed here. CBD was detected in negative mode, with a precursor ion at mass-to-charge ratio (m/z) 357.4 (CV = 4 V), a quantifier ion at m/z 359.39 (CE = 20V), and two qualifier ions at m/z 245.4 (CE = 28V) and m/z 179.2 (CE = 26V). THC-acid-d3 was used as an IS for CBD and was detected in negative ionization mode with a precursor ion at m/z 352.5 (CV = 68V) and a quantifier ion at m/z 308.44 (CE = 20V). CBD-d5 was used as an IS for CBD and was detected in positive ionization mode, with a precursor ion at m/z 315.2 (CV = 14V), a quantifier ion at m/z 313.0 (CE = 22V), and two qualifier ions at m/z 259.1 (CE = 20V) and m/z 134.9 (CE = 20V).

Results
Body weights for the rabbits ranged from 2.22 to 2.57 kg. CBD was administered successfully to all rabbits at the commencement of phases 1 and 2. No clinically significant changes were appreciated in the CBC variables before and after the study period. Any serum biochemistry changes before and after the

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study period were considered minor and not clinically significant. No adverse effects were observed in any of the rabbits in this study.

The pharmacokinetic data for CBD and CBDA are reported for each rabbit and as a mean (range) for phases 1 and 2 (Tables 1 and 2). Pharmacokinetic data were normally distributed, with uniform variance. Statistically significant differences \( (P < .05) \) occurred in the CBD \( C_{\text{max}} \), AUC\( \text{∞-0} \), and \( t_{\text{1/2}λ} \) and for the CBDA AUC\( \text{∞-0} \) between phases 1 and 2. The post hoc power analyses for the \( t_{\text{max}} \) for CBD, and the \( C_{\text{max}} \), \( C_{\text{max}} \), and \( t_{\text{1/2}λ} \) for the CBDA were < 0.8 and therefore are not reported. The arithmetic mean (SD) plasma profiles were evaluated (Figure 1).

### Table 1—Noncompartmental pharmacokinetics of plasma cannabidiol (CBD) concentration in 6 clinically normal, specific pathogen (Pasteurella sp)-free New Zealand White rabbits (Oryctolagus cuniculus) after a single oral dose of 15 mg/kg CBD and 16.4 mg/kg cannabidiolic acid in hemp oil when administered alone (phase 1) or immediately followed with a food slurry (phase 2), with a 2-week washout period between treatments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC( \text{∞-0} ) (h X ng/mL)</td>
<td>179.8 (119.1–255.5)</td>
<td>102 (55–143)</td>
<td>.0150</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>30.4 (17.8–49.8)</td>
<td>15.0 (9.8–25.4)</td>
<td>.0151</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>3.78 (2.00–6.00)</td>
<td>3.25 (2.00–6.00)</td>
<td>N/A</td>
</tr>
<tr>
<td>( t_{\text{1/2}λ} ) (h)</td>
<td>7.12 (5.24–8.49)</td>
<td>3.80 (2.29–6.74)</td>
<td>.0104</td>
</tr>
</tbody>
</table>

AUC\( \text{∞-0} \) = AUC extrapolated to infinity; \( C_{\text{max}} \) = Maximum plasma concentration; N/A = Not applicable; \( t_{\text{max}} \) = Time to \( C_{\text{max}} \); \( t_{\text{1/2}λ} \) = Terminal half-life. Data are presented as geometric mean (range).

### Table 2—Noncompartmental pharmacokinetics of plasma cannabidiolic acid concentration in phase 1 (without food slurry) and phase 2 (with food slurry) for the rabbits described in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC( \text{∞-0} ) (h X ng/mL)</td>
<td>12,286 (8,608–18,717)</td>
<td>6,176 (5,298–7,944)</td>
<td>.003</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>2,573 (1,122–5,039)</td>
<td>1,196 (556–4,027)</td>
<td>N/A</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>1.07 (0.50–6.00)</td>
<td>1.12 (0.25–4.00)</td>
<td>N/A</td>
</tr>
<tr>
<td>( t_{\text{1/2}λ} ) (h)</td>
<td>3.26 (2.53–3.93)</td>
<td>3.49 (3.14–3.93)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

AUC\( \text{∞-0} \) = AUC extrapolated to infinity; \( C_{\text{max}} \) = Maximum plasma concentration; N/A = Not applicable; \( t_{\text{max}} \) = Time to \( C_{\text{max}} \); \( t_{\text{1/2}λ} \) = Terminal half-life. Data are presented as geometric mean (range).
Discussion

This study described the pharmacokinetics of CBD and CBDA administered orally in the domestic rabbit, and compared the pharmacokinetics of CBD and CBDA administration with and without a food slurry. Contrary to the hypothesis, CBD and CBDA both had longer plasma concentrations and half-lives when administered without food, compared to administration with a food slurry. CBDA reached greater plasma concentrations and had a longer half-life than CBD both with and without a food slurry. In other species, the most commonly documented adverse effect of CBD administration is gastrointestinal upset, including ptyalism and loose stool. No adverse effects were noted in the rabbits involved in our study.

CBDA was likely better absorbed than CBD, as evidenced by its greater concentration and exposure compared with CBD at nearly equal dosages. Alternatively, the volume of distribution could be much larger for CBD than for CBDA. Another study on the pharmacokinetics of CBD and CBDA in a canine model also found greater CBDA concentrations and retention when compared to CBD. There is a scarcity of data on the potential therapeutic effects of CBDA, but it has been demonstrated to have greater potency and superior ability to reduce emesis in rats and shrews compared with CBD.

Plasma concentrations of both CBD and CBDA were greater in phase 1 than in phase 2, suggesting that CBD and CBDA are both better absorbed without food than in a food slurry in the domestic rabbit. Based on the AUC0–∞ ratios, the relative bioavailability of both CBD and CBDA was about 50% less in fed versus fasted animals. The concept of food–drug interaction, or “food effect,” considers the impact that food may have on orally administered medication rates and extent of absorption. Many variables contribute to the food effect, including the physiologic status of the patient and the composition of the food ingested, including the quantity of food, solid-to-liquid ratio, fat content, and gastrointestinal pH, as well as factors relating to the drug’s physiochemical properties and specific foods ingested. In human studies, the FDA recommends creating meal conditions that will have the most profound effects on gastrointestinal physiology, and thus will reflect the most significant impacts that a meal may have on drug absorption. Thus, FDA suggests using a high-fat, high-calorie diet in food-effect studies in humans, although, interestingly, dietary lipids have been demonstrated to increase the absorption of orally administered CBD in rats. For our study, the implications of rabbits’ inherently high-fiber diets must be considered. Rabbits are hind-gut fermenters, and most of their ideal diet is hay-based, and thus high in fiber. The food slurry used in phase 2 was intended to simulate a normal, appropriate diet for a rabbit. Drugs may adsorb to high-fiber substances, and the fiber can therefore carry the drug with it through the digestive system, precluding the drug from absorption. The results from our study suggest that the high-fiber food slurry may have reduced CBD and CBDA absorption substantially. A previous pharmacokinetic and pharmacodynamic study on torsemide in horses attributed some of the variability in Cmax and tmax on the variable amounts of ingesta consumed by the horses, commenting that ingesta may have impeded drug absorption.

The prolonged plasma concentrations and half-life of CBD when administered without food bears importance for the clinical use of CBD in rabbits. Rabbits eat almost constantly (roughly 20 times in a 24-hour period), and because prolonged periods without eating can lead to negative energy balances quickly in small mammals, fasting is not advised. Furthermore, it is well known that without nearly constant food intake, concern for gastrointestinal stasis syndrome can be a significant problem in rabbits. Rabbits are coprophagic lagomorphs, and coprophagy may have a substantial impact on drug absorption profiles in rodents. The rabbits in our study were not precluded from performing their natural behaviors, so it is reasonable to conclude that coprophagy may have occurred between samples points and may have impacted the pharmacokinetic profiles presented here.

Although negative correlations have been appreciated between plasma CBD concentrations and seizure frequency and between CBDA or CBD concentrations and emesis, the results have been variable and the specific target plasma concentrations have not been established in any species. The therapeutic concentration may change based on the condition being treated or due to unaccounted active ingredients in some preparations. Further research is needed to elucidate whether a correlation exists between a therapeutic effect for a given condition and plasma concentrations.

One strength of our study is that its design allowed for a comparison of these compounds’ pharmacokinetics both with and without a food slurry. Our study also presents information on the pharmacokinetics of CBDA, for which there is a paucity of data in the literature for any species. The formulation of the drug administered here (in hemp oil) allows for ease of dose adjustment by the practitioner, as the volume of distribution could be much larger for CBD than for CBDA. Another study on the pharmacokinetics both with and without a food slurry. The rabbits in our study were not precluded from performing their natural behaviors, so it is reasonable to conclude that coprophagy may have occurred between samples points and may have impacted the pharmacokinetic profiles presented here.

Limitations of our study include a small sample size (n = 6) and single-dose administration. A longer duration of administration would be beneficial to assess trends in CBD and CBDA concentration over time, and to monitor for adverse effects with longer term or chronic administration. As a result of the lack of an IV formulation of CBD and CBDA, we cannot comment on the oral bioavailability of these compounds. Furthermore, studies assessing a multidose regimen and evaluating the pharmacodynamics of CBD and CBDA in the domestic rabbit would provide important insights into the clinical use of these compounds in rabbits.
additional clinical significance in the domestic rabbit. The effectiveness of CBD and CBDA in treating specific conditions should be investigated further. The results of this study will inform future studies.

Our study describes the pharmacokinetic properties of CBD and CBDA in 2 single-dose studies (in a hemp oil suspension alone and administered with a food slurry) in the domestic rabbit. Furthermore, this study provides pharmacokinetic data for CBDA, of which there is a paucity in the literature across species. These data suggest that CBD and CBDA are both better absorbed without food than with a food slurry, and that CBDA is absorbed more readily than CBD in the domestic rabbit. These results contribute information about the pharmacokinetics of CBD and CBDA to a growing body of literature surrounding CBD use in the medical field. These results may be useful in determining appropriate dosing of cannabinoids in the domestic rabbit and may be extrapolated further to other mammalian species. Controlled clinical trials focusing on the elucidation of the therapeutic properties of both CBD and CBDA are needed, and studies that focus on specific disease processes may be applicable for both veterinary and human medicine. Compounded products were prepared from FDA-approved products to create a liquid suspension that would provide ease of administration to rabbits, as non-FDA products were 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. Medical cannabis treatment holds promise for both human and veterinary medicine, and data pertaining to its use may be of benefit to both fields.

Acknowledgments

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The authors declare that there were no conflicts of interest.

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