Pharmacokinetics of cannabidiol in a randomized crossover trial in senior horses

Shelley E. Turner, MSc1; Heather K. Knych, DVM, PhD2,3; Amanda A. Adams, PhD4

1M. H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY
2K.L. Maddy Equine Analytical Pharmacology Laboratory, School of Veterinary Medicine, University of California-Davis, Davis, CA
3Department of Veterinary Molecular Biosciences, School of Veterinary Medicine, University of California-Davis, Davis, CA
4M. H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY

*Corresponding author: Ms. Turner (Shelley.turner@uky.edu)

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OBJECTIVE
To determine the pharmacokinetics, bioavailability, and pharmacological effects of cannabidiol (CBD) in senior horses.

ANIMALS
8 university-owned senior horses.

PROCEDURES
In this randomized, crossover study, horses were assigned to receive either a single oral dose of 2 mg/kg CBD in oil or a single IV dose of 0.1 mg/kg CBD in DMSO between August 10 and September 4, 2020. Blood samples were collected before and then 0.5, 1, 4, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 264 hours after CBD administration. Serum biochemical analyses and CBCs were performed. Plasma concentrations of CBD and its metabolites were determined with the use of liquid chromatography-tandem mass spectrometry.

RESULTS
Concentrations of CBD and metabolites (7-COH CBD and 7-COOH CBD) were detected in all plasma samples up to 8 hours after dosing (oral and IV), with 7-COOH CBD being the most predominant metabolite. Pharmacokinetic results for CBD oral dosing at 2 mg/kg were mean ± SD half-life of 7.22 ± 2.86 hours, maximum concentration of 18.54 ± 9.80 ng/mL, and time to maximum concentration of 2.46 ± 1.62 hours. For both oral and IV administrations, 7-COOH CBD did not fall below the limit of quantification for the times reported. Oral bioavailability for CBD was 7.92%. There was no meaningful effect of CBD on results for CBC, serum biochemical analyses, or vital signs for any horse.

CLINICAL RELEVANCE
Pharmacokinetics and bioavailability of CBD in senior horses were determined, and there were no adverse effects of administering either the oral or IV dose of CBD evaluated.
In vivo, it has been shown, both in vitro and in aging horses, that the process of inflammaging occurs. The term inflammaging is described as a chronic, low-grade inflammation that occurs over time as the individual ages and can be defined as elevated proinflammatory cytokines, including tumor necrosis factor-α (TNFα), interleukin-8 (IL-8), or interleukin-6 (IL-6) as well as chemokines. In humans that CBD may have the ability to mitigate the inflammaing process by reducing proinflammatory cytokines, such as TNFα or IL-6, and reintroducing a balance on oxidative stress. Therefore, it is of relevance to determine whether CBD may reduce some of the effects of inflammaging in senior horses.

In fact, we have recently shown that CBD has an effect on inflammaging in vitro. In that study, peripheral blood mononuclear cells collected from senior horses were incubated in vitro with various concentrations of pure CBD. We showed that at increasing concentrations of CBD there were reduced concentrations of proinflammatory cytokines TNFα and interferon-γ (IFNγ), and results indicated that CBD has the potential to modulate inflammation in horses; however, in vivo studies are warranted. Moreover, before efficacy studies are conducted in senior horses, pharmacokinetic studies should be undertaken. Thus, the objectives of the study reported here were to measure the pharmacokinetics, bioavailability, and pharmacological effects of CBD in senior horses.

Materials and Methods

All experimental procedures were approved by the University of Kentucky’s Animal Care and Use Committee.

Animals

Eight healthy university-owned senior horses (2 geldings and 6 mares) of mixed breed and mean age of 24 ± 3 years were used in this randomized, unbalanced crossover design study conducted from August 10 to September 4, 2020. All horses were housed in their respective paddocks, at the University of Kentucky, C. Oran Little farm. Before, during, and after all sample collections, the horses had access to pasture and water; however, grain was withheld prior to all dosing. All of the horses exhibited no clinical abnormalities (no nasal discharge, coughing, etc) at the time of blood collection. Rectal temperature, heart rate, and respiratory rate were monitored in all horses before and 0.5, 1, and 4 hours after CBD administration.

CBD preparation for oral and IV administration

Horses were randomly allocated into either oral dose group (n = 6) or the intravenous (IV)-dose group (2). The first oral or IV dosing occurred on the same day with a washout period of 14 days before the crossover treatment with CBD. For each of the 6 horses randomly chosen to receive oral administration of CBD, a single oral dose of 2 mg/kg CBD was made by adding the needed amount of CBD, delta-9-tetrahydracannabidiol (THC) free distillate oil (AgTech Scientific Corp) to 15 mL of soy oil. The use of 2 mg/kg CBD for oral administration was determined with a pilot study using 4 horses. In this pilot study, a 2-mg/kg CBD oral dose was compared with a 1-mg/kg CBD oral dose, and it was determined that the 2-mg/kg dose was better detected after administration (results not shown). For each of the 2 horses randomly chosen to receive IV administration, a single IV dose of 0.1 mg/kg CBD was made by dissolving the needed amount of CBD powder (AgTech Scientific Corp; 99% purity) in 0.5 mL of DMSO (99.7% purity; Sigma-Aldrich). Both the CBD distillate oil and CBD powder were analyzed for CBD at a third-party laboratory and were confirmed to have been THC-free and 98% or 99.9% CBD, respectively.

Sample collection

Blood samples were collected via aseptic jugular venipuncture into EDTA-prepared tubes (Covetrus). Collection time points for both treatments were immediately before administration of CBC (time 0) and then 0.5, 1, 4, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 264 hours after CBD administration. All blood samples were placed on ice immediately and within an hour of collection and centrifuged at 800 X g for 10 minutes, and then plasma was aliquoted into 1.5-mL plastic microcentrifuge tubes and stored at −20°C until analyzed. Additional blood samples were collected into 5 mL serum tubes and EDTA tubes for CBCs and basic serum biochemical analyses before and 24 hours after CBD administration. All CBC and basic serum biochemical analyses were analyzed at Rood and Riddle Equine Hospital Laboratory (Lexington, KY). The CBCs and basic serum biochemical analyses included determination of hemoglobin concentration, WBC count, RBC count, PCV, serum concentrations of total protein and albumin, and serum activities of glutamic-oxaloacetic transaminase (SGOT), aspartate aminotransferase (AST),...
alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT).

**CBD and metabolite concentration determination in equine plasma**

All plasma samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for determination of the concentrations of CBD and its metabolites, 7-hydroxy cannabidiol (7-COH CBD) and 7-carboxy cannabidiol (7-COOH CBD), as previously described.\(^13\)

**Pharmacokinetic and bioavailability calculations**

Noncompartmental analysis was performed as described previously.\(^13\) Briefly, plasma CBD and metabolite concentrations were analyzed using commercially available software (Phoenix WinNonlin Version 8.1; Certara).\(^13\) Bioavailability was calculated for the 4 horses receiving both IV and oral doses using the following equation:

\[
\frac{\text{AUC}_{0-\infty \text{Oral}} \times \text{Dose IV}}{\text{AUC}_{0-\infty \text{IV}} \times \text{Dose Oral}} \times 100
\]

where AUC\(_{0-\infty}\) is the area under the concentration vs time curve from time 0 to infinity and calculated using the linear up log down trapezoidal rule. Dose is the actual oral or IV dose that was used as defined previously.

**Statistical analysis**

Statistical analyses were completed using commercially available statistical software (SAS version 9.4; SAS Institute Inc). Paired t tests with horse as the fixed effect were used to test the effect of CBD on CBC and basic serum biochemical analyses for samples obtained immediately before CBD administration at time 0 and at 24 hours after CBD administration. Normality of data were assessed using Q-Q plots. Nonnormally distributed data were log transformed to adjust for normality and significance for all horses was defined as \(P < .05\).

**Results**

All of the horses used in this study had no adverse effects from the doses of CBD either orally or IV administered. Data for all horses dosed with IV CBD (0.1 mg/kg) were log transformed to adjust for normality, and data from all horses orally dosed (2 mg/kg) were normally distributed as reported. For pharmacology analysis, all plasma samples were sent to the same laboratory for analysis by LC-MS/MS. The assay’s accuracy and precision were determined through analyzing the quality control samples in replicates (\(n = 6\)). Accuracy was stated as a percent nominal concentration and precision as a percent relative SD (Table 1). The limit of quantitation (LOQ) was 0.1 ng/mL for all analytes, and the limit of detection (LOD) was approximately 0.05 ng/mL for all analytes.

**Plasma concentrations**

Plasma concentrations of 7-COH CBD were much lower overall for all the time points in both oral and IV administration groups. For oral administration, 7-COH CBD concentrations at 24 hours after dosing were under the LOQ for 7 horses, and by 48 hours after dosing, 7-COH CBD concentrations were under the LOQ for all horses. Following IV administration, 7-COH CBD concentrations in plasma were below LOQ for 1 of the 4 horses. By 8 hours after IV administration, CBD concentration was below LOQ for all horses.

Plasma concentrations of 7-OH CBD were much lower overall for all the time points in both oral and IV administration groups. For oral administration, 7-OH CBD concentrations at 24 hours after dosing were under the LOQ for 7 horses, and by 48 hours after dosing, 7-OH CBD concentrations were under the LOQ for all horses. Following IV administration, 7-OH CBD concentrations in plasma were below LOQ for 1 of the 4 horses. By 8 hours after IV administration, CBD concentration was below LOQ for all horses.

**Bioavailability calculations**

Bioavailability was calculated for all horses as described previously. CBD, 7-COOH CBD, and 7-OH CBD were detected in the plasma after CBD administration with 7-COOH CBD being the predominant metabolite (Figure 1). Plasma concentrations of the most predominant metabolite 7-COOH CBD were detectable throughout all of the collection time points presented in this study. Concentrations of CBD were nondetectable for 2 of the horses by 24 hours after oral administration. At 48 hours after oral administrations, concentration of CBD was nondetectable for half of the horses. By 72 hours after oral administration, plasma CBD concentration was either below the LOQ of the assay or nondetectable in all horses. Concentrations of CBD were below LOQ by 24 hours after IV administration for half of the horses. By 48 hours after IV administration, CBD concentration was below LOQ for all horses.

**Pharmacokinetic parameters**

Plasma concentrations of 7-OH CBD were much lower overall for all the time points in both oral and IV administration groups. For oral administration, 7-OH CBD concentrations at 24 hours after dosing were under the LOQ for 7 horses, and by 48 hours after dosing, 7-OH CBD concentrations were under the LOQ for all horses. Following IV administration, 7-OH CBD concentrations in plasma were below LOQ for 1 of the 4 horses. By 8 hours after IV administration, CBD concentration was below LOQ for all horses.

The approximate mean half-life (\(t_{1/2}\)) CBD was 7.88 ± 2.86 hours for oral administration (2 mg/kg) or 8.55 ± 2.19 hours for IV administration (0.1 mg/kg), and the mean time to reach maximum concentration (\(T_{\text{max}}\)) was 1.62 ± 0.21 hours after oral administration (Tables 2 and 3). For both the oral and IV administration, the metabolite 7-COOH CBD did not fall below the LOQ at any of the time points reported. Mean ± SD oral bioavailability (F) for CBD administration in senior horses was 7.2% ± 0.5%

There were no meaningful differences in results of any of the CBC or serum biochemical analyses.

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**Table 1—Accuracy and precision values for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of cannabidiol (CBD), 7-carboxy cannabidiol (7-COOH CBD), and 7-hydroxy cannabidiol (7-OH CBD) concentrations in horse plasma (n = 6/concentration).**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration (ng/mL)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBD</td>
<td>0.3</td>
<td>8</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>104</td>
</tr>
<tr>
<td>7-OH-CBD</td>
<td>0.3</td>
<td>6</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>9</td>
<td>105</td>
</tr>
<tr>
<td>7-COOH-CBD</td>
<td>0.3</td>
<td>4</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>5</td>
<td>98</td>
</tr>
</tbody>
</table>

Samples were tested in replicates.
Table 2—Mean ± SD pharmacokinetic parameters for CBD and 7-COOH CBD following a single oral administration of CBD (2 mg/kg) to 12 university-owned, healthy, senior horses during a randomized unbalanced crossover study that had a 2-week washout period between treatments (2 treatment periods, each with 6 horses receiving oral dosing and 2 horses receiving IV dosing) conducted from August 10 to September 4, 2020.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CBD</th>
<th>7-COOH CBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC extrap (%)</td>
<td>8.14 ± 7.52</td>
<td>0.53 ± 1.04</td>
</tr>
<tr>
<td>AUC_{0-\infty} (h·ng/mL)</td>
<td>132.44 ± 64.21</td>
<td>11,500 ± 6,609</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>18.54 ± 9.80</td>
<td>307 ± 186</td>
</tr>
<tr>
<td>t_{1/2\lambda} (h)</td>
<td>7.22 ± 2.86</td>
<td>43.10 ± 16.10</td>
</tr>
<tr>
<td>λ_{z} (1/h)</td>
<td>0.130 ± 0.10</td>
<td>0.017 ± 0.00</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>2.46 ± 1.62</td>
<td>5.08 ± 2.31</td>
</tr>
<tr>
<td>F (%)</td>
<td>7.92 ± 2.85</td>
<td>—</td>
</tr>
</tbody>
</table>

All values were generated using noncompartmental analysis. AUC extrap = Percentage of area under the concentration-versus-time curve (AUC) extrapolated. AUC_{0-\infty} = Area under the plasma-concentration curve from time 0 to infinity. C_{max} = Maximum concentration. F = Oral bioavailability. t_{1/2\lambda} = Terminal half-life. λ_{z} = Terminal slope. T_{max} = Time to maximum concentration. — = Not calculated.

Table 3—Mean ± SD pharmacokinetic parameters for CBD and 7-COOH CBD following a single IV administration of CBD (0.1 mg/kg) to 4 university-owned, healthy, senior horses.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CBD (n = 3)</th>
<th>7-COOH CBD (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC extrap (%)</td>
<td>4.74 ± 2.47</td>
<td>1.46 ± 0.77</td>
</tr>
<tr>
<td>AUC_{0-\infty} (h·ng/mL)</td>
<td>57.6 ± 18.19</td>
<td>2,000.3 ± 2,422.2</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>—</td>
<td>72.6 ± 98.20</td>
</tr>
<tr>
<td>t_{1/2\lambda} (h)</td>
<td>3.15 ± 2.19</td>
<td>67.5 ± 10.8</td>
</tr>
<tr>
<td>λ_{z} (1/h)</td>
<td>0.29 ± 0.15</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Vd_{area} (L/kg)</td>
<td>—</td>
<td>72.6 ± 98.20</td>
</tr>
<tr>
<td>C_{max} (ng/mL)</td>
<td>—</td>
<td>3.5 ± 3.3</td>
</tr>
</tbody>
</table>

All values were generated using noncompartmental analysis. Vd_{area} = Volume of distribution calculated using the AUC method. λ_{z} = Volume of distribution at steady state. See Table 2 for the rest of the key.
Table 4—Mean ± SD serum biochemical analyses and CBC results for all 8 horses immediately before and 24 hours after receiving their assigned CBD treatments (time 0) described in Tables 2 and 3, stratified by treatment group (2 mg/kg, PO [n = 12]; or 0.1 mg/kg, IV [4]).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral Time 0</th>
<th>Oral Time 24</th>
<th>IV Time 0</th>
<th>IV Time 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>3.2 ± 0.3</td>
<td>3.3 ± 0.3</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>239.5 ± 33.3</td>
<td>241.1 ± 27.1</td>
<td>251.3 ± 30.2</td>
<td>266.3 ± 28.7</td>
</tr>
<tr>
<td>Alkaline phosphate (U/L)</td>
<td>129.6 ± 33.0</td>
<td>135.5 ± 34.4</td>
<td>137.8 ± 19.7</td>
<td>140.8 ± 22.5</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>84.7 ± 8.8</td>
<td>94.0 ± 10.9</td>
<td>88.8 ± 13.9</td>
<td>100.0 ± 17.8</td>
</tr>
<tr>
<td>γ-Glutamyl transferase (U/L)</td>
<td>17.3 ± 7.6</td>
<td>17.5 ± 7.7</td>
<td>18.3 ± 8.3</td>
<td>17.8 ± 7.5</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.5 ± 1.2</td>
<td>11.7 ± 1.5</td>
<td>12.1 ± 1.2</td>
<td>11.8 ± 0.5</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>34.6 ± 3.6</td>
<td>35.7 ± 3.9</td>
<td>36.0 ± 3.4</td>
<td>35.7 ± 1.8</td>
</tr>
<tr>
<td>RBCs (X 10⁶ cells/µL)</td>
<td>6.7 ± 0.7</td>
<td>6.8 ± 0.8</td>
<td>6.9 ± 0.2</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>WBCs (X 10³ cells/µL)</td>
<td>7.7 ± 1.7</td>
<td>8.4 ± 1.7</td>
<td>7.9 ± 1.7</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.43 ± 0.29</td>
<td>7.5 ± 0.6</td>
<td>7.5 ± 0.2</td>
<td>7.6 ± 0.1</td>
</tr>
</tbody>
</table>

measures prior to or 24 hours after oral or IV administration of CBD (Table 4). There were no meaningful effects of CBD dosing on any of the vital signs measured (rectal temperature, pulse, and respiratory rate) at 30 minutes and 1 hour after administration (data not shown).

Discussion

The objective of the present study was to describe the pharmacokinetics, bioavailability, and pharmacological effects of CBD in senior horses. After oral or IV administration of CBD, the CBD parent concentrations were shown at low levels 30 minutes after administration with a peak at 4 hours after administration. Similar findings were reported in young exercising Thoroughbreds at the same oral dose (2 mg/kg). The t₁/₂ in the present study was shorter at 7.22 ± 2.86 hours for oral dosing of CBD at 2 mg/kg than what was previously reported in young horses (10 hours). The mean t₁/₂ was much lower for the IV dose (3.15 ± 2.19 hours) versus the oral dose (7.22 ± 2.86 hours). One possible explanation for this difference was the way in which the compound is formulated. For IV administration, CBD was formulated in DMSO, whereas the oral formulation was in oil. Delivery of oral CBD in an oily formulation can lead to a phenomenon termed flip-flop kinetics, whereby the rate of absorption is substantially slower than the rate of elimination due to slow release of the drug from the vehicle. In this case, t₁/₂ is affected by the process of absorption, whereas with the IV formulation, the slope is primarily determined by the rate of elimination. This ultimately leads to differences in the t₁/₂ between routes of administration. The carrier for the oral CBD formulation used in the current study was different from previous studies, in which sesame or olive oils were used. Cannabidiol is a very lipophilic molecule, and when combined with fats or a highly fatty diet absorption is improved in animal and human studies. In the presently reported study, a soy oil that was a typical part of the horses daily feeding routine was used as the carrier as it is known to be very palatable for these horses. The horses used here were not fasted during this study as they were in their respective paddocks with grass ad libitum. Feed was withheld immediately following administration of the CBD for 30 minutes. In a recent pharmacology study, a pelleted CBD product was used, top dressed over the horses feed and monitored extensively to make sure it was consumed. The delivery method for CBD is still under investigation to determine the best formulation for optimal absorption in horses.

Cannabidiol is extensively metabolized in the liver and has been studied extensively in other species as well as in vitro. Although these species all produce metabolic profiles with similar metabolites, they vary with how they metabolize CBD. Cannabino- noid molecules have multiple sites for hydroxylation and carboxylation to occur; however, these reactions are carried out by cytochrome P450 enzymes differently in each species. The major metabolites that were investigated in this study 7-OH CBD and 7-COOH CBD were identified in humans and have a low prevalence in other species. In previous studies, concentrations of 7-OH CBD were extremely low from the initial dosing and were quickly below the lower LOD for most horses, within the first 24 hours following both oral or IV dosing. However, 7-COOH CBD was the predominant metabolite in horses and in this study was determined to be present in the blood plasma 11 days after administration of CBD. With little research having been completed at this time with CBD and horses, complete elimination of CBD and its respective metabolites is only partly defined. Results of the present study indicated that 24 hours after administration of CBD (2 mg/kg, PO; or 0.1 mg/kg, IV), plasma CBD was below the detection limit. Furthermore, even 264 hours after such a single dose, the 7-COOH CBD metabolite is still above detection limits in senior horses. More research with additional collection time points is needed to define an absolute clearance of CBD and its metabolites in horses.

To our knowledge, the present report was the first to determine absolute oral bioavailability in horses, in particular senior horses. The bioavailability (F) of 7.92% ± 2.85 in the present study was comparable to the 13% to 19% oral bioavailability reported...
in dogs.\textsuperscript{10} The lower bioavailability in horses of the present study could have been due to the first-pass metabolism. Cannabidiol has a very high extraction ratio in dogs.\textsuperscript{10} If the same is true in horses, low concentrations of CBD following oral administration may be a result of extensive first-pass metabolism. It is also important to note that in the current study, only a small number of horses were administered the IV formulation of CBD and therefore the bioavailability reported represented that for just 4 horses. However, using few animals when measuring bioavailability is oftentimes not uncommon.\textsuperscript{10,32,33} As therapeutic concentrations of CBD in horses are as of yet unknown, the low bioavailability does not necessarily equate to poor efficacy. Low bioavailability of CBD in horses could be affected by absorption time due to the amount of forage in the gastrointestinal tract, pH variations in the gastrointestinal tract, absorptive surface areas, protein binding, and enzymes specific to the equine digestive tract due to them being hindgut fermenters.\textsuperscript{34–36} Another potential explanation for differences in kinetic absorption is that horses have different enzymes and protein binding that could have interfered. A protein binding study may be beneficial to be conducted with oral CBD, where it can be determined whether CBD is being bound by the enzymes in senior horses and maybe the free CBD is the only portion being absorbed. More research with CBD and protein binding in horses should be investigated in future studies. There may also be an impact of age on bioavailability.\textsuperscript{37,38} More research is warranted to further our understanding of the effects of CBD on health and determine therapeutic concentrations. Studies in human medicine and small animals have investigated alternative dosing routes.\textsuperscript{7,13,39} and different formulations of CBD,\textsuperscript{26} encapsulated by liposomes to help improve bioavailability.\textsuperscript{11,40} Modifying the mode of delivery and CBD formulations could be future areas of research for horses.

After administering CBD as a single oral dose (2 mg/kg) or a single IV dose (0.1 mg/kg), there were no observable adverse effects in the horses based on results of CBC, basic serum biochemical analyses, and measurements of heart rate, respiratory rate, and rectal temperature. It has been reported in dogs that ALP, AST, and SGOT enzymes have been slightly elevated\textsuperscript{26} possibly due to the first-pass metabolism in the liver.\textsuperscript{32} However, monitoring of serum biochemical analyses before and after the single doses administered to the horses of the present study did not show any elevation in prominent liver enzymes such as ALP, AST, and SGOT.

Overall, the present study indicated the oral half-life of CBD to be 7.22 ± 2.86 hours and the oral bioavailability of CBD to be 7.92% ± 2.85%, following the administration of a 2 mg/kg CBD dose in horses. The source of drug was distillate oil CBD, suspended in soy oil and was well tolerated by all horses. Furthermore, there were no adverse effects of administering either an oral dose or an IV dose of CBD to the horses. Future studies are warranted to determine the efficacy of CBD on health parameters in horses.

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### References


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