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# Pharmacokinetics of single feeding of cannabidiol in cattle: A pilot study

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Pharmacokinetics of single feeding of cannabidiol in cattle: A pilot study

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for the Murray State University Honors Diploma

Haley E. Cornette

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**Abstract**

Cannabidiol (CBD) is a substance that has been used in complementary medicine for many years. However, modern medicine has little knowledge of how this substance is utilized and metabolized in ruminant animals. Regulations on quality assurance and use in animals are lacking, and CBD supplementation in livestock is not approved. If CBD supplements can be shown to be safe and effective, detection in the animal will be important for determining regulation of use. A withdrawal period can then be established to allow time for deterioration of product to safe levels before livestock products enter human markets. This study sought to determine the pharmacokinetics of an oral CBD supplement in cattle. An oral gel cannabinoid-containing product, formulated for equine, designed to be absorbed through mucosal membranes was used in this study. Based upon current knowledge, appearance of cannabinoids in plasma should occur at one-hour post administration and increase to peak concentrations at approximately ten hours. Blood collections from the jugular vein of two mature cows occurred at 0 (post-treatment), 0.5, 1, 2, 4, 8, 12, 16, and 24 hours. Subjects were housed in outdoor working pens for the duration of the study, with free access to water and hay. Multiple cannabinoids were detected, which aligned with the guaranteed analysis stated by the manufacturer. Detection of cannabinoids was inconsistent between subjects. Cannabinoids were first detected in plasma at 1-hour post treatment in one subject, and at 12 hours post treatment in the other. Only CBD and 7-hydroxy cannabidiol (CBD-7 acid) were detected during the collection period. Plasma cannabinoid concentrations were still rising at the end of the

collection period, indicating that peak concentrations had yet to be reached. Appearance of cannabinoids in plasma indicated that oral gel cannabinoid product was able to be metabolized and absorbed by the ruminant animal. A half-life of the product was unable to be determined. Future studies should consider expanding sampling numbers and extending collection period.

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**Chapter 1: Review of Related Literature**

*Introduction*

Innovations are vital to the survival of the agriculture industry, as they have the ability to increase productivity. Industrial hemp is an area of innovation that should be considered for a place in the cattle industry. Industrial hemp products have been shown to provide nutrition and therapeutic benefits when used in livestock production (Kleinhenz, 2020). Predating prohibition in the 1930s, industrial hemp, *Cannabis sativa,* was used to mitigate a variety of issues including pain, anxiety, anorexia, nausea, and inflammation in humans. Today, the extract from hemp flowers is often concentrated into an oil for its therapeutic properties to be obtained (della Rocca & Di Salvo, 2020).

Application of cannabidiol-containing products in western medicine is surrounded by controversy due to association with the psychoactive compound tetrahydrocannabinol (THC), which is associated with marijuana. In many countries and U.S states, THC is illegal to use or possess. In 2018, hemp was defined by the United States Department of Agriculture as "plant *Cannabis sativa L.* and any part of that plant... with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis." (*Agriculture Improvement Act of 2018,* 7 U.S.C § 1639o *et. seq.*). The legal separation of hemp and marijuana allows for potential hemp use in medicine and livestock production.

*Cannabis*

Cannabis is a plant originating from Central Asia with a long-standing history of application for medicine, fiber, and dietary supplementation. However, use of cannabis in western medicine declined in the 1930s due to increased popularity of opium and unreliable product quality (Klumpers & Thacker, 2019). Today, cannabis use is limited and restricted under federal regulations. The United States Department of Agriculture (USDA) requires license for all hemp production and research facilities. Guidelines for obtaining a project permit are outlined in the Agricultural Improvement Act of 2018. States are permitted to establish and enforce regulations stricter than those set federally. As part of regulations, all facilities that cultivate or manufacture hemp containing products are subject to annual inspection of crops at random. These inspections ensure that crops are remaining within the legal concentrations of chemical composition.

Cannabinoids are a group of lipid-soluble compounds derived from cannabis plants. In the plant, cannabinoids account for the majority of the bioactive compounds. In the body, they exert their effects through the endocannabinoid system, bonding to cannabinoid receptors types one and two. The fat-soluble compound is broken down into water soluble metabolites so that it is able to exit the body system (Klumpers & Thacker, 2019). With fat solubility, higher body fat percentages should increase the rate at which the cannabinoids are metabolized. This can result in varying absorption and excretion times across subjects with higher or lower body-fat composition. Common cannabinoids include cannabidiol (CBD), cannabidivarinic acid (CBDVA), cannabidivarian (CBDV), cannabichromenic acid (CBDA), cannabigerolic acid (CBGA), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), cannabichromene (CBC), and various forms of

THC and its acids. There have been over 100 naturally occurring phytocannabinoids detected in *C. sativa* (Landa et al., 2016).

Cannabinoids have been explored in areas of modern medicine for many applications. Examples in human medicine include their potential use as an antiemetic, an appetite stimulant, an analgesic, and in the treatment of neurological diseases such as multiple sclerosis (Costa, 2007). Research in animals is more limited than that in human medicine. Rodents have been used to explore the possibility of treatment for pain, inflammation, and other chronic conditions. There have been reported possibilities of beneficially reducing depression or changing feed intake related conditions (Landa et al., 2016).

*Tetrahydrocannabinol*

There are many different forms of THC. With THC, psychoactive components are largely associated with it at large, yet it is **Δ**9-THC that exhibits the major psychoactive component. While the psychoactive effect of THC products is a cause for concern, benefits from some of the cannabidiol metabolites merit exploration for potential therapeutic uses. These products are effective as an anti-inflammatory, as they suppress cytokine and chemokine production which are involved in the inflammatory response. In humans, reported therapeutic effects have not been isolated to THC and are likely due to the combined administration of all cannabinoids present in marijuana products (Costa, 2007). In addition to being therapeutic, THC toxicity has been observed in companion animal medicine, yet does not have an identified level at which it becomes toxic to the animal's system.

*Combined Administration*

The effects of THC and CBD administered together have been preliminary examined in mice. In one laboratory study, when CBD was administered alongside THC, there were changes in physiological actions. Changes included locomotor suppression, reduced anxiogenic effects, and reduced hypothermic effects. This study also noted that different areas of the brain were activated with singular versus combined administration. Limited effects on the brain were observed with only CBD in low doses. It is suggested that CBD inhibits the effects of THC to an extent, bringing the system near its baseline (Todd & Arnold, 2015). There is little research available on the effects that THC and CBD have on the absorption of the other.

*The Endocannabinoid System*

The Food and Drug Administration and the Association of American Feed Control Officials have encouraged research of cannabinoids in hopes of formatting established dosing guidelines to ensure safety for the food supply (Draeger, 2020). This call to research stems from knowledge that cannabinoids play an important role in bodily functions through naturally occurring endocannabinoids. The endocannabinoid system contains many receptors, the first of those named being $CB_1$ and $CB_2$.

Activation of the $CB_1$ receptor, retrograde inhibition of the neuronal release of chemicals, including acetylcholine, dopamine, gamma aminobutyric acid (GABA), histamine, serotonin, glutamate, cholecystokinin, D-aspartate, glycine and noradrenaline, is achieved. Retrograde inhibition is unique to the endocannabinoid system. In retrograde inhibition, the ligand is fired back from the postsynaptic neuron to the presynaptic

neuron. Activation of the $CB_2$ receptor is closely associated with immune response and regulation of the inflammatory response. These receptors, regulation of bodily responses such as pain, memory, mood, and stress are achieved (Landa et al., 2016). Similarly, phytocannabinoids derived from hemp plants and synthetically created cannabinoids closely resemble naturally occurring endocannabinoids. Phytocannabinoids can activate receptors like endocannabinoids, exhibiting their effects on the system.

Primary cannabinoids, THC and CBD, have the greatest binding affinity for the $CB_1$ and $CB_2$ receptors. Several other cannabinoids possess some affinity for the receptor, yet not as strong as those exhibited by THC and CBD. Cannabinoids with affinity include CBDA, CBDV, CBDVA, THCA, THCV, THCVA, CBNA, CBGA, and CBCA (Draeger, 2020).

### *Product Detection*

Pharmacokinetic knowledge indicates that THC and CBD are metabolized by the body very similarly and show comparable reported absorption times in human studies. Reported absorption through mucosal surfaces indicates that cannabinoids will start to appear in the first plasma sample one-hour post administration. After cannabinoids begin to appear, plasma levels should increase over the next several hours to peak concentration around ten hours post administration (Karschner et al., 2007). Metabolites of primary cannabinoids should start to decrease as its metabolites increase, indicating that the subject is metabolizing the product. A higher dose of product may result in a longer observed half-life in the subject. Cannabinoids and their metabolites have been detected in plasma up to 13 days post oral administration (Grotenhermen, 2003).

*Purpose of Study*

This study sought to evaluate the pharmacokinetics of an oral cannabinoid gel product in cattle in a single dosing. It is expected that CBD should first start to appear in the first plasma sample, at 30 minutes post administration, and increase to peak concentrations around 10 hours before declining. Following the first detection of CBD, metabolites should appear 30 to 60 minutes later.

**Chapter 2: Methods**

This protocol was approved by the Murray State University Institutional Animal Care and Use Committee (Protocol number 21-032, Appendix A).

*Subjects and Management*

Two open cows were selected for use in study. The first subject was a mature five-year-old unregistered Black Angus. The second was a mature six-year-old unregistered Black Angus cross. Subjects weighed 453.59 kg with a body condition score of 4 (Subject One) and 682.66 kg with a body condition score of 6 (Subject Two). During the study, subjects were housed in small dirt pens outside. Subjects were maintained with free access to water and provided fresh hay each morning. Between each collection subjects were given free choice hay and water access.

This study was conducted on May 6, 2021. Reported temperature during administration and collection periods fluctuated between 68-48°F with an average temperature of 57.53°F. No precipitation was recorded. Humidity ranged between 45%-90% over the course of the day with the average falling at 71.26%. Wind speeds peaked at 14 miles per hour. Day length was recorded at 13 hours 53 minutes (Barkley Regional Airport Station, 2021).

Cattle were worked through a chute system under a covered pavilion containing fluorescent lighting. They were moved through an alleyway into a crowding pen. Sorting sticks were used and gentle pressure applied utilizing flight zones to control the subject's

movement. Subjects were moved from the crowding pen and through the raceway into a head gate with a squeeze chute for treatment and collection. A rope halter was used to secure the head to give access to the jugular vein for sample collection. Following treatment and/or collection, the squeeze chute was released and head gate was opened, allowing each subject to return to the post-working pen until the next collection time.

*Cannabinoid Dosing*

An oral gel supplement with naturally occurring phytocannabinoids designed for equine was used. Product was refrigerated in darkness prior to use. This product was a thin gel absorbed mucosally through the buccal surfaces. The manufacturer dosage recommendations for equine was 2.5 mL/453.59 kg, which resulted in delivering 0.0055 ml/kg body weight to each subject. Based on body weight, Subject One received 2.5 mL, and Subject Two received 3.8 ml. Each dose was pre-drawn in a single-use syringe. The syringe tip was inserted into the side of the mouth, with the tip in the middle of the subject's tongue. Delivery was initiated by depressing the syringe plunger and followed by observation of subjects in head gate to ensure that all product remained in the mouth. The subjects were monitored for any product loss out of the mouth for approximately 45 seconds.

The product manufacturer did not have a recommendation on the amount of time that the product should remain in the oral cavity of the animal. The manufacturer recommended that the product be administered approximately 30 minutes before a stress inducing event, or immediately after exercise or injury occurs. For maintenance, the

manufacturer suggests that the product be administered daily in addition to the subject's feed.

### *Sample Collection*

All blood plasma samples were collected from the jugular vein using 18-gauge needles and a Vacutainer® system (Vacutainer®, BD, Mississauga, Ontario, Canada). A total of 60 mL of blood was drawn each collection, three 10 mL red top serum tubes followed by three 10 mL lavender top ethylenediaminetetraacetic acid (EDTA) tubes. All collected tubes were labeled with the subject number and collection time. After labeling, plain tubes were stored upright in a test tube rack and allowed to clot. Each EDTA tube was gently inverted ten times. These tubes were immediately placed in a test tube rack to be stored in a chilled cooler above frozen gel ice packs. Following this, samples were transported immediately after the completion of the collection to an on-campus laboratory to be processed.

Tubes were loaded and balanced into a centrifuge and spun at 3400 RPM for ten minutes. Centrifuged tubes were removed and placed on a sanitized lab bench in test tube racks. Tubes were uncapped one at a time so that the supernatant, serum (red top tubes) and plasma (EDTA tubes), could be removed by a single-use pipette. The extracted liquid was placed in 1.5 mL microcentrifuge tubes. Samples were labeled with subject, date, serum/plasma, and collection time. Microcentrifuge tubes were sealed and stored at -40°C until shipping. This process was repeated for each sample collection: pre-dose (baseline), 30 minutes, one hour, two hours, four hours, eight hours, 12 hours, 16 hours, and 24

hours. Prepared samples were sent to Kansas State University to be analyzed along with samples of the oral cannabinoid gel product.

### *Lab Detection Limits*

Plasma samples and oral gel product were analyzed for the presence of 21 substances (Table 1). These substances are common cannabinoids and their metabolites. Substances tested for include the cannabinoid THC and its metabolites. The compounds and their respective lower limits of detection effective for results reported in plasma and oral gel product can be found in Table 1. Compounds measuring above the lower detection limit appear in reported results. If the compound concentrations fell below the lower limit of detection, they were reported as not detected.

**Table 1**

*Lower Limit of Cannabinoid Detection*

| Compound | Lower Detection Limit (ng/ml) |
| --- | --- |
| CBC-7-acid, CBCA, CBDVA, CBGA, CBLA, THC-acid, THCV | 2.5 |
| CBC, CBD, CBDA, CBDV, CBG, CBL, CBN, THC-acid-glu, THC-glu, THC-OH, THCA-A, THCP | 1.0 |

*Laboratory Method of Analysis*

Kansas State University quantified cannabinoids in oil and serum using ultra-high-pressure liquid chromatography with mass spectroscopy. Methods were adapted from Zhang et al (2016).

Frozen plasma samples were collected and stored at Kansas State University until all samples were received. On the day of analysis, plasma samples were allowed to thaw. From thawed samples, proteins were precipitated by mixing 0.1 mL of thawed plasma with 0.1 mL of internal standard mixture at a concentration of 200 ng/mL and 0.1 mL of acetonitrile with 0.1% formic acid. This mixture was vortexed for five seconds, followed by centrifuging at 7000 g for five minutes. Produced supernatant was diluted with 0.4 mL of ultra-pure 18 $\Omega$ water and loaded onto a solid phase extraction plate with positive pressure nitrogen. Sample wells were washed twice with a 0.25 mL of 25:75 mixture of methanol-water. Samples were then eluted with two-25 μL aliquots of 90:10 acetonitrile-methanol and then diluted by 50 μL of water before analysis. Chromatographic separation was performed to generate plasma concentrations (Kleinhenz et al., 2020).

Cannabinoid oil concentrations were determined by obtaining one gram of product and hydrating it on a vortex for 15 minutes in a 50 mL polypropylene tube with 10 mL of 18W water. To each sample, 10mL of 2% formic acid in acetonitrile was added. This was followed by the addition of 4 g $MgSO_4$ and 1 g NaCl and one ceramic stone. These mixtures were transferred into a shaker and centrifuged at 3,000 g for 15 minutes. The supernatant was extracted and centrifugation was repeated for 5 minutes. This extract was further diluted by 100 and 10,000 by adding a 40:60 mixture of acetonitrile and water. Diluted samples were cleansed by solid-phase extraction. Cleansed

samples were loaded into the solid-phase-extraction sorbent and forced through with a nitrogen-96 processor. The sorbent was washed with 0.2 mL of 5% aqueous ammonium hydroxide and 0.2 mL of a 50:50 mixture of water-methanol. From this, cannabinoids were eluted in 0.15 mL of methanol, containing 1% formic acid. Before analysis, 0.15 mL of water was added to each well, letting it mix while covered for 10 seconds. Cannabinoid concentration analysis was then performed by triple quadrupole mass spectrometry and chromatographic separation (Kleinhenz et al., 2020).

### *Statistical Analysis*

Based on the design of the study, including a limited number of animals and minimal results, statistical analysis of the data was not performed. Detected metabolites were noted and tabulated.

## Chapter 3: Results and Discussion

*Subject Handling*

The two subjects used during this study were separated from their herd for the duration of collection periods. The subjects were handled as little as possible to prevent inducing excess stress. While working subjects, gentle pressure by the utilization of flight zones was important in movement from holding pens to working areas. While all researchers present had experience working with cattle, it should be considered in future studies that all involved researchers undergo training for study expectations in order to better provide consistency in handling expectations. The administration of the product takes an experienced hand to avoid underdosing. It is also important that blood collections be clean draws to prevent potential errors in laboratory analysis. This combination leads to the potential of results being impacted by slight differences based upon the technique for administration and collection.

The design of this pilot study did not account for individual behavior of cattle, as it was solely focused on detection of cannabinoids in plasma samples post administration rather than possible effects of cannabinoid dosing. The design of this pilot study was set to only determine if the oral gel product was able to be detected in the subject's plasma before continuing to the possible observed impacts of the dosing.

*Company Product Analysis*

The manufacturing company had product lot information linked to product packaging. Samples from each ingredient used were obtained and analyzed. The displayed sample results were broken down into CBD, CBC, CBG, and several product additives. In these analyses, safety information regarding residue and a cannabinoid profile analysis were provided (Table 2, Table 3). The method of liquid chromatography diode array detectors was utilized in the company lab; procedural methods used are not published to the public.

**Table** *2*

*Cannabinoid Concentration Reported from Company Sample Analysis*

| Cannabinoid | Sample Number | Concentration (mg/g) | Conversion Factor (g/ml) |
|:---:|:---:|:---:|:---:|
| CBG | 1061934[a] | 6.27 | 1.01 |
| CBC | 1076066[b] | 829.82 | Not reported |
| **Δ**9-THC | 1066032[c] | <LOQ | 1.06 |
| CBD | 1066032[c] | 132.65 | 1.06 |
| CBDV | 1066032[c] | 0.86 | 1.06 |
| CBG | 1066032[c] | <LOQ | 1.06 |

*Note. Values reported as not detected are omitted from this table.*

[a] *Reported on 10/29/2020.*

[b] *Reported on 03/15/2021.*

[c] *Reported on 12/11/2020.*

**Table 3**

*Cannabinoid Lower Detection Limits Reported from Company Sample Analysis*

| Cannabinoid | Lower Limit of Detection (µg/g) | Lower Limit of Quantification (µg/g) |
|:---:|:---:|:---:|
| CBC | 0.27 | 0.8 |
| CBD | 0.067 | 0.2 |
| CBG | 0.4 | 1.0 |
| THC | 0.067 | 0.2 |

### *Study Product Analysis*

In the gel cannabinoid product, laboratory results detected CBD, CBG, CBN, CBC, and CBDV (Table 4). This aligns with packaging advertising that the product contains CBD, CBC, and CBG, along with other non-cannabinoid additives that were not tested for in this study. Product packaging directly stated that the oral cannabinoid gel product did not contain THC. There were detectable amounts of 9-THC discovered (Table 4), however, this amount of THC was below the manufacturers LLOD of 0.067 mg/g and well below United States legal specifications for hemp (0.03%) (*Agriculture Improvement Act of 2018,* 7 U.S.C § 1639o *et. seq.)*. That being said, neither hemp nor marijuana is yet approved for administration in livestock on a commercial basis.

The cannabinoid product was very thin in consistency. This thin product is designed to be best absorbed through the mucosal membranes of the animal's oral cavity. This leads to potential loss of product easily occurring, as its low viscosity does not assist in keeping the product in the animal's oral cavity. Due to this, its appeal to commercial consumers may be limited, as it slows working speed so that each subject can be

individually dosed and monitored to make sure that they do not let the product immediately leak out of their mouth. If product is allowed to leak from the mouth post administration an incomplete dosage leading to decreased absorption potential will be observed. This designed route of administration prohibits the use of a drench for this product as it needs to stay in the oral cavity as long as possible for maximum absorption. If the product is immediately allowed to continue down the digestive tract, its absorption has the potential to be reduced and/or delayed. In this study, the subjects were observed moving the product around in their oral cavity after administration, leading to increased chances of absorption.

Not examined in this study is the alternative manufacturer labeling of long-term use in the form of a feed additive. Typically, cattle are fed in group settings, not individually. If the product was used for long term maintenance in this situation, then its dosage to each animal in a herd would not be measured. However, this would make administration to large herd sizes more efficient. The product would also be less likely to fall from the oral cavity as it would be blended with grain rations.

In comparison to the Kansas State lab, the method of detection used by the company appears to be less sensitive in cannabinoid detection levels (Table 1, Table 3, respectively). This is likely from the difference in protocol by the laboratory and method used. Methods used for this same standard are not available from the company.

Finally, the question of shelf stability is brought into play, as there is a gap of research in the discipline. With the earliest samples from manufacturing being obtained 13 months before final product analysis, there is potential for cannabinoid concentrations to differ from original reports.

**Table 4**

*Study Cannabinoid Concentration in Gel Cannabinoid Product*

| Cannabinoid | Concentration | | |
| --- | --- | --- | --- |
| | μg/ml | mg/g | % |
| CBD | 43,944 | 43.944 | 4.394 |
| CBG | 1,279 | 1.279 | 0.128 |
| CBN | 3.9 | 0.004 | 0.0004 |
| CBC | 2,700 | 0.407 | 0.041 |
| CBDV | 407 | 0.407 | 0.047 |
| 9-THC | 36.6 | 0.037 | 0.004 |

*Note. Gel product was administered on May 6, 2021. Gel product analysis was performed on June 5th, 2021.*

### *Plasma Analysis*

In plasma samples, laboratory analysis revealed only two compounds present, CBD and CBD-7-acid, over the 24-hour sampling period (Table 5). In Subject Two, CBD was detected at 1-hour post administration, making it the first compound to appear in samples. This is the only time that CBD was detected in Subject Two. Contrastingly, Subject One sample analysis revealed CBD only at hour 24 post administration. There was no THC detected in plasma samples from the 24-hour collection period. Studies on THC and CBD pharmacokinetics indicate that THC and CBD are metabolized in such similar ways, so their appearance in subject samples should be observed at similar timing if it were going to occur (Grotenhermen, 2003).

**Table 5**

*Cannabinoid Concentrations in Plasma of Subjects from 24-hour Sampling Period*

| Time (Hour) | Cannabinoid | Concentration (ng/mL) | |
|:---:|:---:|:---:|:---:|
| | | **Subject 1** | **Subject 2** |
| 1 | CBD | – | 6.1 |
| 8 | CBD-7-acid | – | 3.4 |
| 12 | CBD-7-acid | 2.9 | 12.8 |
| 16 | CBD-7-acid | 7.7 | 27.8 |
| 24 | CBD-7-acid | 32.2 | 62.9 |
| 24 | CBD | 5.8 | – |

*Note. Substances reported from the lab as not detected are omitted. Only detectable concentrations are listed above. Reference lower limit of detection listed in Table 1.*

As shown in the table above, there is inconsistent data from what was expected based upon literature. The appearance of CBD in Subject One at the 24-hour mark only contradicts the earlier appearance of CBD-7-acid. With CBD-7-acid being a metabolite of CBD and not being detected in product samples, it raises the question of if plasma samples were mislabeled in processing or at the laboratory. If the plasma sample from hour 24 was switched with a sample time before hour 12, the results would adhere to expected predictions.

The appearance of CBD-7-acid likely occurred between the four- and eight-hour collection period, as it first becomes detectable at 2.5 ng/ml. As seen in Table 5, the concentration of CBD-7-acid was on an upward trend at the end of the 24-hour pilot study. This indicates that the CBD had likely reached peak concentrations and was

actively being metabolized by the subjects. The appearance and rise of CBD-7-acid is the result of hydroxylation and oxidation of CBD, indicating that metabolism was occurring (Mechoulam & Hanuš, 2002).

While subjects were administered a comparable dose for their respective weights, the cannabinoid product seems to be absorbed at differing rates between the subjects. This is to be expected as the subjects presented at different body condition scores and CBD is a fat-soluble compound. In the results, the detection seemed to be inconsistent with first appearance and continued detection between the subjects. With the varying body condition scores, this may be an influence on the metabolism of cannabinoids. Fat soluble compounds need available fat to bind with in order to enter the bloodstream. The difference in scoring across subjects has the potential to alter the time that is required for absorption and excretion.

The potential of impaired absorption from a recent feeding should be explored. With the cannabinoids being fat soluble, subjects with a fatty meal in their system may have more rapid metabolism. In order to examine if this has an impact on the pharmacokinetics, fasting subjects and subjects on routine diet should be compared.

Similarly, time spent in contact with buccal surfaces could also lead to discrepancies in observed absorption. This is a challenge with livestock, as they cannot be instructed to hold the product in their mouth for a set amount of time. With this product there is no way to keep the product in place for a measured amount of time, as the animal is free to swallow at any point. It is possible that favorable palatability of the product could lead to increased time spent being worked in the mouth. In addition,

improved consistency and detection would likely be observed with long term maintenance dosing of the product in feed rations.

*Implications*

The use of industrial hemp usage comes with an ongoing concern of possibly containing THC. This raises the question of product quality control: however, as long as companies are willing to conduct proper testing and publish results there should be no issues. In this study, one 2.5 mL package of product was sent to be analyzed. Subjects were dosed with 2.52 packets of product in total. In future studies a sampling of product from different lots should be sent in to obtain an average of cannabinoid concentrations prior to subject dosing.

Limited research is published in regard to the shelf stability of products containing cannabinoids. It would be beneficial to have allotted product to store in various locations and conditions and pull samples from over time. This provides insight to how and if the cannabinoid levels fluctuate. Environmental storage conditions and time are both likely factors to product life. Possible product deterioration could result in improper dosing.

When administering the product, absorption has the potential to vary depending on how long the product remains in contact with the mucous membrane, possibly affecting cannabinoid levels reported in plasma. The time that the product remained in contact with the mucous membranes was not measured in this study. As subjects were only monitored after administration to ensure that product was not lost out of the mouth

immediately. In future studies the potential to measure the time the product spends in the oral cavity on average should be explored.

Further, future studies sampling period should be increased in order to expand tracking of concentrations in the animals after administration. This combined with more frequent sample collections will allow for a better analysis of how cannabinoid levels fluctuate after administration. This could possibly be achieved by a continuous blood sampling pump that is often used in human studies to create a continuous monitoring curve. Future studies should explore this option as a possibility.  In comparable studies orally administered cannabinoids can be detected up to two weeks after administration (Grotenhermen, 2003). In order to establish how long product remains detectable in the subjects' plasma, sampling period should be extended until no cannabinoids are detected for an extended period. Due to the short nature of this study, an observed half-life was unable to be established.

In addition to an explained study length, sampling variety should be increased to achieve data from a wide range of body condition scores to assess how varying condition affects the metabolism of the cannabinoid product. Subjects' condition should be recorded over the course of longer studies to account for growth and fluctuation. In this study, two mature cows were used, age and stage of development has the potential to affect the metabolism of cannabinoids and should be explored to better evaluate.

The combined alterations of the study would allow a better understanding of the pharmacokinetics of cannabinoids in cattle. Without these changes it is likely that studies will result in inconclusive data.

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# Appendix A

**MURRAY STATE UNIVERSITY**
**Institutional Animal Care and Use Committee**

April 26, 2021

Dr. A. J. Davis
Animal/Equine Science
Murray State University
306 S. Oakley Applied Science Building
Murray, KY 42071

Dear Dr. Davis:

The Murray State University Institutional Animal Care and Use Committee (IACUC) has
approved your protocol for the project titled, "Effects of Cannabinoid Supplementation on
Weaning and Transportation Stress in Beef Cattle."

The protocol timeline is approved through December 31, 2021. 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

We are Racers.                                                                murraystate.edu

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