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Pharmacokinetics of a Single Feeding of Pelleted Cannabidiol in Horses

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Cover Page Footnote
I would like to extend my gratitude to all that made this project possible. There was an unparalleled level of support and guidance throughout. Thanks to Dr. Tony Hicks and Equine Veterinary Services for the donation of time and product. I also extend my thanks to Dr. Robert Silver of Folium Biosciences, Dr. Daniel Gustafson of the CSU Cancer Center Pharmacology Core, and Dr. Travis Mays of TVMDL for your collaboration and shared knowledge regarding CBD analysis. The incredible support of my advisor and mentor Dr. Shea Porr has been invaluable during the entirety of the project, providing the support and encouragement to ensure success. Additional thanks goes out to Dr. Amanda Davis, Dr. Laura Hoffman, Pat Godwin, and the wonderful staff and students of the Murray State Equine Center.

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About the Author

Anna Draeger, MSc in Agriculture, Class of 2020

My decision to return to Murray State for my master's was simple thanks to my positive undergraduate experience. During that time, I was given the opportunity to participate in research and attend my first conference. After some time spent in the industry training Icelandic Horses and providing riding lessons, I discovered that teaching was a career path I wanted to pursue. This research project began with the investigation of foundational knowledge of CBD in the horse and worked towards evaluating its impact on both behavior and movement. Not only have I gained valuable knowledge and understanding of the subject matter and research process, but also I have thoroughly enjoyed experiencing the collaboration and teamwork essential to success. Following graduation, I hope to earn a lecturer position within an equine science program.

C.A. Shea Porr, PAS, Ph.D., Associate Professor

C.A. Shea Porr, PAS, Ph.D. is an Associate Professor and the Department Head of Murray State University’s Animal/Equine Science Department, Dr. Porr teaches courses and advises undergraduate and master's students, guides the animal/equine science program, and oversees the farms and facilities. She is also the Racer 1 Faculty Coordinator and a co-advisor of the MSU Horsemens’s Association. Dr. Porr received degrees from Texas A&M University, the University of Florida, and Virginia Tech. Her research focus in graduate school was on nutrition and exercise effects on bone development in horses. She has held several positions in both academia and industry, including working for the Ohio State University, Buckeye Nutrition, and Virginia Tech before joining Murray State University. In addition to her other duties, Dr. Porr supports the horse industry through presentations at various professional and industry meetings. While her research topics vary, she is particularly interested in education in emergency response and preparedness with horses in mind.
Abstract

Claims about cannabidiol (CBD) supplementation improving health and behavior are extensive, but research is lacking. Some studies have shown decreased anxiety behavior in rats, and increased activity in osteoarthritic dogs supplemented with CBD, but even less research exists on horses. This study monitored pharmacokinetics and short-term safety for 3 CBD dosages. Eighteen Quarter Horse geldings were randomly assigned to 3 treatment groups: 50 mg (TXT1), 100 mg (TXT2), and 250 mg (TXT3). Dosage was derived from manufacturer recommendations and existing literature on other species. Horses were fed a single dose of CBD pellets. Blood was collected pre- and post-treatment at 0.5, 1, 2, 4 and 12 hr. Serum was analyzed for CBD and serum chemistry, and plasma was analyzed for a complete blood chemistry (CBC) evaluation. Statistics were completed on serum chemistry using PROC MIXED procedure of SAS. Serum chemistry and CBC results were within normal parameters; however, treatment differences were observed for BUN (TXT1=15.50, TXT2=16.52, TXT3=18.61; P≤0.03) and creatinine (TXT1=1.41, TXT2=1.22, TXT3=1.49; P≤0.01). In other species, peak CBD concentrations occur approximately 2 hr post treatment. Peak serum concentrations were detected in 1 of 6 TXT2 horses and 5 of 6 TXT3 horses at 2 hr post treatment. This data can be used to support further research to determine correct and safe doses of CBD in horses.

Keywords: Pharmacokinetics, Cannabidiol, Equine
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Introduction

*Cannabis sativa* L. has piqued the interest of both medical- and industry-based research (Callaway, 2004; Zuardi, 2006; Russo, 2007; Hartsel et al., 2016). The recent passing of the 2014 and 2018 Farm Bill Acts served to legally separate hemp from marijuana, requiring the concentration of the psychoactive component, Δ-9 tetrahydrocannabinol, to be less than 0.3% (Sec. 297A Federal Farm Bill Act, 2018). Cannabinoids derived from the hemp plant, such as cannabidiol (CBD), are classified as lipid-signaling molecules that bind to and interact with appropriate receptors (Freund et al., 2003). While research has been directed towards CBD as a physical and behavioral therapeutic agent in mice (Malfait et al., 2000), rats (Hammell et al., 2016; Philpott et al., 2017), felines (Ellis & Contino, 2019), canines (Gamble et al., 2018), and humans (Wade et al., 2004), efforts specific to the equine species is lacking.

All vertebrates have an endocannabinoid system that serves as the master regulator of bodily homeostasis (McPartland et al., 2005; De Laurentiis, 2014; Sallaberry and Astern, 2018; Hartsel et al., 2019). Dependent on the interaction of cannabinoids, regulatory enzymes, and receptors, the system functions through a negative feedback loop (Pertwee, 2006; Mackie, 2008). There are three primary classifications of cannabinoids, including endocannabinoids, phytocannabinoids, and synthetics (Scuderi et al., 2009). Endocannabinoids naturally exist in the body, phytocannabinoids derive from the plant *C. sativa*, and synthetic cannabinoids are purified isolates with increased potency. Both phytocannabinoids and synthetic cannabinoids mimic natural endocannabinoid effects and may impact receptor interaction (Landa et al., 2016). Two primary receptors identified include cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). Primarily, CB1 receptors are located within the central nervous system, while CB2 receptors are associated with the immune system and cytokine release (Howlett et al., 2002; Pertwee, 2006). Existing research supports this system’s role in a wide range of central nervous system and endocrine functions. Specific roles include immune system and inflammatory responses, influence on heart rate and blood pressure, inhibition of tumor cell growth, nociception modulation, and influence on reproductive function (Di Marzo et al., 1998; Kogan et al., 2004).

Medicinal Claims

Proposed medicinal benefits of CBD range from physical healing to behavioral modification. Specific conditions marketed for humans include epilepsy, multiple sclerosis, anxiety, rheumatoid arthritis, inflammation, and anxiety disorders. Relative to horses specifically, behavioral modification seems to be a popular focus for CBD use. Despite lay claims from owners and supplement companies, species specific pharmacological understanding of CBD and other cannabinoids is relatively low. Factors such as receptor location and absorption rates within certain species may vary, and could potentially increase the risk of overexposure (Hartsel et al., 2019).
Detection techniques are still advancing, particularly in reference to cannabinoid detection in equine serum. A pilot study demonstrated that both pelleted and oil-based CBD products appear to be bioavailable to the horse; however, detection concentrations at the current dosage recommendations are relatively low and difficult to detect (Jones et al., 2020). One of the current methods of serum cannabinoid detection is through the use of liquid chromatography-mass spectrometry (LC/MS) (Gamble et al., 2018; Davis, 2019; Deabold et al., 2019). In this method, physical separation must occur first through dissolving the CBD-containing serum sample in a solution, followed by mass analysis. In LC-MS/MS instruments, there is a second mass spectrometry detector attached. Though a standard for testing has not yet been implemented due to lack of regulation, some of the most sensitive HPLC-LC/MS methods are reading serum cannabinoid concentrations down to a 0.05 ng/mL lower limit of quantification (LLOQ). However, LLOQ for research reports has typically been above 1 ng/mL (Davis, 2019; Deabold et al., 2019). Product analysis may occur through both LC-MS/MS and high performance liquid chromatography diode-array detectors (HPLC-DAD). Following physical separation, DAD allows researchers to examine samples for organic compounds including CBD based on wavelengths (Hädener et al., 2019). Though sensitivity can be dependent on the metabolite being analyzed and the specific method used, LC-MS/MS is considered to contain greater sensitivity and accuracy (Theodoridis et al., 2013).

Most medical based studies on CBD have been completed in murine models, dogs, and humans (Malfait et al., 2000; Lodzki et al., 2003; Blake et al., 2006; Jeong et al., 2014; Hammell et al., 2016; Devinsky et al., 2018). Current literature demonstrates potential for various administration methods of CBD to contain medicinal capabilities within controlled research settings (Lodzki et al., 2003; Blake et al., 2006; Scuderi et al., 2009; Devinsky et al., 2018). However, a product under consideration for pharmaceutical qualities must undergo species and dose-specific trials in order to determine the safety and efficacy for each labeled use. It is possible that other components within CBD products could be the true catalyst for improved health (Simopoulos et al., 2002). Determination of potential positive and negative consequences of CBD medicinal use, if any, is a necessary step for consideration as a form of treatment. Research is particularly needed in regards to the effects of oral and transdermal doses within horses.

Pharmacokinetic research completed in other species typically demonstrates a blood concentration maximum between 1-2 hours (Tmax), and a half-life of 4 to 4.5 hours (Martin-Santos et al., 2012; Gamble et al., 2018). However, some species, such as cats, have demonstrated a Tmax as long as 4 hours (Deabold et al., 2019). A deeper understanding of pharmacokinetic measures such as this could assist in providing the proper dosing amount and frequency. A case study on a horse suffering from neuropathic pain demonstrated alleviation of pain with an oral dose of 0.5 mg/kg bodyweight twice daily for 2 months without reported
negative consequences (Ellis and Contino, 2019). To avoid symptom recurrence, the horse was required to remain on a lower maintenance dose. Dosing was based loosely on human medicine. Though it turned out to be safe, the author reaffirmed the need for research to better understand drug interactions and dose guidelines. While negative effects were not observed in this study, blood sample results were not reported. Data on other species treated with CBD has demonstrated a rise in certain enzymes, such as alkaline phosphatase (ALP) (Gamble et al., 2018; Deabold et al., 2019).

Despite the lack of supporting research, the use of CBD products in humans and animals has grown rapidly. The primary intent of this project was to evaluate the properties of oral CBD products in horses to increase understanding of product availability, pharmacokinetics of CBD in the horse, and the physiological results of a single treatment. Ultimately, this project provided foundational material for equine CBD dosage and use recommendations for the safety and welfare of the animal.

**Methods**

The Murray State University Institutional Animal Care and Use Committee approved the research protocol submitted for this study.

**Animals & Management**

Eighteen Quarter Horse geldings from a university riding program were selected. Weekly exercise demand was categorized as low to moderate for all horses (1 h/wk, 3 h/wk, respectively; National Research Council, 2007). Average age was 15±4.2 yrs, and horses had an average weight of 555±40.9 kg. Groups were balanced by typical housing, stalled (n=7) versus pasture kept horses (n=11), and age. Young horses were considered ≤14 yrs (n=10) and aged ≥15 yrs (n=8). Subsequently, subjects were randomly divided into one of three treatment groups (n=6): 50 mg (TXT1), 100 mg (TXT2), or 250 mg (TXT3). Manufacturer dosing recommendations were 25-50 mg 1-2 times daily. In a pilot trial, the 50 mg dose did not reach sufficient serum concentrations at 2 h to guarantee consistent detection for pharmacokinetic analysis. A high dose of 250 mg was derived from a case study that demonstrated efficacy and safety for pain management in a horse with extended treatment (Ellis and Contino, 2019). Horses were stalled for the duration of the study (total time approximately 30 hr), and fed their standard rations of bermudagrass hay and concentrate with free choice water. Subjects known to have negative behavioral reactions associated with being stalled were avoided. Concentrate included a once daily feeding of Southern States® (Southern States, Cadiz, KY) HSS Reliance 12% protein, 6% fat bulk horse pellets (n=15) or Kalm’N EZ® (Tribute Equine Nutrition®, Upper Sandusky, OH), (n=3). Rations included the following, depending on the individual’s needs: 4 quarts (n=15), 2 quarts (n=1), or 6 quarts (n=2). Horses were handwalked regularly, during stall cleanings. One test subject was on a Vitamin E supplement. Intravenous jugular catheters were placed by a veterinarian within 2 hr of stall confinement. Following study conclusion, catheters were removed and horses were observed for 12 hr for signs of adverse reactions prior to their return to standard housing.
Data Collected

Blood collection for each horse consisted of four 10 ml serum Vacutainers® for cannabinoid analysis and serum chemistry, as well as one 10 ml EDTA Vacutainer® for a complete blood count (CBC) (Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ). The first blood collection (pre-treatment) allowed subjects to serve as their own control.

Within 30 min of the pre-treatment collection (0 h), all horses were administered a single dose of full-spectrum, ground and pelleted CBD from industrial hemp (Equine Veterinary Services Pharm, Paducah, KY, USA) in accordance with their treatment group. Other ingredients mixed into the pelleted product included the flavoring agent, Apple Ade (Nutriad, Inc, Hampshire, IL, USA) and mold inhibitor, Myo CURB® (Kemin Industries, Inc.,©, Verona, MO, US). Dosages were based on manufacturer recommendations and research available on other species. Subsequent catheter drawn cannabinoid samples were taken at 0.5, 1, 2, 4, and 12 hr post treatment. Additionally, a second sample for CBC was taken at 12 hr post treatment. Subsequent serum chemistry collections occurred at 4 hr and 4 d post treatment.

Serum samples were centrifuged at 3500 RPM (LWS-Combo-V24 Centrifuge LW Scientific, Atlanta, GA). A minimum of 5 cc of serum was pipetted into 5 mL Eppendorf storage tubes (Eppendorf Tubes® 0030119452, online-shop.eppendorf.us, USA) and chilled in a refrigerator at 4.4º C until serum chemistry analysis. Comparative lab analysis through accredited labs was incorporated to validate blood concentrations and testing procedures. Serum chemistry and CBC samples were evaluated at the Breathitt Veterinary Center (Murray State University Breathitt Veterinary Center, Hopkinsville, KY, USA). Cannabinoid samples were frozen in a -20º C freezer until shipment for analysis. Pharmacokinetics were evaluated in order to determine the effect of a single dose. Serum cannabinoid potency was analyzed by the Pharmacology Shared Resource (University of Colorado Cancer Center) at Colorado State University (P30 CA046934) using liquid chromatography-mass spectrometry (LC-MS/MS) technology. An Agilent 1200 Series Binary Pump SL HPLC system was coupled to a 3200 Q-TRAP triple quadruple mass spectrometer (Applied Biosystems, Inc., Foster City, CA). Each lab is an accredited institution.

Analysis

Statistical analysis for serum chemistry was completed through PROC MIXED Procedure of SAS (SAS, Cary, NC, USA). The experimental unit was horse, with each serving as its own control. Results were analyzed under the effects of treatment and time. Dependent variables included blood urea nitrogen (BUN), albumin, creatinine, and alkaline phosphatase (ALP) to monitor kidney and liver function per dose rate. Statistical significance was defined as $P \leq 0.05$ while tendencies were identified as $P \leq 0.10$.

Results and Discussion

No negative behaviors or effects were observed in any horse during this project.
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Serum CBD and complete blood count

Results suggested that the time of CBD concentration maximum in the equine species was 2 hr, which is similar to what has been documented in other species (Table 1; Gamble et al., 2018; Deabold et al., 2019). Only horses administered the 250 mg dose showed serum concentrations at or above the lower limit of quantification (LLOQ) of 1 ng/ml, with the exception of one horse from the 100 mg treatment group. One out of six horses on the 250 mg dose did not reach LLOQ detection levels. This horse consumed only 120 mg of the 250 mg dose. Despite a portion of the horses (n=6) reaching a quantifiable CBD serum concentration, all levels remained quite low (Table 1). Additionally, dosing was not adjusted per bodyweight, though horses were of comparable breed type and contained limited variability in weight (mean=555±40.9 kg). Maintaining consistent CBD presence above LLOQ via more efficient and higher dosing by bodyweight could assist in achieving a heightened understanding of CBD pharmacokinetics. Results from CBC analysis returned within normal parameters.

Table 1. Equine CBD serum concentrations that reached standard LLOQ.

<table>
<thead>
<tr>
<th>Horse ID</th>
<th>CMax (ng/mL)</th>
<th>Tmax (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.87</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>2.17</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>2.9</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>1.74</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>6.9</td>
<td>2</td>
</tr>
<tr>
<td>18*</td>
<td>3.66</td>
<td>2</td>
</tr>
</tbody>
</table>

Note. Asterisk indicates single horse from TXT2.

Data for some species, including horses, suggests Tmax could extend beyond 2 hr (Davis, 2019). One explanation for this discrepancy could be that the current study had gaps within blood collection times where a maximum could have occurred without opportunity for detection. Although concentrations dropped at the subsequent 4 hr collection, it is not possible to know if those concentrations continued to rise because samples were not collected between 2 and 4 hr post treatment. It is also possible that the CBD bioavailability depends on the method of administration (Figure 1). In a trial using oral CBD oil (Davis, 2019), horses were administered a lower dose rate (0.10 mg/kg) than the maximum dose of a pelleted product (0.22 mg/kg) used in this study. While Tmax was achieved more quickly in this trial, the actual maximum concentration (Cmax) geometric mean observed in Davis’ study was higher (27.2 ng/mL versus 3.04 ng/mL, respectively). Therefore, although the amount administered was less, and it potentially took longer to absorb, a greater percentage of the product was ultimately absorbed. This is likely attributable to the first pass effect at the liver and highly variable bioavailability observed with transmucosal absorption of oral oil products (Hammel et al., 2003; Lodzki et al., 2003; Davis, 2019).

Figure 1. Comparison of the geometric mean for CBD concentrations detected in the blood and time that the maximum concentration was reached between a pelleted and oil oral CBD product in horses.
**Serum Chemistry**

Though serum chemistry results indicated all concentrations were within reference ranges, significant changes within those ranges were observed (Figure 2, Figure 3, Figure 4). A relationship summary of concentrations based on treatment is provided in Table 2.

![Figure 2. Serum concentrations for blood urea nitrogen (BUN) based on treatment group. Data lines represent reference ranges for BUN (10-25 mg/dL).](image1)

![Figure 3. Serum concentrations for creatinine based on treatment group. Data lines represent reference ranges for creatinine (0.45-1.8 mg/dL).](image2)

![Figure 4. Serum concentrations for albumin based on treatment group. Data lines represent reference ranges for albumin (2.4-3.8 g/dL).](image3)

Table 2. Enzyme concentration differences based on treatment in horses fed a single dose of cannabidiol pellets.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>50mg</th>
<th>100mg</th>
<th>250mg</th>
<th>P value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>15.50</td>
<td>16.52</td>
<td>18.61</td>
<td>≤0.03</td>
<td>0.66</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.41</td>
<td>1.22</td>
<td>1.49</td>
<td>≤0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>ALP</td>
<td>101.56</td>
<td>100.44</td>
<td>114.22</td>
<td>0.12</td>
<td>5.20</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.41</td>
<td>3.35</td>
<td>3.24</td>
<td>0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: Different letters within the same row differ by P ≤ 0.05. Different symbols within the same row indicate tendencies that differ by P ≤ 0.10. Units are as follows: BUN (mg/dL), creatinine (mg/dL), ALP (IU/L), albumin (g/dL).

Observations pertaining to the impact of time on enzyme concentrations are summarized in Table 3. Though changes were seen based on treatment and time for some measures, each horse ultimately remained well within reference ranges. A low albumin concentration and high ALP could indicate poor liver function. Observations from this study contained limited concentration shifts, with all being within expected reference ranges. Typically, signs of kidney dysfunction would demonstrate a concurrent rise of BUN and creatinine. Within the documented ranges, BUN consistently increased with treatment concentration and progression of time, but creatinine did not (Figure 5). While there could be physiological impacts from CBD treatments that stimulated concentration fluctuation, such changes did not reach concentrations of concern. There can be alternative factors that produce concentration changes, such as high protein diets or dehydration. Given the subjects of this study were maintained on bermudagrass hay with free choice water, these particular effects are unlikely. Furthermore, both brands of concentrate offered contained ≤14% crude protein and remained consistent with what the horse had been receiving prior to the start of the investigation. Ideally, all horses on the study would have received a consistent diet. However, the focus of this study was to monitor the safety of a single dose and
CBD uptake, not nutritional value or efficacy. Future studies concerning CBD efficacy should be attentive to factors such as differences in diet, age and use to avoid inaccurately attributing treatment differences to CBD alone. Ultimately, the single treatment of each dose represented in this study appeared to produce minimal changes in blood chemistry concentrations. However, as slight variations were observed, it would be appropriate to monitor such parameters with extended CBD treatment.

Table 3. Enzyme concentration differences based on time in horses fed a single dose of cannabidiol pellets.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Pre</th>
<th>4 hours</th>
<th>4 days</th>
<th>P value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>15.89</td>
<td>16.00</td>
<td>18.74</td>
<td>&lt;0.01</td>
<td>0.66</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.39a</td>
<td>1.46b</td>
<td>1.34c</td>
<td>≤0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>ALP</td>
<td>104.22</td>
<td>107.06</td>
<td>104.94</td>
<td>0.92</td>
<td>5.20</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.28</td>
<td>3.39</td>
<td>3.34</td>
<td>0.27</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Note: Different letters within the same row differ by P ≤ 0.05. Different symbols within the same row indicate tendencies that differ by P ≤ 0.10. Units are follows BUN (mg/dL), creatinine (mg/dL), ALP (U/L), albumin (g/dL).

Figure 5. Serum concentrations of blood urea nitrogen (BUN) and creatinine based on time of blood collection.

Conclusion

Ultimately, the results suggest relative safety of dosing horses with a single treatment of 50 mg, 100 mg, or 250 mg of an oral pelleted CBD product. Though slight shifts in blood parameters occurred, each remained within normal reference ranges. From the given observations, horses appear relatively consistent with other species in the time of concentration maximum in the blood. Primarily, the only horses reaching above standard LLOQ were in the 250 mg group, and those readings were still fairly low. Therefore, there is the potential that future pharmacokinetic trials will either require higher dose rates for consistent CBD detection, or need to seek more readily absorbed forms. While existing literature contains a base for canines (Gamble et al., 2018), humans (Wade et al., 2004), felines (Deabold et al., 2019), and murine models (Malfait et al., 2000), this project provided a foundation to expand upon concerning the relative safety of CBD and dosing recommendations specifically in the horse. Future research should attempt to fill the time gaps of this project to more accurately represent the pharmacological nature of CBD in the horse.
Referenced Materials


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