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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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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 broken down 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, then detection will be important for determining regulations of use. A withdrawal period can then be established to allow time for excretion before livestock products enter human markets. This study sought to determine the pharmacokinetics of a single dose of an oral CBD supplement in ruminant animals. Based upon current knowledge, first appearance of cannabinoids should occur at one hour post administration and increase to peak concentrations at approximately ten hours. An oral gel cannabinoid-containing product designed to be absorbed through mucosal membranes was used in this study. The product was labeled as containing no tetrahydrocannabinol (THC). 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. The product was found to contain THC, contradicting product labeling. 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. In

this study, 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. One area of innovation that should be considered for a place in the future of the cattle industry is industrial hemp. 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, and others. 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 application is surrounded by controversy due to close relation to the psychoactive compound tetrahydrocannabinol (THC), which is associated with marijuana. In many areas, THC is illegal to use or possess and can result in legal charges. In 2018, hemp was defined 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.*). This separation of hemp and marijuana is critical to use in medicine and livestock production, as both plant varieties have naturally occurring THC.

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 fell off in the 1930s due to increased popularity of opium, as well as unreliable product quality (Klumpers & Thacker, 2019). Today, cannabis use is very limited and restricted under federal regulations. The United States Department of Agriculture (USDA) requires that all hemp research and production must be submitted for approval in order to obtain a license. Guidelines for what to be included in request for a project permit is outlined in the Agricultural Improvement Act of 2018. States are permitted to establish and enforce regulations more strict than those set federally. As part of regulations, all facilities are subject to annual inspection of crops at random. These inspections ensure that crops are remaining within the definitive concentrations of chemical composition.

Cannabinoids are a group of fatty compounds derived from cannabis plants. In the plant, cannabinoids account for the majority of the bioactive compounds. In the body, they can be found exerting their effects through the endocannabinoid system, bonding to cannabinoid receptors type 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. 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.

Cannabinoids have been explored in areas of modern medicine for a wide array of desirable effects. Effects worth noting in human medicine include the potential use as an antiemetic, an appetite stimulant, an analgesic, and the treatment of neurological diseases such as multiple sclerosis (Costa, 2007). While 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 a variety of other chronic conditions. There have been reported possibilities of beneficially reducing depression or 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 component associated with the use of THC products is a cause for concern among many, benefits that can come along with some of the cannabidiol metabolites raise a need for exploration of their potential therapeutic uses. There is reason to believe that these products can be used as an anti-inflammatory, as they suppress the 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 cannabinoids present in marijuana products (Costa, 2007).

Combined Administration

The possibility of THC and CBD when administered together has been preliminary examined in studies with mice. In one laboratory study, when CBD was administered alongside THC reported changes in physiological actions. When the compounds were administered together, locomotor suppression, reduced anxiogenic effects, and reduced hypothermic effects were observed. In addition this study noted that different areas of the brain were activated with singular versus combined administration. Further, 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 Organization have encouraged research of cannabinoids in hopes of formatting an established dosing guide to promote safety for the consumer and food supply, which have prompted several studies in recent years (Draeger, 2020). This call to research stems from knowledge that cannabinoids play an important role in bodily functions through naturally occurring endocannabinoids. This endocannabinoid system contains many receptors, the first of those named being CB_1 and CB_2.

Through the activation of the CB_1 receptor, retrograde inhibition of the neuronal release of chemicals, including acetylcholine, dopamine, GABA, histamine, serotonin, glutamate, cholecystokinin, D-aspartate, glycine and noradrenaline, is achieved. The activation of the CB_2 receptor is closely associated with immune response and regulation of the inflammatory response. With the activation of these receptors, the 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 the naturally occurring endocannabinoids. These phytocannabinoids can activate receptors like endocannabinoids, exhibiting their effects on the system.

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 sample one hour post administration, as it should be rapidly absorbed through the mucosal membranes. After cannabinoids begin to appear, levels should increase over the next several hours to peak concentration around ten hours post administration (Karschner et al., 2007). Metabolites of originally detected cannabinoids should start to decrease as its metabolites are detected and increase, indicating that the subject is breaking down the product. It has been discussed that a higher dose of product will result in a longer observed half-life in the subject. Cannabinoids and their metabolites have been discovered remaining traceable in plasma concentrations 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 the ruminant animal in a single dosing. In this study, it is expected that cannabinoids should first start to appear in the first collection, at 30 minutes post administration, and continue to peak concentrations around 10 hours before trending downward. Following the first detection of CBD, metabolites should appear 30 to 60 minutes later, indicating that the product is being metabolized by the ruminant animal.

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 subject was a mature six year old unregistered Black Angus mix. 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 next to the working area. Subjects were maintained with free access to water through a ball watering trough and provided additional fresh hay each morning. Between each collection subjects were given free choice hay and water access. Environmental data was recorded using the Barkley Regional Airport Station.

Cattle were worked through a chute system that was located under a covered pavilion containing fluorescent lighting. Cattle were moved through an alleyway and into a crowding pen. To assist in movement, sorting sticks were used and gentle pressure was applied by utilizing flight zones to control which way the subjects wanted to move. Subjects were moved through the crowding pen and into the raceway leading into a head gate with a squeeze chute, where they entered individually for treatment and collections. A rope halter was used to secure the head to the side while giving access to the jugular vein for sample collection. Following treatment or collection, squeeze chute was released and head gate was opened, allowing subjects to return to the post-working pens until the next collection time.

Cannabinoid Dosing

An oral gel supplement with naturally occurring phyto-cannabinoids was used. Product was stored in a residential fridge unexposed to light. This product was a thin gel designed to be absorbed mucosally through the buccal surfaces. Manufacturer product dosage recommendations was 2.5 mL/453.59 kg, which resulted in delivering 0.0055 ml/kg body weight. Based on body weight, Subject One received 2.5 mL, and Subject Two received 3.8 ml. To deliver the product, each calculated dose was predrawn for accuracy in a single-use syringe. The syringe tip was inserted into the side of the mouth, with the tip landing 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 time that the product remained in the oral cavity was not measured, but 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. The product was administered with a syringe with the goal of placing the product in the cheek or underneath the tongue. For maintenance, the manufacturer suggests that the product be dosed daily by addition to the subject's food rations.

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 period to an on campus laboratory to be processed.

In the laboratory, tubes were loaded and balanced into a centrifuge and spun at 3400 RPM for ten minutes. Once complete, 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. Filled tubes were labeled with subject, date, serum/plasma, and hours after dosage administration. Microcentrifuge tubes were sealed and placed within plastic bags inside of a -40℉ freezer to prepare for 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, 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 should 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 Ω 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

Environmental Conditions

This study was conducted on May 6, 2021. Reported temperature during administration and collection periods fluctuated between 68-48℉ with an average temperature of 57.53℉. 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).

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.

Product Analysis

In the gel cannabinoid product, laboratory results detected CBD, CBG, CBN, CBC, and CBDV (Table 2). 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. There were also detected amounts of 9-THC reported (36.6 mg/ml, 3.6%). Product packaging directly states that the oral cannabinoid gel product does not contain THC. This amount of THC contained in a product that is being marketed as hemp contradicts guidelines imposed by USDA. That being said, neither hemp nor maijuana is yet approved for administration in livestock on a commercial basis.

Table 2

Cannabinoid Concentration in Gel Cannabinoid Product

Cannabinoid	Concentration (mg/ml)
CBD	43,944
CBG	1,279
CBN	3.9
CBC	2,700
CBDV	407
9-THC	36.6

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

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, it can be noted that 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.

Company Product Analysis

The manufacturing company has Lot information linked to product packaging. Samples from each ingredient used were obtained and analyzed. The displayed sample results are 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 3). The method of Liquid Chromatography Diode Array detectors were utilized in the company lab; procedural methods used are not published to the public.

Table 3

Cannabinoid Concentration Reported from Company Sample Analysis

Cannabinoid	Concentration (mg/g)	Conversion Factor (g/ml)
	Sample 1061934[a]	
CBG	6.27	1.01
	Sample 1076066[b]	
CBC	829.82	Not reported
	Sample 1066032[c]	
Δ9-THC	<LOQ	1.06
CBD	132.65	1.06
CBDV	0.86	1.06
CBG	<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.*

Contrary to product marketing, THC did appear in one sample, falling between the Limit of Detection (LOD) and Limit of Quantification (LOQ). Company claims indicate that there are 250 mg of CBD per unit of the product, with one unit being listed as 3.5 mL. With the manufacturing process and ingredient quantities not available, it is unknown if the reported sample concentrations and oral gel product contain comparable amounts of cannabinoids.

In comparison to Kansas State, the method of detection used by the company appears to be less sensitive in cannabinoid detection levels (Table 4). This is likely from the difference in protocol by the laboratory and method used. The possibility of residual THC from outside samples does exist both for Kansas State analysis and for product manufacturing. While the contamination of the product at Kansas state is possible, it is not probable, as their methods demonstrate procedural cleaning to prevent cross contamination. Methods used for this same standard are not available from the company.

Additionally, 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. While cannabinoid metabolites may increase in concentration if the original cannabinoid starts to deteriorate, an increase from baseline concentration should not be observed.

Table 4

Company Cannabinoid Lower Detection Limits

Cannabinoid	Lower Limit of Detection (mg/g)	Lower Limit of Quantification (mg/g)
CBC	0.27	0.8
CBD	0.067	0.2
CBG	0.4	1.0

Plasma Analysis

In plasma samples, laboratory analysis revealed only two compounds present, CBD and CBD-7-acid, over the 24 hour sampling period. 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. Despite THC being present in product, there was no THC detected in plasma samples from the 24 hour collection period. In accordance with studies on THC and CBD pharmacokinetics, THC and CBD are metabolized in such similar ways that their appearance in subject samples should be observed at similar timing (Grotenhermen, 2003). The large difference between the amounts of THC and CBD found in the oral cannabinoid product (Table 1) is likely the cause behind no THC being found in the subject samples.

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.

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 3, 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 occuring (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.

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 usage of industrial hemp usage comes with an ongoing concern of possibly containing THC. As seen in this study, product testing revealed that THC concentrations in the oral cannabinoid product used were over legal limitations. This raises the question of product quality control, as published company sample analysis demonstrates that there is no THC contents. 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. If limits still exceed USDA regulations then consumers would need to seek an alternative cannabinoid product.

Limited research is published in regards 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.

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Appendix A

IACUC documentation