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Pharmacokinetics of CBD Supplementation in Horses: Single Dose vs. Long-Term Feeding

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HONORS THESIS

Certificate of Approval

Pharmacokinetics of CBD Supplementation in Horses:

Single Dose vs. Long-Term Feeding

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Pharmacokinetics of CBD Supplementation in Horses:

Single Dose vs. Long-Term Feeding

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Abstract

Research about supplemental feeding of cannabidiol (CBD) to horses is still in its early stages, but many horse owners are feeding CBD supplements for supposed benefits, including behavior and pain management. Cannabinoids work through the endocannabinoid system to modulate nervous and hormonal actions in the body, working to maintain a healthy homeostatic balance. Safety and efficacy have been tested for dosages of up to 500 mg of CBD per day for horses, but further research about the pharmacokinetics based on dosage amount and form of CBD are still needed. The objectives of this study were to evaluate the pharmacokinetics of single dose versus long-term feeding of a pelleted CBD supplement. In Project 1, eighteen Quarter Horse geldings were given a single dose of 50 mg (n=6), 100 mg (n=6), or 250 mg (n=6) of pelleted CBD. Blood samples were collected before treatment (0 hrs), and at 0.5, 1, 2, 4 and 12 hrs post treatment. The highest concentration of CBD in the blood was seen in the 250 mg group at 4 hrs post-treatment. The highest concentration of the metabolite 7-COOH CBD was again seen in the 250 mg group at 4 hrs post-treatment. However, both CBD and 7-COOH CBD concentrations appeared to still be rising at 12 hrs in the 50 mg group while they were decreasing in the other two groups. Project 2 utilized 24 horses (12 CON and 12 TRT), where TRT horses were fed 100 mg of CBD once a day for 8 weeks (wks). Blood samples were collected pre-treatment (0), and at 2, 4, 6 and 8 wks. The highest CBD concentrations were seen at 2 wks, but concentrations decreased at 4 wks before increasing again at 6 wks and remaining elevated at 8 wks. Results from these

projects agree with some publications but differ from others, though no negative side effects were noted in any horse. More research is needed to better understand CBD metabolism and its effect in horses.

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Chapter 1: Introduction

Human and animal supplementation is a growing industry. As consumers learn more about what it takes to live a healthy lifestyle for themselves, owners have started doing it for their companion animals as well. The majority of the reasons why owners are feeding their pets supplements are to increase performance, fix nutrition deficiencies and as a preventative measure for future issues. Cannabidiol (CBD) is a newer supplement that does not have much research done on how it works in the body. So far, it is known to have its own neuroimmune system in humans and many animals called the endocannabinoid system. Cannabidiol attaches to receptors that cause certain effects in the body. For humans, CBD can relieve pain for cancer patients. The anti-inflammatory property has also been proved in dogs with arthritis. The research for understanding how CBD works when fed to horses has been started but there is much more to learn. The purpose of this study is to evaluate the pharmacokinetics of feeding different amounts of CBD over time to horses. Project 1 took eighteen Quarter Horse geldings and randomly assigned them to 3 groups of 6. The first group was fed a single dose of 50 mg of pelleted CBD. Group 2 was fed 100 mg and group 3 was given 250 mg of CBD. Blood was collected pre-treatment (0 hrs), and at 0.5, 1, 2, 4, and 12 hrs post-treatment. The purpose was to determine if there were significant differences seen between differing amounts of CBD fed and at what time peak concentrations happened as well as how long CBD stayed in the blood. Project 1 also tested for one of CBD's metabolites, 7-COOH CBD which is known for having the highest concentrations seen in the blood serum. Project 2 took 24 university horses and randomly assigned them to 2 groups of 12. One was the

control group and the other was given 100 mg of CBD once a day for 8 wks. Blood was collected pre-treatment (0 wks) and at 6 wks for the CON group, while the TRT group had blood drawn every two weeks for the 8 wks. The purpose of Project 2 was to see if CBD concentrations fluctuate or plateau from week to week. Since many organizations that oversee equine competitions have prohibited CBD use, this information is useful for many equine industry groups from veterinarians to owners. Knowing when CBD concentrations peak and how long they remain in the horse's system will help both veterinarians and owners to determine withdrawal times in order to allow the horse to be able to compete legally.

Chapter 2: Review of Related Literature

Introduction

The supplement market, for both humans and the equine industry, is gaining popularity as different types of products are being introduced. Cannabidiol (CBD) products are a newcomer to these markets and have gained a wave of popularity despite their short- and long-term effects being largely undocumented. This review will focus on what it means to be a safe and effective supplement in the animal industry, the potential clinical uses of CBD products, and the beginning of their journey in application to the equine industry.

Supplement Use

Supplements are designed to be added to a diet when there is a certain nutrition lacking in the body. Humans use dietary supplements to not only balance their diet, but also improve athletic performance, for example, increasing stamina before a workout, or mitigating health problems (Swirsley, 2015). Supplements have also been marketed towards companion animal use. Pet supplement sales in 2016 estimated $580 million (Packaged Facts, 2017). Horses are not necessarily considered companion animals like cats and dogs, but the equine industry does use supplements as well. In Swirsley's 2015 survey of horse owners in the U.S., 84% of the respondents fed a supplement to at least one horse. Just like human supplements can be marketed based on what the supplement can do for the body, horse supplements are categorized based on the body condition it is trying to fix, maintain, or prevent. According to Swirsley (2015), supplement categories

reported included joint support (90%), hoof care (83%), to provide nutrients not provided in other feedstuffs (80%), digestive issues other than colic (68%), colic issues (60%), improve performance (50%), improve skin/coat appearance (49%), and treat or prevent behavioral problems (31%). Clearly, horse owners are willing to spend a fair amount of money on supplements to make sure their horse is at a peak performance and nutritional level.

Supplement Efficacy

The efficacy of animal supplements are currently in question. There is little research on whether or not the main ingredients actually help fix or prevent issues in the body. The Food and Drug Administration (FDA) does not heavily regulate companion animal supplements like it does with human supplements since pet supplements do not fall under the Dietary Supplement Health Education Act of 1994. While it is the job of the FDA to strictly regulate foods and drugs, the administration claims it will not investigate the safety or efficacy of supplements for people, pets, or humans until a report from the manufacturers or distributors is made that shows the supplement has adverse effects. In 2001, the National Animal Supplement Council (NASC) was formed to place safety standards on manufacturers to promote the use of safe ingredients. The products get a NASC seal but do not require the companies to verify the product's efficacy with scientific data (Finno, 2021).

At the moment, it is up to the consumer to test the supplement's efficacy by taking the supplement themselves and leaving a review for others online. According to the 2018 survey by the Council for Responsible Nutrition, 87% of U.S adults reported

they were confident in the quality, safety, and effectiveness of the dietary supplements. Further scientific research is needed to analyze the effectiveness of supplements.

Disadvantages of Supplements

Many people who use supplements have the belief that it will relieve or cure an issue, but sometimes too much of a good thing can be bad. Some common adverse effects seen in the emergency room (ER) due to supplements are chest pain, choking, and allergic reactions. It was estimated that over 23,000 ER visits a year were due to adverse effects after taking a supplement (Geller et al., 2015). In people aged 20-34, 71.8% of visits were due to taking supplements related to weight loss and increased energy. Children had incidences of opening supplement containers while unsupervised and people aged 65 and older came in frequently for choking on pills (Geller et al., 2015). In many instances, the consumer may have to try multiple supplements to see which has the best efficacy. Supplement use that results in an ER visit, or those that do not work in general, is money and time wasted. The same concepts can be carried over to horses. One study (Wagner et al., 2008) gave 3 horses a single dose of 0.17 mg/kg of BW of a weight gaining/muscle building cattle supplement called Zilpaterol to see if it would be safe for equines. This drug is approved by the FDA in the United States (U.S.) for feeding to cattle to enhance carcass leanness. Within 40 minutes after the single dose, the horses' heart rates increased by up to 475% from the baseline rate before taking the supplement and the horses were sweating profusely on their necks, shoulders, backs and flanks. Within 90 minutes, the horses had muscle tremors in their necks, shoulders and forelegs. These tremors continued up to one week after the supplement was given. Clearly, not

every horse will have a positive result from taking a supplement so consumers should be mindful that there are possible risks when changing the horse's diet.

Cannabidiol

Cannabidiol works in the body of humans and animals through the endocannabinoid system. This is a system of fatty acid neurotransmitters (Cohen, 2021). There are cannabinoid-based ligands which are endogenous chemically synthesized phytocannabinoids and the endogenous enzymes controlling their concentrations (Kupczyk et al., 2022). Their job is to control neuroimmune homeostasis in the body. The endocannabinoid system has two known cannabinoid receptors, CBR1 and CBR2. These receptors are found throughout the central, peripheral and immune system, synovium, reproductive cells, adipose tissue, lungs, kidneys, liver, and heart in humans (Cohen, 2021). The endocannabinoid system in horses is still being studied. So far, researchers have found the receptors in the dorsal root ganglion through equine genome sequencing analysis and most recently in the ileum and sperm.

Cannabidiol products have made their way into the health and wellness market for humans but are not currently legal in livestock feeds. In 1000 B.C., cannabis was used in India for headaches, anxiety, gastrointestinal problems, asthma, as an anticonvulsant, hypnotic, anesthetic, appetite stimulant, and aphrodisiac (Touw, 1981). Now that modern-day technology can give a better understanding of how the human and animal body works, researchers can go back and test if CBD can actually help with these issues. Cannabidiol is included in the first anti-seizure medication for epilepsy, which was approved by the FDA, called Epidiolex®. Gamble et al. (2018) found that 2mg/kg of

CBD twice a day helped increase comfort and activity in dogs with osteoarthritis, showing that CBD can be used as an anti-inflammatory. The dog's detectable lameness on visual gait assessment and painful to the touch joint palpitations lessened after just 2 weeks. It is also an option to extract fatty g-linoleic acid from cannabis seeds to create a topical cream for eczema (Zuardi, 2006). If CBD has anticonvulsant properties, it could possibly be used to help keep horses from having muscle spasms. Galiazzo et al.'s 2020 research study found that an equine's glial cells in the ileum have CB1R and CB2R found in enterocytes and subepithelial connective tissue of the ileum. This showed that the receptors assist in epithelial wound healing when there is inflammation in the ileum. In human cancer patients, tetrahydrocannabinol (THC) is used to induce glioma cell death (Salazar et al., 2009) which could possibly be transferred over to killing equine cancer cells in the future. Bute and banamine are two heavily used drugs to help with pain but they can cause gastric ulcers if used for chronic cases (Turner, 2020). Cannabidiol could potentially take the place of one or both of these painkillers and not cause stomach ulcers in equids. Lastly, Arroyo-Salvo et al.'s 2021 research found that CBR1 was located on the post-acrosomal region and flagellum of equine sperm while cannabinoid receptor 2 was in the post-acrosomal region and middle piece. This finding can help stallion breeders whose sperm has issues with incomplete in vitro sperm capacitation which is needed to penetrate the zona pellucida.

To be able to do further research on these ideas, farmers would have to be allowed to grow the cannabis plant. Thankfully, due to the 2018 Farm Bill, the production of cannabis sativa L. (hemp) became legal as an agricultural commodity and can be grown in 46 of the 50 states. The cannabis plant can be grown in both temperate and tropical

climates and is a very adaptive and hardy annual plant. It grows for 4-6 months. The reason the cannabis plant used to be on the controlled substance list was due to the fact that it has a psychoactive component, deltahydrocannabidiol (THC). The FDA says the crop product cannot contain more than 0.3% THC on a dry weight basis. The female cannabis plant produces an abundance of small flowers at the top of the plant (Barbagallo, Finocchiaro, Ahmadi, 2019). The highest concentration of THC is in the buds and leaves while the stalks and seeds have lower concentrations. The flowers have a single curled leaf covered with hair-like gland cells called trichomes. When these trichomes are ruptured, a resinous oil is released. The oil has high quantities of active compounds including THC. Here, there is 60% THC concentration versus 1-10% in the other parts of the plant. Every strain of cannabis varies in CBD to THC concentrations. Storage can also affect the cannabinoid concentration as well. Too much exposure to light, heat and oxygen can accelerate the process of converting carboxylic acid-containing precursors. This means that the plant that was harvested could have been compliant to FDA standards but the final product could have noncompliant delta-9 THC concentration after storage (Aizpurua-Olaizola et al., 2016; Draeger, 2020).

CBD Equine Supplement Studies

To better understand the pharmacokinetics of how an equine's endocannabinoid system works, some preliminary single dose CBD feedings had to be done. Cohen et al. (2021) gave horses a single CBD oil dose of either 0.3 mg/kg of body weight (BW) or a higher dose of 0.6 mg/kg of BW. Heart rates were recorded every 15 minutes for 3 hours (hrs). The study concluded that heart rates overall tended to be higher in the lower dose (0.3 mg/kg) horses, but were still within normal resting range. Feed and water intake

were also recorded for 24 hrs. Feed intake tended to be higher in horses fed the higher dose, but still were not abnormal. Blood was drawn at hour 0, 1, 4, and 24. The low and high dose cannabidiol concentrations peaked at hour four at 0.51 ng/mL and 2.55 ng/mL respectfully. Turner et al. (2021) gave stalled horses a single oral dose of either 0.3 mg/kg BW or a higher dose of 0.6 mg/kg of BW of cannabidiol oil as well. The purpose of the study was to observe to see if giving a single oral dose of CBD oil would decrease the occurrence of bored behaviors like pawing, cribbing, and aggression when stalled. It was found that the occurrence of these behaviors was lower in horses given CBD versus the control horses. Draeger (2020) also had a preliminary project where she fed a single dose of 50 mg of CBD oil to one horse and 50 mg of CBD pellets to another horse. Both forms of CBD had no THC. At hour one after administration, the cannabinoid concentrations were not high enough to be detected. At hour 2, the oil was detected at 0.11 ng/mL and the pellets detected at 0.163 ng/mL. A more recent study by Turner, Knych et al. (2022) gave senior horses a single 2 mg/kg dose of CBD without THC either orally as an oil or intravenously (IV). Blood was drawn every day for 11 days. By 72 hrs, plasma CBD concentrations were below LOQ or undetectable in all horses who were given the oral CBD oil. By 48 hrs, CBD by IV was below LOQ for all horses. 48 hrs after oral administration, 7-COOH CBD concentrations were under LOQ in all horses. Draeger (2020)'s second project gave horses a single dose of either 50 mg, 100 mg, or 250 mg of pelleted CBD to test the safety. Blood was collected at 0, 0.5, 1, 2, 4, and 12 hours after a single dose was administered. At hour 2, cannabidiol concentrations peaked. Draeger claimed these amounts of CBD in the pelleted form were considered safe. Hill and Byrne also did a study using the single 250 mg dose of CBD to assess behavior changes, heart

rate, blood pressure, body weight, general condition of the horse, appetite, urine color, feces color and consistency, amount of salivation, skin reactions, mucosae color, signs of dehydration, and amount of food consumption for 10 days. Blood samples were taken a 0, 2 minutes (mins), 10 mins, 20 mins, 30 mins, 1 hr, 2 hours (hrs), 4 hrs, 6 hrs, 8 hrs, 12 hrs, 24 hrs, and 48 hrs after the single dose on day 1. The average peak concentration of cannabidiol in the blood was 2.47 ng/mL. The average concentration at 48 hrs post treatment was 0.567 ng/mL. Behavior was measured on day zero, 2 hrs, 4 hrs, 6 hrs, 8 hrs, 12 hrs, 23 hrs, 47 hrs, 71 hrs, and 95 hrs after administration. The findings were that the horses kept a good appetite, normal heart rate, normal blood pressure and body weight. No adverse behaviors or effects. After these single dose studies, researchers started performing long-term feeding tests. Turner, Guay et al. (2022) gave horses 0.6 mg/kg of BW of CBD oil once a day for 5 days. This study was to test how CBD would affect the behavior of transported horses. The horses were transported in groups of 3, 2 days after the last dose for 3 hours. There were no differences shown in the analysis of cortisol levels, blood lactate, or rectal body temperature. Williams et al. (2022) fed horses either 0.35 mg/kg or 2 mg/kg of BW once a day for seven days. This CBD did have THC in it that was tested in the blood samples as well. The 0.35 mg/kg of BW dose was found to be too low to test efficacy. For the 2 mg/kg of BW, both the CBD and THC peaked at 2 hrs after the last dose at almost 6 ng/mL and 1 ng/mL respectfully. Steady concentrations of CBD were seen by day 3. Draeger (2020) did a third project that gave a daily dose of 100 mg of pelleted CBD to horses for 6 weeks (wks) and evaluated changes in movement, reactivity, and heart rates. At the walk, treatment (TRT) horses had a longer stance and swing phase. Heart rates stayed within normal ranges. Treatment horses

had lower reactivity scores more frequently than control (CON) horses. Treatment horses also had more positive behaviors when standing tied and while being tacked up. Treatment horses were in the high suppleness category but CON horses were more frequently rated higher for suppleness when moving on a circle and rated high for their ability to track up. St. Blanc et al. (2022) fed horses 150 mg (0.3 mg/kg of BW) per day of pelleted CBD for 56 days (8 wks) to see if there were any significant instances of ataxia or sedation seen. He noted that the CBD pellets were palatable and safe for that long of dosing. Doses of CBD of up to 500 mg/day have been shown to be both safe and effective. Hill and Bryne (2022) showed no negative safety or behavioral effects in their project. Draeger et al. (2020) found no negative effects on serum chemistry or CBC results after the single feeding as part of the larger study that this project was part of. Ellis and Contino (2019) gave 250 mg of pure crystalline CBD orally twice a day to a 4 yr old Quarter Horse mare that had violent behavior due to pain near the withers. After 36 hrs of CBD treatment, the mare allowed touch in that area without adverse behavior. After 60 days, the dose was cut in half but clinical signs returned within 24 hrs, so the 250 mg dose twice/day was resumed. The amount of CBD given was gradually decreased over two months and now the horse is maintained on 150 mg once a day. This study shows that feeding up to 500 mg/day was safe and effective.

Conclusion

While the previously mentioned studies showed that a CBD supplement is safe and causes no adverse effects to the horse, there was not a lot of pharmacokinetic analysis of the blood cannabinoid concentrations seen for the long-term feedings. The objective of

this project was to analyze the pharmacokinetics of short and long-term CBD supplementation on horses from data collected in a previous, larger study.

Chapter 2: Methodology

All horses were chosen from the Murray State University Equine Center's population. Standard management practices were maintained throughout each project. All procedures were approved through the Institutional Animal Care and Use Committee at Murray State University (Appendix A and B).

Project 1

Horse Selection and Management

Eighteen Quarter Horse geldings (avg age = 15±4.2 yrs, avg body weight (BW) = 555.2 ±40.8 kg) were randomly assigned to one of three treatments based on age category (≤14 yrs = young, ≥15 yrs = older), level of use (low = 1-2 hrs/wk, moderate = 3 hrs/wk, or intense ≥4 hrs/wk); (National Research Council, 2007), and housing (pasture or stalled). One older horse used for this study also received daily long-term Vitamin E supplementation; however, no other horses received any additional supplementations except their respective treatment.

During the 24-hour feeding trial, horses were confined to stalls, fed concentrates (including their respective treatment) and recommended amounts of Bermuda grass hay, with ad libitum access to water. Concentrate consisted of either Kalm'N EZ® (Tribute Equine Nutrition®, Upper Sandusky, OH, USA), (n=3), or Reliance® (Southern States®, Cadiz, KY, USA) (n=15). Rations were dependent on the horse's individual demands as follows: 1 kg (n=1), 2.2 kg (n=15), or 3 kg (n=2) per day. Horses were hand walked two times during the study at time of stall cleanings.

Treatments and Data Collection

All horses were fitted with an indwelling catheter to reduce stress associated with blood collections. A licensed veterinarian inserted catheters within 2 hrs of horses being brought into stall confinement. Jugular blood was collected immediately before supplemental feeding began, allowing each horse to serve as their own control.

Treatment consisted of feeding a pelleted (PEL) CBD supplement containing 25 mg of cannabinoids per serving (10 g/tbsp) (Equine Veterinary Services Pharmacy, Paducah, KY, USA). The product was labeled as having 0 THC. A single administration was given in one of three dosage rates (50 mg = TRT1, n=6; 100 mg = TRT2, n=6; 250 mg = TRT3, n=6). To guarantee horses consumed the entire treatment dose of CBD, a small amount of their daily concentrate was mixed with the treatment dosage. An observer stood with the horse until all the treatment dose was consumed.

Blood collections for cannabinoid analysis occurred at 0.5, 1, 2, 4, and 12 hrs post treatment. Samples were collected into four 10 mL, no additive serum vacutainers (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ). Catheters were removed after the final blood collection. Horses remained stalled and monitored for signs of adverse reactions for 12 hrs before being returned to their pre-trial housing.

Blood samples were transported within 20 min to a university laboratory and 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 stored in a -20° C freezer until being shipped on dry ice for analysis by Industrial Laboratories (Denver, CO).

Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) analysis was conducted for CBD and the metabolite 7-COOH CBD. The lower limit of detection (LOD) was 0.1 ng/mL.

Statistical Analysis

Statistical analysis was performed for effects of treatment and time upon CBD and 7-carboxy cannabidiol (7-COOH CBD) serum concentrations using Microsoft Excel (version 16.65). Descriptive statistics were processed at 95% confidence interval. Following the summary statistics, a One-Way ANOVA was used for identification of differences within all the time groupings. Then from the One-Way ANOVA results, two-sample t-tests were used to identify the specific differences that existed between different groupings. Both the One-Way ANOVA and the t-tests utilized a p-value set at 0.05. Significance was reported at $P \leq 0.05$. Experimental units were the horse, with each horse serving as its own control, as well as the amount of CBD given (50, 100 or 250 mg).

Project 2

Horse Selection and Management

Twenty-four horses from the same Murray State University equine population were randomly selected to undergo an extended CBD treatment based off of results from Project 1 (TRT=12, CON=12). All horses remained in their normal housing, consisting of either stall with pasture turnout or pasture only. Diet consisted of Bermuda hay and concentrate with ad libitum access to water. Two forms of concentrate were fed based on standard rations for individual needs: Kalm'N EZ® (n=3) Tribute Equine Nutrition®, Upper Sandusky, OH) or Reliance® (Southern States®, Cadiz, KY, USA) (n=21). The

majority of horses (n=20) received 2.2 kg of concentrate/day. Other rations fed include 1 kg/day (n=1) and 3 kg/day (n=3). One TRT horse received Previcox® (firocoxib) throughout the study period.

Treatment and Data Collection

Twelve horses were fed 100 mg CBD PEL (Equine Veterinary Services Pharmacy, Paducah, KY, USA) once a day for 8 wks. Treatment dosage rate was determined based on Project 1 results and committee consultation. Blood samples for cannabinoid concentration were taken every 2 wks in the treatment group while control horses' blood samples were only collected at 0 and 6 wks. All blood samples were collected and handled as described in Project 1.

Statistical Analysis

Statistical analysis performed was the same as Project 1. Significance was reported at $P \leq 0.05$. Experimental unit was the horse and the control horses were compared against treatment horses at time 0 and 6 wks.

Product Sampling

Two batches of CBD pellets were used during the 8 wk feeding of Project 2, COHP19-03 (EVS Pharmacy) and COHP19-01 (Folium Biosciences). Both were produced through Folium Biosciences (Folium Biosciences, Colorado Spring, CO). For the first 6 wks, the batch from EVS Pharmacy was fed. For the last 2 wks, the batch from Folium was fed. An original cannabinoid concentration at the point of original packaging was obtained from an in-house lab for each batch. Subsequent analysis through two independent labs occurred throughout the study to monitor any changes in CBD

concentration. Manufacturer claims for COHP19-03 ensured a minimum of 25 mg phytocannabinoids per tbsp scoop. Other components of the product included 20% plant protein, 30% insoluble fiber, 50% complex carbohydrates, a flavoring agent (Apple Ade) and an FDA approved mold inhibitor (Myo Curb ®). Including the distributing company's original internal analysis, four subsequent samples were tested through two separate accredited labs, including the Murray State University Analytical Chemistry Department (MSUAC) and Botanacor TM (Botanacor Laboratories, Denver, CO). Analysis completed by the MSUAC was completed in triplicate, using ultrasonication for analysis. The CBD pellet samples were first ground. Subsequently, ~0.5g was extracted and analyzed for CBD and THC content using a validated LC-MS/MS method. Concentrations from BotanacorTM were achieved through Agilent HPLC-DAD instrumentation compliant with Good Laboratory Practices and current Good Manufacturing Practices requirements.

Chapter 3: Results and Discussion

Project 1

One horse had CBD concentration much higher than the rest of the population. The ANOVA and t-tests confirmed that horse's values were an outlier, therefore, the data was removed from the results before final statistics were calculated.

All 18 horses showed the presence of CBD and 7-COOH CBD in serum at 0 hrs. This was surprising, as no CBD supplementation had occurred at that time. The laboratory's LOD was 0.1 ng/mL, so any results below that level are suspect and should not be considered as a positive result. Only 2 of the 18 had CBD concentrations above the LOD (0.12 and 0.116 ng/mL). 6 of the 18 had 7-COOH CBD concentrations above the LOD (0.102-0.689 ng/mL). T-tests were run to check for any significant differences between each mg group at time 0 and none were found.

There were no significant differences in CBD concentrations within the 50 mg treatment group over time (P=0.3789). This could be due to the fact that there was no CON group in this project that could be used for comparison. However, there were differences within the 100 mg and 250 mg groups over time (P=0.0370 and P=0.0022, respectively; Table 1 and Table 2). Mean concentration values peaked at 4 hrs for the 100 mg and 250 mg groups, while the 50 mg group suggested that concentration levels were still rising at 12 hrs post-treatment. Significant differences of CBD concentrations in the blood were not seen until an amount greater than 50 mg was given for a single dose.

Table 1: Pharmacokinetics of a single feeding of 100 mg of cannabidiol to horses were different over time based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 hrs	0.0256	0.0007	
0.5 hrs	0.4419	0.1840	
1 hr	0.804	0.9382	
2 hrs	2.7879	11.2694	
4 hrs	3.47	0.4839	
12 hrs	2.3609	18.2520	
			0.0370

Table 2: Pharmacokinetics of a single feeding of 250 mg of cannabidiol to horses were different over time based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 hrs	0.0550	0.0015	
0.5 hrs	1.5624	1.1758	
1 hr	3.3606	2.6486	
2 hrs	7.258	21.1252	
4 hrs	8.24	26.9886	
12 hrs	2.813	7.6906	
			0.0022

Williams et al. (2022) fed horses a pelleted CBD supplement at 0.35 mg/kg and 2.0 mg/kg. Mean high concentrations were 6.6±2.1 ng/ml and 51±14 ng/ml, respectively (Williams et al., 2021). Based on calculations, Williams et al. (2022) fed between 165 mg and 210 mg CBD to the 0.35 mg/kg treatment group. In comparison to this study,

Williams et al. (2022) fed a little more on the low end and a little less on the high end as compared to the current project. Comparing mean serum concentrations on the low end, it makes sense that Williams et al. would have higher concentrations than this study (6.6 ng/mL vs 2.8 ng/mL in this study) due to having fed more CBD. However, on the high end, Williams et al. (2022) showed a much higher mean concentration at a lower dose than was seen in this study (51 ng/mL vs 8.2 ng/mL in this study). Williams et al. (2022) also reported the presence of THC in their pelleted supplement. A review by Urits et al. (2020) reported a relationship between CBD and THC, suggesting that a combination of both compounds may result in an increased efficacy. This could explain why Williams et al. (2022) showed higher concentrations at a lower dose rate. Serum CBD concentrations in the 0.35 mg/kg group peaked at 2 hrs post-treatment (Tmax 1.8±1.2 hrs). These results differ from those seen in this project, where CBD concentrations in the 100 mg and 250 mg groups (comparable in dose amounts) both peaked around 4 hrs after the single dose feeding (Table 1 and Table 2). Differences in the manufacturer could result in differences in digestibility of the product, potentially impacting absorption of the CBD within the pellets. Williams et al. (2022) also reported the presence of THC in their pelleted supplement.

Hill and Bryne (2022) evaluated safety and behavior effects of feeding a single dose of 250 mg CBD to eight healthy horses. Mean CBD peak concentration was 2.47 ng/mL. Results from this study, 3.8851 ng/mL, were higher than the means found by Hill and Bryne (2022). That study did not specify what form the CBD was in when fed orally. In Project 1, CBD was fed in a pelleted form. The difference in the form of CBD could be a possibility for the difference in concentrations found.

Ryan et al. (2021) fed horses a single dose of 0.5 mg/kg (268 mg), 1 mg/kg (537 mg) and 2.0 mg/kg (1,074 mg) of CBD, and times peaked at 2.8 hrs, 4.7 hrs and 3.2 hrs, respectively. The 0.5 mg/kg dose was closest to Project 1's 250 mg group for comparison. While the 250 mg group in this study had a peak at time 4 hrs, Ryan et al.'s (2020) study showed concentrations peaking sooner. The main difference between these two studies is the form of CBD. Ryan et al.'s (2020) study fed CBD that was originally in a powdered form, then pulverized and put in sesame oil. Project 1's pellet form of CBD could have had a slower absorption rate than CBD in oil. Ryan et al. (2020) had peak CBD concentrations in the blood of 1.69±0.830 ng/mL for the 0.5 mg/kg group. This is a much lower concentration than Project 1's 250 mg group even though Ryan et al.'s (2020) study fed slightly more CBD. One potential reason for this difference could be that CBD in oil is absorbed more quickly than a pelleted form. However, this was not shown to be true in a pilot study where a pelleted and oil-based CBD supplement were compared (Draeger, 2020). In that study, blood samples were only collected at 1- and 2-hrs post administration, and the pelleted supplement resulted in higher concentrations in the serum. Had data collection been continued to 4 hrs or more, the results may have been different.

Turner, Knych et al. (2022) gave horses a single dose of 0.1 mg/kg CBD by IV injection or 2.0 mg/kg of CBD oil fed orally. Weights of the horses were not included, so the total dose given to each horse could not be calculated. Peak time and concentration for the CBD by IV was not included, so no comparisons were made to the current study. Serum CBD concentrations peaked at 2.46±1.62 hrs for the oral administration. Compared to Project 1's peak times, this study showed an earlier CBD peak. Results of

peak concentration times from Turner, Kynch et al. (2022) were similar to Ryan et al. (2020) discussed previously. This could be related to the fact that both studies fed CBD in oil. It is also possible that Turner, Kynch et al. (2022) may have fed a total amount of CBD higher than the 250 mg fed in Project 1. Turner, Kynch et al. (2022) showed a peak CBD concentration of 18.54±9.8 ng/mL for the 2.0 mg/kg group. These results were much higher than Ryan et al. (2020), where the 2.0 mg/kg treatment peaked at 6.14±3.52 ng/mL. While these results cannot be compared to the current study due to dose differences, it does call to question what might be occurring in horses fed the same concentration of CBD in oil.

While Project 1 only collected blood up to 12 hrs after a single dose of CBD, both Ryan et al. (2021) and Turner, Knych et al. (2022) found that CBD concentrations did not fall below the LOQ or were completely undetectable until 72 hrs after a single oil oral treatment of 2.0 mg/kg. Turner, Knych et al. (2022) also had an IV form of 0.1 mg/kg where CBD concentrations fell below LOQ after only 48 hrs (Table 3 and Table 4).

Table 3: Summary of previously published methodology compared to those of Project 1 and Project 2.

Source	Project 1	Hill and Bryne, 2022	Williams et al., 2022	Ryan et al., 2021	Turner, Kynch et al., 2022	Project 2
Breed	Quarter Horses	Czech Warmbloods	Mixed	Thoroughbreds	Mixed	Mixed
Age (yrs)	15±4.2	2-18	6-23	4-7	24±3	9-26
Weight (kg)	555.2±40.8	600-800	497-600	537±33.9	N/A	440.91-754.55
Time of Blood Collection	0, 0.5, 1, 2, 4, 12 hrs post-treatment	0, 2, 10, 20, 30 mins, 1, 2, 4, 6, 8, 12, 24, 36, 48 hrs post-treatment	0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 168 hrs post 7th day of treatment	15, 30, 45 mins, 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, 72 hrs post-treatment	0.5, 1, 4, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264 hrs post-treatment	0, 2, 4, 6, 8 wks
Form of CBD	Pelleted	N/A (Oral)	Pelleted	Powder pulverized then put in sesame oil	Oil or IV	Pelleted
Amount of CBD	Single dose of either 50 mg (0.09±0.10 mg/kg), 100 mg (0.18±0.10 mg/kg), or 250 mg (0.45±0.04 mg/kg)	250 mg (0.31-0.42 mg/kg)	0.35 or 2.0 mg/kg once a day for 7 days	Single dose of either 0.5, 1.0, or 2.0 mg/kg	Single oral dose of 2.0 mg/kg or single IV dose of 0.1 mg/kg	100 mg once a day for 8 wks

Table 4: Summary of previously published cannabidiol concentration results for comparison to Project 1 and Project 2.

Parameter	Turner, Knych, et al., 2022		Ryan et al., 2021			Williams et al., 2022	
	2.0 mg/kg (Oral)	0.1 mg/kg (IV)	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	0.35 mg/kg	2.0 mg/kg
CBD Cmax (ng/mL)	18.54±9.8	-	1.69±0.83	3.22±2.18	6.14±3.52	6.62±2.1	51±14
CBD Tmax (hrs)	2.46±1.62	-	2.81±0.98	4.74±3.77	3.18±0.98	1.8±1.2	2.4±1.1
Below LOQ/LOD	72 hrs	48 hrs	-	72 hrs	72 hrs	-	-
THC Cmax	-	-	-	-	-	0.7±0.6	-
THC Tmax	-	-	-	-	-	2.5±1.0	-

Parameter	Hill and Bryne, 2022	Project 1			Project 2
	250 mg (total)	50 mg (total)	100 mg (total)	250 mg (total)	100 mg/day (total)
CBD Cmax (ng/mL)	2.47	2.0916	3.47	8.24	2.5742
CBD Tmax	-	12 hrs	4 hrs	4 hrs	2 wks
Below LOQ/LOD	-	-	-	-	-
CBD AVG (ng/mL)	-	0.4773	1.3814	3.8815	1.21996

After running a two-sample t-test assuming unequal variances for 50 mg vs 100 mg, no significant differences were seen (P=0.0718). However, there were significant differences between 50 mg vs 250 mg (P=0.0128), and between 100 mg and 250 mg (P=0.0289). The most variance (V) and the highest mean was seen in the 250 mg group (V=3.0642; mean=3.8815 ng/mL). It was unsurprising that the highest mean was seen in the 250 mg group, as higher doses would typically be associated with higher concentrations in the blood.

Based on a one-way ANOVA, there was a significant difference in the metabolite 7-COOH CBD concentrations over time for all groups combined (P<0.0001). The highest mean and variance were at 4 hrs post-treatment (mean=95.2848 ng/mL; V=6168.9284). An ANOVA was then run for each individual group (50 mg, 100 mg and 250 mg). Significant differences were seen in all three groups (P=0.0018; P<0.0001; P<0.0001, respectively) (Tables 5, Table 6 and Table 7). Ryan et al. (2021) also showed significant differences in the peak concentrations of 7-COOH CBD at all amounts of CBD fed (0.5 mg/kg, 1 mg/kg and 2 mg/kg). The greatest variances in Project 1 were observed at 12 hrs post-treatment for the 50 mg and 100 mg groups (V=490.5324 and V=3670.5164, respectively), but at 4 hrs post-treatment for the 250 mg group (V=5195.5). The greatest means were mainly seen at 4 hrs post-treatment for 100 mg and 250 mg groups (mean=97.6 ng/mL and 181 ng/mL, respectively). The greatest mean for the 50 mg group was seen at 12 hrs post-treatment (mean=29.6583 ng/mL), and concentrations appeared to still be rising. For all groups (50 mg, 100 mg and 250 mg), when the CBD concentrations were peaking, so were the 7-COOH CBD concentrations. This was unexpected because the 7-COOH CBD metabolite is a byproduct of CBD breaking down,

so theoretically 7-COOH CBD concentrations should peak after the CBD concentrations peak. This was not the case found in Project 1 and was different from other published research. Turner, Kynch et al. (2022) saw CBD concentrations peak at 2.46±1.62 hrs and 7-COOH CBD concentrations peaked at 5.08±2.31 hrs, after feeding 2.0 mg/kg of CBD. Ryan et al. (2021) saw CBD concentrations peak at 2.8 hrs, then 7-COOH CBD concentrates peaked at 5.67±3.08 hrs, after feeding 0.5 mg/kg of CBD. Another interesting find from comparing the 100 mg vs 250 mg 7-COOH CBD concentration mean values was that the 250 mg 7-COOH CBD concentrations increased at a greater amount between blood collection times and also fell at a greater amount after peaking than the 100 mg 7-COOH CBD concentrations (Table 4 and Table 5).

Table 5: Pharmacokinetics of 7-COOH CBD after a single feeding of 50 mg of cannabidiol to horses were different over time based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 hrs	0.1349	0.0116	
0.5 hrs	0.5217	0.0680	
1 hr	2.2111	4.2662	
2 hrs	10.7242	176.0975	
4 hrs	21.5403	423.8075	
12 hrs	29.6583	490.5324	
			0.0018

Table 6: Pharmacokinetics of 7-COOH CBD after a single feeding of 100 mg of cannabidiol to horses were different over time based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 hrs	0.0554	0.0040	
0.5 hrs	1.0803	1.6572	
1 hr	3.7233	6.8688	
2 hrs	46.8	1583.104	
4 hrs	97.6	1280.94	
12 hrs	91.0583	3670.5164	
			<0.0001

Table 7: Pharmacokinetics of 7-COOH CBD after a single feeding of 250 mg of cannabidiol to horses were different over time based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 hrs	0.208	0.0746	
0.5 hrs	4.6116	14.4798	
1 hr	25.506	282.2322	
2 hrs	79.04	2343.733	
4 hrs	181	5195.5	
12 hrs	142.98	4609.502	
			<0.0001

Ryan et al. (2021) had a peak 7-COOH CBD concentration of 46.3±19.9 ng/mL at time 5.67±3.08 hrs for the 0.5 mg/kg (268.5 mg) group. Project 1's 250 mg group is the closest amount of CBD for comparison. The 250 mg group had peak concentrations of 181 ng/mL at 4 hrs. The mean concentration of 7-COOH CBD is nearly 4 times that of Ryan et al.'s (2021) study even though the amount of CBD fed was less than Ryan et al. (2021). This may again be linked to feeding an oil versus a pelleted product. Though the time of peak concentration was numerically different between Ryan et al. (2021) and this study, it cannot be concluded that they were statistically different based on the standard deviation in their study.

Turner, Knych et al. (2022) showed a max concentration of 7-COOH CBD of 307±186 ng/mL seen at 5.08±2.31 hrs after feeding a single dose of 2.0 mg/kg of CBD. This concentration of 7-COOH CBD was the highest of all the research found. The IV treatment (0.1 mg/kg) peak concentration of 7-COOH CBD was only 72.6±98.2 ng/mL at 3.5±3.3 hrs. It is expected that the less CBD fed would result in less 7-COOH CBD metabolite seen in the blood. Turner, Knych et al.'s (2022) outcomes could infer that the less CBD fed would have 7-COOH CBD concentrations peak sooner, and if the amount of CBD fed was higher, then 7-COOH CBD concentrations would peak later. Similar to Project 1, Ryan et al. (2021) and Turner, Knych et al. (2022) studies never had 7-COOH CBD concentrations fall under the LOQ, even with Turner, Knych et al. (2022) study testing out to 72 hrs post the single dose (Table 8).

Table 8: Previously published 7-COOH CBD concentration results for comparison to Project 1.

Parameter	Turner, Knych, et al., 2022		Ryan et al., 2021			Project 1		
	2.0 mg/kg (Oral)	0.1 mg/kg (IV)	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	50 mg (total)	100 mg (total)	250 mg (total)
7-COOH CBD Cmax (ng/mL)	307±186	72.6±98.2	46.3±19.9	85±42.4	210.8±157.5	29.6583	97.6	181
7-COOH CBD Tmax (hrs)	3.5±2.31	3.5±3.3	5.67±3.08	8.42±5.88	6.45±3.86	12	4	4
Below LOQ/LOD	Above	Above	Above	Above	Above	-	-	-
7-COOH CBD AVG (ng/mL)	-	-	-	-	-	10.7984	40.0529	72.2243

T-tests were run to compare each group against the other. A significant difference was seen between the 50 mg vs 100 mg group as well as between the 50 mg vs 250 mg group (P=0.0087 and 0.0054, respectively). There was no significant difference between the 100 and 250 mg group (P=0.0686). The greatest variance and mean were seen in the 250 mg group (V=793.5504 ng/mL and mean=72.2243 ng/mL). When comparing t-test times within the 50 mg group, significant differences were not seen in time until comparing the 0 vs 4 hrs (P= 0.0117), and 0 vs 12 hrs (P=0.020). When comparing the 0.5 hrs post-treatment to the other times, a significant difference was only seen when compared to 4 hrs post-treatment (P=0.0260). T-tests for comparing the times of the 50 mg group were only completed to 1 hr vs 12 hrs. These t-tests back up the previously discussed evidence that 7-COOH CBD concentrations increased the most starting at 4 hrs post treatment.

Project 2

Based on previously published literature and the results of Project 1, a dose of 100 mg of CBD was selected for the long-term project. In Project 2, 100 mg of pelleted CBD was administered daily for 8 wks. Unlike Project 1, which had only geldings as part of the TRT groups, there were mares in the study population. Published research suggests that there may be a difference between male and female animals and their response to CBD and/or THC products (Viudez-Martinez et al., 2018; Blanton et al., 2021). T-tests were run to compare the mares and geldings at 0, 2, 4, 6 and 8 wks to see if there was a significant difference between the sexes in horses used in this study. No significant difference was seen (P>0.0914), so sex was dropped from the model. Arkel et al. (2021) did not see a difference between sexes when a CBD product containing THC was

vaporized and suggested that route of administration (vape vs oral oils vs other edibles) may produce different results. At least in this study, with a limited number of mares, a pelleted CBD supplement did not result in differences between mares and geldings.

Based on a one-way ANOVA, there were significant differences seen between all weeks ($P<0.0095$). The highest variance and mean values were found at 2 wks of treatment (V=11.6179; mean=2.5742 ng/mL). At 4 wks, the CBD concentrations dropped by more than 30%. This was very unexpected as the horse's exercise amount and feed regiment remained unchanged for the entire duration of Project 2. None of the mares were bred during that time and none of the population came down with an illness. At 6 wks, CBD concentrations were back up and remained there until the end of the 8 wks (Table 9). Next, t-tests were run to compare the CON vs TRT groups. There were no significant differences seen between the CON and TRT groups at 0 wks (Table 10). The mean values and variances were almost identical (CON, V=0.0043; mean=0.0476 and TRT, V=0.0031; mean=0.0399 ng/mL). That being said, 5 of the CON horses showed CBD concentrations of 0.0536-0.182 ng/mL at 0 wks. Three of those 5 horses were above the LOD of 0.1 ng/mL. Also, 5 TRT horses showed CBD concentrations of 0.0294-0.155 ng/mL at 0 wks, two of which were above the LOD of 0.1 ng/mL. At 6 wks, 2 of the CON horses showed no CBD concentration in the serum, 1 showed an increase that was above the LOD of 0.1 ng/mL, and 2 showed a decrease but both were below the LOD of 0.1 ng/mL (Table 11). The possibility of residual CBD from Project 1 was considered, however, there was a 7-week washout between studies. It is unlikely that a single dose of CBD would result in detection 7 weeks later. Also, 3 of the 5 horses were not involved in Project 1. All of the TRT horses showed increased CBD concentrations at 6 wks, which

is unsurprising. There was a significant difference seen between the CON and TRT groups at 6 wks (P=0.0005). The variance was clearly coming from the TRT group (Table 12).

Table 9: Pharmacokinetics of feeding 100 mg of cannabidiol to horses once a day for 8 weeks based on a one-way ANOVA with alpha of 0.05.

Time	Mean	Variance	P-value
0 wks	0.0399	0.0031	
2 wks	2.5742	11.6179	
4 wks	0.834	0.2399	
6 wks	1.6086	1.156	
8 wks	1.0413	0.1976	
			0.0095

Table 10: Pharmacokinetics of control and treatment groups at 0 weeks based on a two-sample t-test assuming unequal variances.

Group	Mean	Variance	P-value
CON	0.0476	0.0043	
TXT	0.0399	0.0031	
			0.7653

Table 11: Control and treatment groups CBD concentrations (ng/mL) at 0 weeks and 6 weeks of feeding 100 mg of CBD once a day.

Control Group			Treatment Group		
Name	0 wks	6 wks	Name	0 wks	6 wks
Bruce	0.088	0.0432	Baldy	0	2.26
Charlie	0.182*	0	Beamer	0.0569	0.618
Ellie	0.0536	0	Cujo	0.155*	1.06
Jim	0	0	Jazz	0.0787	4.36
JJ	0	0	Malcolm	0.119*	1.22
Plaino	0	–	Pedro	0	1.01
Marc	0	0	Prophet	0	1.24
Max	0.123*	0.827*	Stella	0	0.615
Shooter	0	0	Henry	0.0294	1.4
Skipper	0.124*	0.0784	Pete	0	2.86
Spanky	0	0	Rapper	0	1.19
Spooky	0	0	Yeller	–	1.47
AVG	0.048	0.086	AVG	0.04	1.609
Range	0-0.182	0-0.827	Range	0-0.155	0.615-4.36

*CBD concentration above LOD of 0.1 ng/mg

Table 12: Pharmacokinetics of feeding 100 mg of cannabidiol to horses once a day were different between control and treatment groups at 6 weeks based on a two-sample t-test assuming unequal variances.

Group	Mean	Variance	P-value
CON	0.0862	0.0610	
TRT	1.6086	1.1557	
			0.0005

Chapter 4: Conclusion

From the 2 projects completed, it is evident that CBD and 7-COOH CBD concentrations in the serum peaked at 4 hrs post-administration of a single dose of CBD. Since blood collection happened at 2 hrs, 4 hrs, and again at 12 hrs post-treatment, further research is needed to pinpoint the exact time CBD and 7-COOH CBD concentrations peaked. It could have happened anywhere between 2-12 hrs. Further research is also needed to determine if the pelleted form of CBD can be detected in the blood serum as long as the oil form. Turner, Knych et al. (2022) study fed 2.0 mg/kg of CBD oil and it did not fall below the LOQ until 72 hrs post-administration. That study also delivered 0.1 mg/kg of CBD in an IV form, and those concentrations were undetected after just 48 hrs. Another project should be done to see how long the 7-COOH CBD metabolite takes to become undetected in the serum after a single dose, since no publication reporting such results was found.

Based on Project 1's results within the 50 mg group, longer term studies with various dosages should be done to determine if long term feedings of lower doses could have beneficial effects. It is possible that CBD may accumulate in tissues, allowing a lower dose to still produce positive effects. Project 2 findings suggested that CBD did not affect mares and geldings differently. It also found that feeding 100 mg of pelleted CBD supplements once a day for 8 wks resulted in peak CBD concentrations 2 wks. Again, more research is needed to better determine if CBD peaked at 2 weeks or if maybe it was week 1 or 3. Further research is needed to figure out what internal or external forces

would cause CBD concentrations to decrease drastically at 4 wks and then stay steady from 6-8 wks. No links to exercise workload, weather and health conditions for the population in Project 2 were seen and the amount of feed and CBD fed stayed the same throughout the project. Further research is needed to determine if CBD is stored in the body tissues or if it is circulated once, then excreted in the urine. The more research that can be done for feeding horses CBD supplements can help pinpoint exactly how CBD works in the body. This information will bring equine CBD supplements closer to being manufactured for veterinarians to prescribe and for owners to use.

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Appendix A: IACUC Approval for Project 1

MURRAY STATE UNIVERSITY

Institutional Animal Care and Use Committee

328 Wells Hall
Murray, KY 42071-2393
270-809-3534 • 270-809-3535 fax

December 10, 2019

Dr. C.A. Shea Porr
Animal/Equine Science
Murray State University
Murray, KY 42071

Dear Dr. Porr:

The Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol with revisions for the project titled, "Pharmacokinetic Study of Oral CBD use in Horses."

The protocol timeline is approved through January 2020. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

If you have any questions, please contact me at 270-809-3534

Sincerely,

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File

Appendix B: IACUC Approval for Project 2

MURRAY STATE UNIVERSITY

Institutional Animal Care and Use Committee

328 Wells Hall
Murray, KY 42071-2393
270-809-3534 • 270-809-3535 fax

January 6, 2020

Dr. C.A. Shea Porr
Animal/Equine Science
Murray State University
Murray, KY 42071

Dear Dr. Porr:

The Murray State University Institutional Animal Care and Use Committee (IACUC) has approved your research protocol with revisions for the project titled, "Safety and Efficacy Evaluation of Extended Oral CBD Treatment in Horses."

The protocol timeline is approved through May 15, 2020. Please use the Animal Use Report (attached) to keep up-to-date information about the animals. At the termination of the protocol, you will need to complete the Conclusion Report (attached) and list final information concerning the animals.

If you have any questions, please contact me at 270-809-3534

Sincerely,

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File

Appendix C: Figures of CBD Concentrations for Project 1

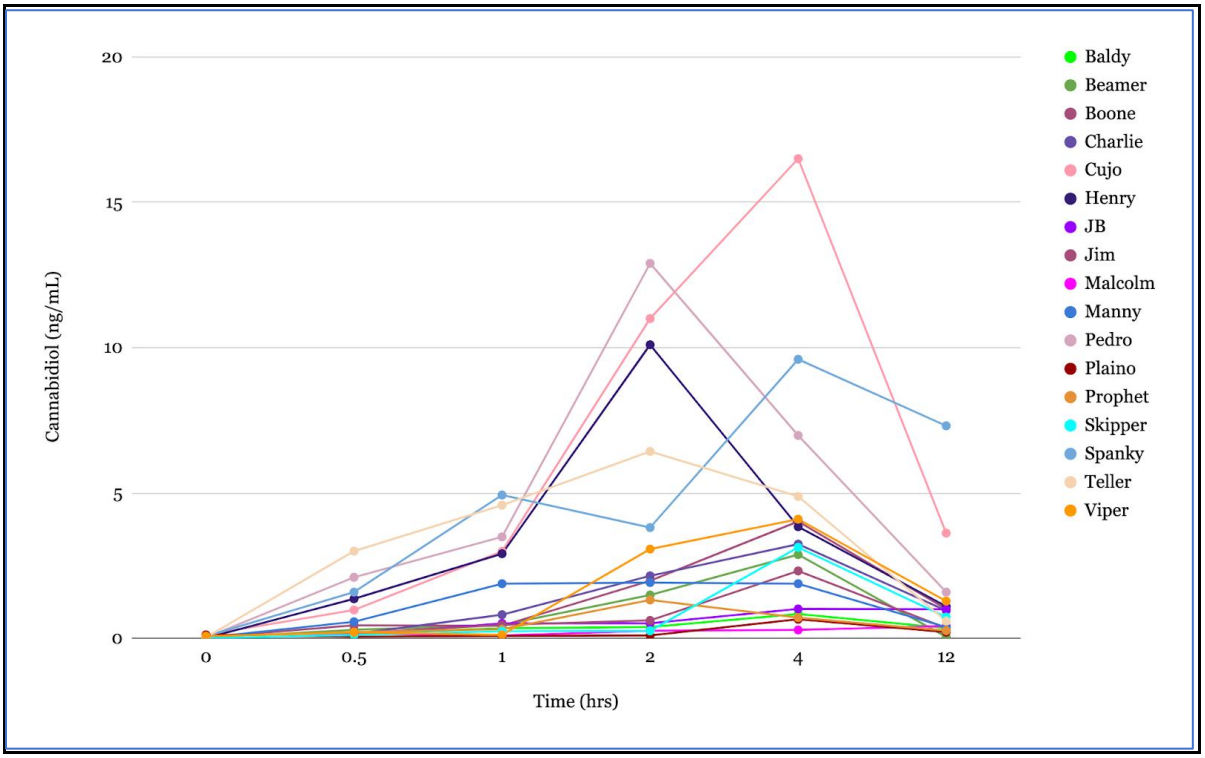

Figure 1. Cannabidiol concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of either 50 mg, 100 mg or 250 mg of CBD.

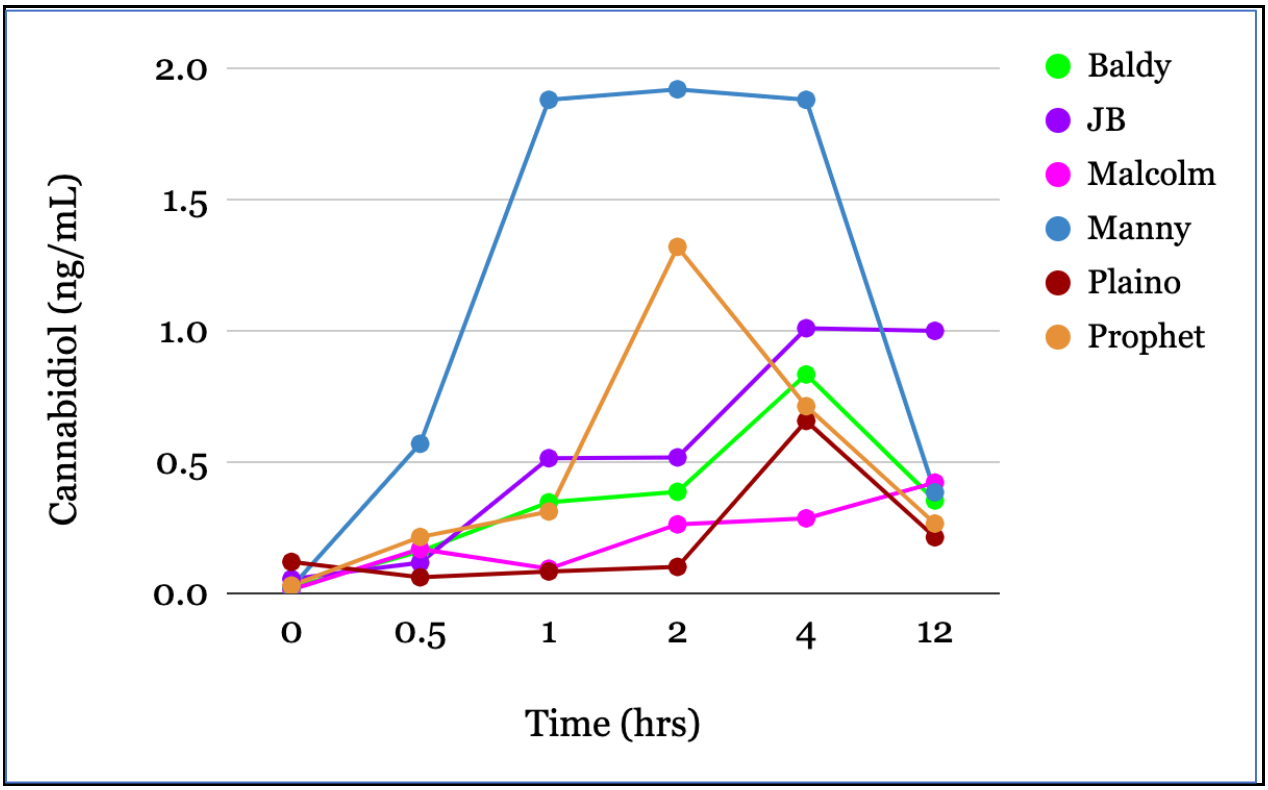

Figure 2. Cannabidiol concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 50 mg of CBD.

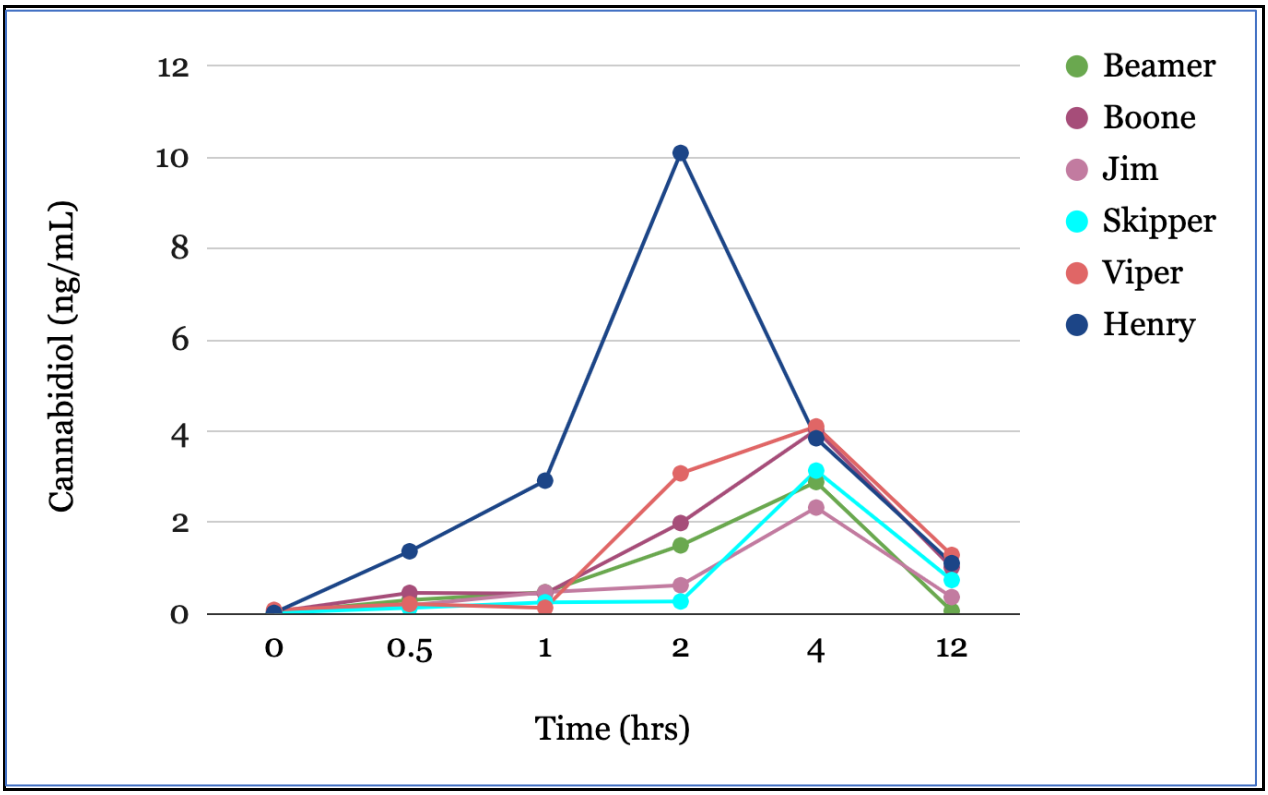

Figure 3. Cannabidiol concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 100 mg of CBD.

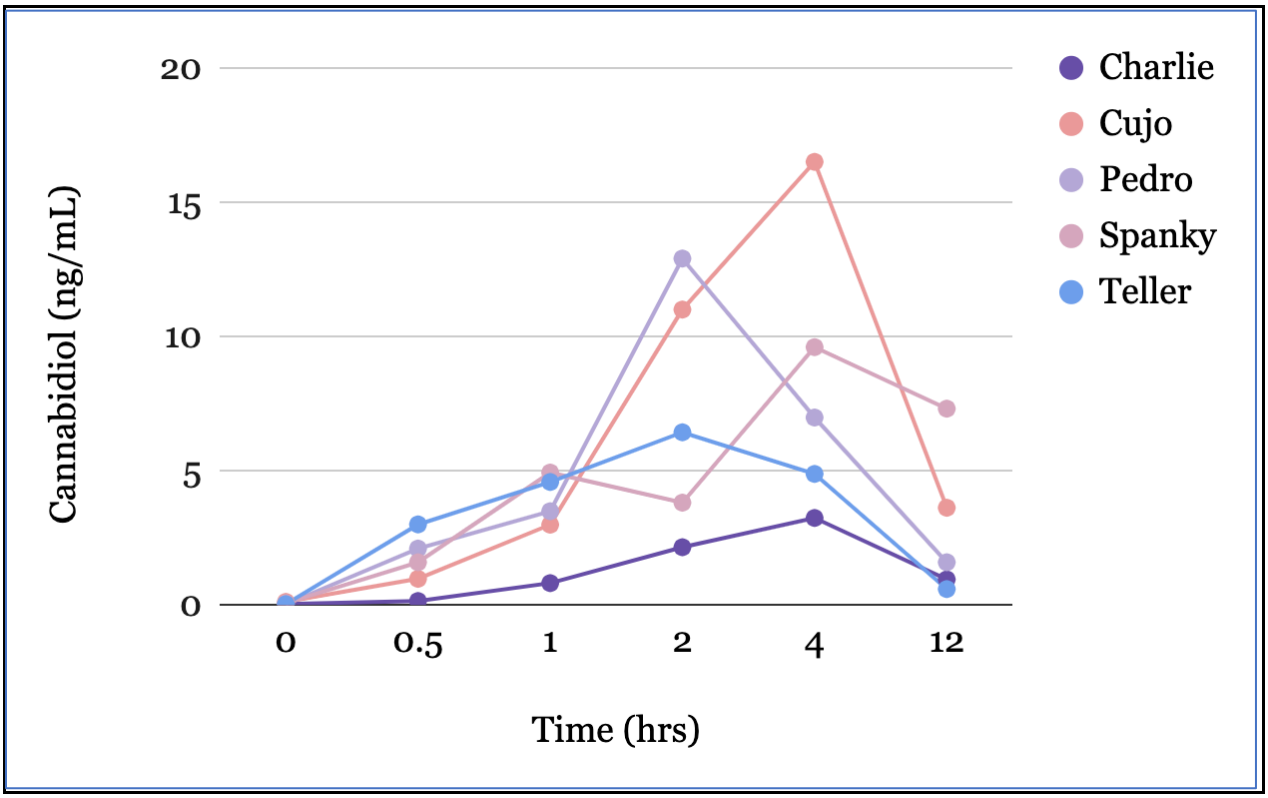

Figure 4. Cannabidiol concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 250 mg of CBD.

Appendix D: Figures for 7-COOH CBD Concentrations for Project 1.

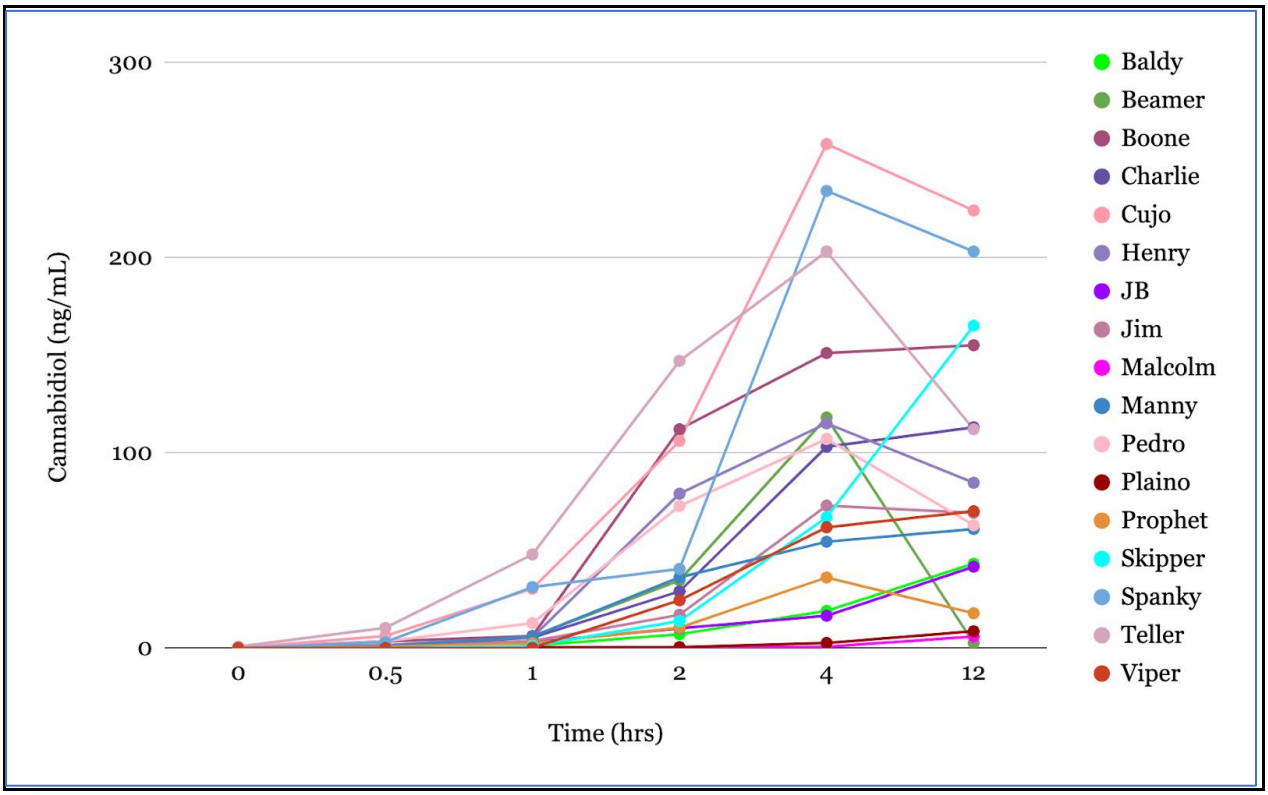

Figure 5. 7-COOH CBD concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of either 50 mg, 100 mg or 250 mg of CBD.

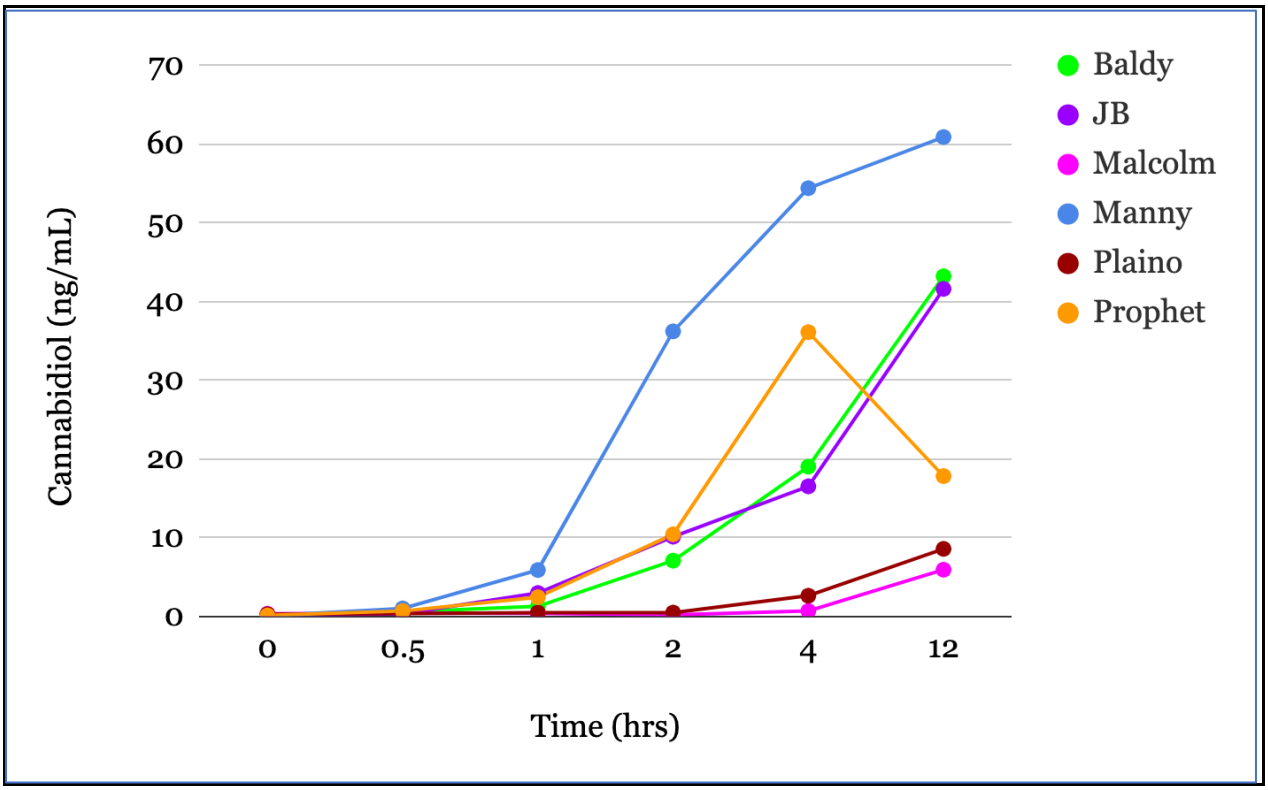

Figure 6. 7-COOH CBD concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 50 mg of CBD.

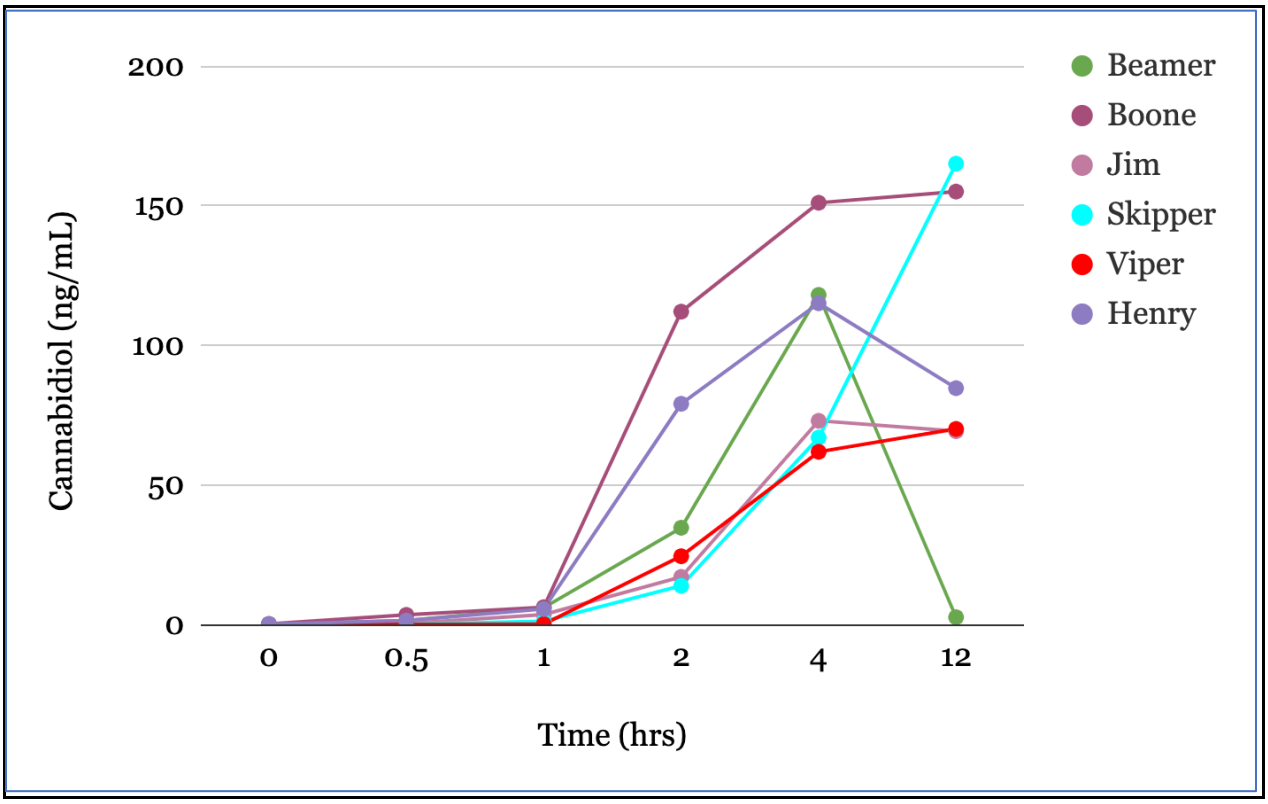

Figure 7. 7-COOH CBD concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 100 mg of CBD.

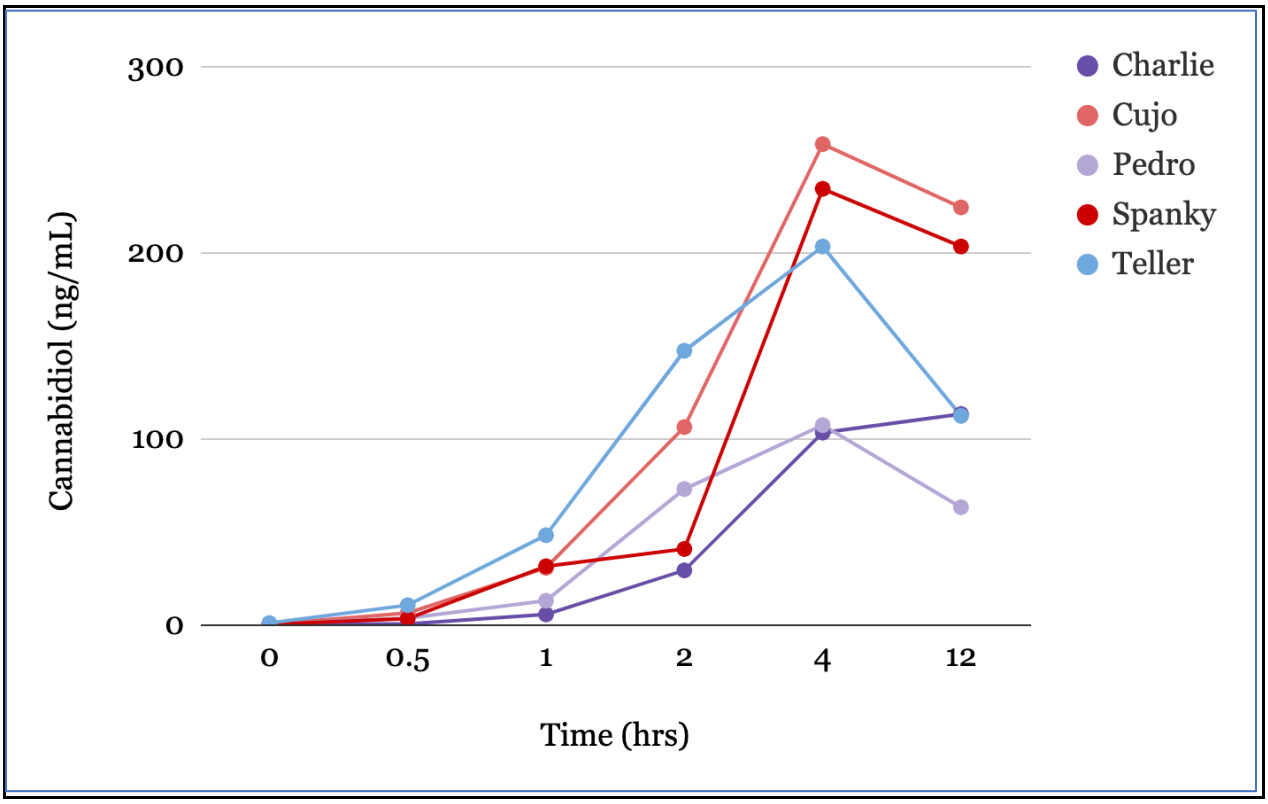

Figure 8. 7-COOH-CBD concentrations in the blood serum at times 0, 0.5, 1, 2, 4 and 12 hours post administration of a single dose of 250 mg of CBD.