MURRAY STATE UNIVERSITY

Murray State's Digital Commons

Murray State Theses and Dissertations | Student Works

2022

EFFECTS OF CANNABINOID SUPPLEMENTATION ON WEANING AND TRANSPORTATION STRESS IN BEEF CALVES

Savannah Kaitlyn Austin
Murray State University

Follow this and additional works at: https://digitalcommons.murraystate.edu/etd

 Part of the Beef Science Commons

Recommended Citation

Austin, Savannah Kaitlyn, "EFFECTS OF CANNABINOID SUPPLEMENTATION ON WEANING AND TRANSPORTATION STRESS IN BEEF CALVES" (2022). *Murray State Theses and Dissertations*. 255.
https://digitalcommons.murraystate.edu/etd/255

This Thesis is brought to you for free and open access by the Student Works at Murray State's Digital Commons. It has been accepted for inclusion in Murray State Theses and Dissertations by an authorized administrator of Murray State's Digital Commons. For more information, please contact msu.digitalcommons@murraystate.edu.

EFFECTS OF CANNABINOID SUPPLEMENTATION ON WEANING AND TRANSPORTATION STRESS IN BEEF CATTLE

A Thesis
Presented to
The Faculty of the Hutson School of Agriculture of Animal Science
Murray State University
Murray, KY

In Partial Fulfillment
Of the Requirements for the Degree
Of Master of Science in Agriculture

Savannah Austin
Spring 2022

Abstract

Transportation stress is a major factor increasing the risk of bovine respiratory disease (BRD) in young calves. Bovine respiratory disease is currently the most economically important disease affecting the cattle industry, costing producers millions of dollars annually. Cannabidiol (CBD) has shown therapeutic benefits in other species, making it a potential tool to use as a supplement to reduce transportation and weaning stress in calves. However, little information is available on cannabinoid bioavailability and disposition of bioactive residue in livestock tissues. The objectives of these studies were to evaluate effective dosage rates of a cannabinoid gel on beef cattle and to determine its effects on weaning and transportation stress. The pilot study served as a short pharmacokinetic study to determine an effective dosage rate for the gel and to observe plasma cannabinoid clearance rate. Two mature cows were dosed at 2.5 mg/kg. Plasma samples were collected at 0, 0.5, 1, 2, 4, 8, 12, 16, and 24 hours following treatment administration. A second, longer study was conducted after the pilot was completed. Phase 1 investigated effects of cannabinoid administration on weaning stress in 27 beef calves. Phase 2 evaluated effects of the same cannabinoid supplement on the same calves during a transportation period. Based on results from the pilot study a dosage rate for the calves was set at 5.0 mg/kg. At weaning, calves were treated and blood was collected at 0, 6, 12, 24, 48 ,72, and 96 hours post treatment and evaluated for complete blood count (CBC) and serum chemistry panels. Video observations were taken using a drone multiple times a day for 4 d to observe weaning stress behaviors. Behaviors observed included bawling, pacing, and standing at fence-line. Phase 2 investigated the effect of treatment on transportation stress in the same 27 calves, approximately a week after the start of phase 1. Calves were dosed again before being loaded onto a trailer for the transportation segment. Treatment was the same as phase 1. Blood samples, body weights, chute scores and exit velocities were collected

pre- and post-transportation. Statistical analysis was conducted on CBC, serum chemistry, and behavior evaluations using the PROC MIXED procedure of SAS. In the pilot study, metabolite CBD7-acid was detected as early as 8 hours and on an upward trend at the end of sampling. Phase 1 CBC and serum chemistry values were within normal reference ranges; however, differences were observed for monocytes (CON=0.92 LSM, TRT=0.69 LSM, P<0.0001). Interactions were observed for white blood cell (P=0.03), red blood cell (P=0.02), hematocrit (P=0.03), and eosinophils (P=0.04). Phase 1 and 2 serum chemistry analysis found significance for CPK (P=0.05), Bilirubin (P=0.05), and glucose (P=0.04). No statistical significance was found for any of the behavior evaluations. Results suggest that cannabinoid treatment may mitigate a stress response in cattle based on the effects on stress biomarkers within blood analysis.

Table of Contents

Introduction

Background and Setting

Cattle experience significant stress during weaning. Under modern cattle production systems, weaning is considered a multifaceted stress event involving nutritional, physical, and psychological elements triggering specific behavioral and physiological responses such as bawling, pacing, and reduced feed intake (Weary et al., 2008; Lynch et al., 2019). In addition to weaning stress, many calves are also exposed to transportation stress during this time. Selling newly weaned calves is a common practice in the U.S. for beef producers. These calves are then transported to other facilities and commingled with other calves. Furthermore, these calves typically experience limited access to feed and water during transport and changes in environmental conditions (Burdick et al., 2011). In a recent review on management practices to improve animal health and well-being for newly weaned calves, Wilson et al. (2017), compiled information from numerous studies establishing a profile for calves most susceptible for developing bovine respiratory disease (BRD). This review indicated that calves at greatest risk of developing BRD were lighter-weight, abruptly-weaned calves that were transported over long distances with limited availability of quality nutrition and water.

BRD has been referred to as the most economically important disease affecting stocker calves and feedlot cattle in the U.S. (Theurer et al., 2013). The economic impact of BRD on the U.S. beef industry has been tremendous. The 2011 National Animal Health Monitoring System's (NAHMS) Feedlot study showed that roughly 21.2% of beef cattle in feedlot settings were affected by BRD, causing approximately 45-55% of the total deaths in feedlots. The study estimated annual economic losses due to BRD for treatment and death

loss to be $800-$900 million, putting the cost of treatment per head at $23.63 (NAHMS Feedlot, 2011; Johnson et al., 2017). Of the stress events mentioned previously, exposure to transportation stress has been identified as the greatest factor increasing the likelihood of a calf developing BRD. Thus, it is imperative for animal health and profitability of producers to investigate novel methods to reduce weaning and transportation stress in beef calves.

Industrial hemp (IH) production has increased in recent years with increasing interest in its use as an alternative feed source for livestock. The 2014 Farm Bill (Agricultural Improvement Act of 2014) legalized cultivation and production of IH for research purposes. Although IH is not approved as a feedstuff for livestock, removal of IH from the Federal List of Controlled Substances and passing of the 2018 Farm Bill opened the door to allow investigation of the effects of feeding IH and IH by-products (IHBP) on health and performance of livestock (Agricultural Improvement Act of 2018; Nepveux et al., 2019).

Little information is available on the pharmacokinetics of IH or IHPB in cattle nor the effects of feeding IH upon transportation stress in cattle. Cannabidiol (CBD) has shown therapeutic benefits in both human and animal medicine, making it a potential supplement that can be used by cattle producers to help diminish the effects of stress associated with weaning and transportation. Therapeutic benefits of cannabinoid supplementation, such as reduced stress, anxiety, and improved behavior have been observed in humans (White et al., 2019), horses (Draeger et al., 2020), and dogs (Deabold et al., 2019). However, little information is available regarding IH or cannabinoid supplementation in cattle. In fact, this author has not been able to find any information regarding use of these products as a means of reducing stress in cattle. Therefore, the objective of this study was to determine the effects of cannabinoid supplementation on weaning and transportation stress in beef calves.

Statement of the Problem

Industrial hemp production has increased in recent years. However, little information has been available concerning cannabinoid bioavailability and disposition of bioactive residue in livestock tissues. The 2014 and 2018 Farm Bills allowed further investigation of IH and its by-products on health and performance in livestock species. (Agricultural Improvement Act of 2014 and 2018). Bovines experience a significant amount of stress during weaning. Due to the stress associated with weaning, transportation, limited availability to feed and water during transport, environmental changes and commingling, calves from the south and southeastern portions of the United States are at a greater risk of developing BRD (Burdick et al., 2011). Thus, methods of mitigating stress involved with weaning and transportation in cattle have become very important.

Purpose of the Study

The objective of this study was to determine if administration of a liquid cannabinoid supplement, prior to weaning and transportation, could be useful in reducing weaning and transportation stress in beef calves.

It is hypothesized that cattle exposed to a liquid cannabinoid supplementation prior to weaning and transportation will demonstrate: 1) reduced weaning stress behavior during and following the event of weaning 2) a calmer behavior following transport indicated by lower chute scores and exit velocities from a chute system, and 3) reduced serum lactate dehydrogenase (LDH) activity. While weaning and transportation of cattle are inevitable, it is more important than ever to find novel ways to minimize stress associated with these basic management practices. Millions of cattle each year are directly affected by BRD with

transportation as the main stress factor contributing to its development. Administration of a single dose liquid cannabinoid supplement to cattle has the potential to be a practical, cost-effective resource available to producers to reduce weaning and transportation stress, ultimately improving animal health and behavior while increasing profitability.

Research Questions/Hypotheses

The following questions were addressed during this study:

1. Does administration of cannabinoid supplementation at manufacturer recommended dosage rates result in detectable concentrations of cannabinoids in bovine blood plasma?
2. Do calves receiving cannabinoid supplementation exhibit changes in body weight (BW), blood parameters (LDH and liver and kidney enzymes), and behavior (vocalizations, pacing, grazing, standing but not grazing) during weaning?
3. Will cannabinoid supplementation influence shrink BW, chute scores, and exit velocity in newly weaned transported calves?

Significance of the Study

Little information is available on effects of supplementing/feeding IH and industrial hemp by-products to cattle. Lack of FDA approval largely limits use of IH as a feedstuff in cattle diets, making most uses of IH illegal. Products used on animals in general are pushed through an unregulated market. Food animals, on the other hand, have not been allowed to consume those products due to lack of knowledge and research concerning bioactive residual effects of cannabinoids in livestock tissue. The pilot study from this research project was intended to determine practical dosage rates for beef cattle in order to evaluate

concentrations and clearance rates of a cannabinoid supplement, which were used in primary study to investigate treatment on stress events in young beef calves. A goal of this study was to provide more insight on IH and cannabinoid research on cattle stress and behavior.

Review of Related Literature

Stress in Cattle

Stress is “a state in which homeostasis is threatened or perceived to be so; homeostasis is re-established by a complex repertoire of behavioral and physiological adaptive responses of the organism. The stress response is stimulated by a stressor and affects the body through activation of the hypothalamic- pituitary-adrenal axis (HPA axis) system and the sympathetic nervous system” (Burdick et al., 2011). Stress can be divided into two aspects, short term or chronic (long term). Short term has been defined as a fight-or- flight stress response, usually lasting minutes, or hours. Short term psycho-physiological stress response, in contrast with long term stress, is more of a survival mechanism serving to enhance protection and performance in stress-inducing events. When a fight-or-flight response has been activated, numerous physiological functions including cardiovascular, neuroendocrine, and musculoskeletal systems help prepare an immune system to fight off infection or other challenges facing a body. Humans and animals experiencing a short-term stress response during a time of immune activation exhibit an enhanced immune response to the stressor (Dhabhar et al., 2018; Dhabhar et al., 2001).

In contrast, chronic or long-term stress adversely affects overall health by modulating biological mechanisms functioning on an immune system (McEwen et al., 1998). These stress actions include weakening health, inhibiting mental and physical performance, and exacerbating disease (Dhabhar et al., 2018). Chronic stress usually persists for several hours to several months or more depending on stress intensity, concentrations of neurotransmitting hormones, and length of exposure to stress inducers.

Stress lasting longer than a few hours is detrimental to an animal's immune system and can have lasting effects whereas short term stress will usually enhance the immune response and mental and physical performance. Short term stress allows animals to adapt to minor stressful situations (Dhabhar et al., 2018).

Weaning Stress

Modern cattle production makes events of weaning and transportation inevitable. Cattle experience significant stress during weaning and transportation. Weaning is considered a multifaceted stress event involving nutritional, physical, and psychological elements triggering specific behavioral and physiological responses such as bawling, pacing, and reduced feed intake (Lynch et al., 2019; Weary et al., 2008). Weaning calves involves transitioning them from dependence on the dam for nutrition and social structure to total independence (Weary et al., 2008). This transition usually changes calf behavior and overall diet, adding nutritional and psychological stressors (Price et al., 2003). In practice, weaning typically occurs gradually when milk production from the dam starts to decline and the calf increases forage and concentrate intake depending on availability of feed resources. A calf will also naturally acquire increasing social independence from their dams and have increased interaction with other calves in the herd.

Most producers begin weaning when calves are six to nine mo of age depending on forage quality and availability (Enriquez et al., 2011), with some producers weaning as early as five mo of age. For more intensive beef production systems, calves are ideally weaned early to maximize reproductive potential of a cow which allows her to cycle sooner, yielding one calf per year (Lynch et al., 2019). During weaning, calves are usually subject to other basic husbandry practices that cause additional stress such as

castration, vaccinations, transportation, marketing, mingling with unfamiliar cattle, changes in environment and diet intake (Lynch et al., 2019). Completely eliminating all weaning-associated stress is impossible. However, there are management practices that can help mitigate stress loads on calves. Fence-line weaning has been a popular management strategy that gradually breaks bonds between cow and calf by separating them by a fence line with calves on one side and cows on the other in separate pens (Suverly et al., 2005). Although cow and calf are separated, they can still see, hear, and have nose-to-nose contact with each other. The only major change is that calves can no longer nurse on their dams. This approach minimizes housing and major dietary changes for calves, helping to reduce stress. Cows and calves are kept on pasture for uninterrupted feed intake that meets both animals' nutritional needs (Suverly et al., 2005).

There are specific physical and physiological indicators of weaning stress in a calf. Behavioral changes that result from weaning stress include increased vocalization and locomotor activity as well as decreased feed and water intake (Price et al., 2003). Changes in behavioral distress of calves have been documented over several days with behaviors being very intense over the first few days of weaning (Enriquez et al., 2011; Weary et al., 2007). Weaning distress has been observed in both cow and calf, however, behaviors were more intense and persisted longer for calves than for cows (Lynch et al., 2019). During initial days of weaning, calves exhibit increased bawling, pacing fence lines. These behaviors result in a decrease in feed and water intake. After a few days, stress behaviors observed in calves gradually decline as they calm down and gain independence. Then calves typically increase grazing and start venturing out into pastures while still exhibiting some mild weaning stress behaviors.

Transportation Stress

After the weaning process has finished, cattle producers in the U.S. will typically sell newly weaned calves through a local stockyard or cattle market. Transportation associated with marketing of these calves is a stress event. Calves are transported from one production facility to a next one where they are commingled with other calves. Then these calves are transported to a feedlot setting, frequently in the Midwest. Therefore, weaned calves being shipped have generally been on trailers for prolonged periods of time. Theurer et al. (2013) explains that in 2010, over 34 million head of cattle were shipped to slaughterhouses in the U.S., with most of them being transported at least once in their lifetime prior to slaughter. Transportation of cattle has been a common management event that is largely unavoidable in the beef industry. Transportation of cattle can generate a multitude of negative factors including physical, psychological, and climactic that can influence these animals with different intensities and duration (Damtew et al., 2018).

Several studies have described factors associated with stress to calves during transportation to include pre-transport management, ambient temperature, humidity, social regrouping, inappropriate driving skills, and handling during loading and unloading (Alam et al., 2018; Damtew et al., 2018; Theurer et al., 2013). Having experienced cattle handlers, drivers, and cool temperatures can mitigate most of these issues. Additionally, cattle routinely experience limited availability of feed and water during transportation and differences in environmental conditions between origin and destination (Burdick et al., 2011). Limited access to feed and water, coupled with high

temperatures during transportation will lead to weight loss, commonly expressed as shrinkage rate.

The impact of weaning and transportation stress on cattle is detrimental if not mitigated effectively. These stressors can lead to poorer quality livestock and economic loss for producers. Weaning and transportation are associated with increased secretion of stress related hormones typical of both physical and psychological stress. This leads to adverse effects on cattle growth on performance, primarily due to disruption of their immune function (Burdick et al., 2011).

Assessment of Stress

Evaluation of stress on a calf's body can be determined through numerous biological markers. Biological markers, as defined by the World Health Organization, are "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological; the measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction." In general, biomarkers are measurable characteristics of biological processes that show objective evidence of a subject's health status (Strimbu et al., 2010). Assessing stress specifically, it has been appropriate to evaluate biomarkers found in blood and urine samples (Smith et al., 2021).

Biomarkers

Biomarkers that can be observed through blood samples are glucocorticoids, catecholamines, lactate dehydrogenase (LDH), and nitric oxide (NO). In a review of the interactions between cattle temperament, stress, and immune function, Burdick et al.

(2011), explains that cattle with more excitable temperaments exhibit greater concentrations of glucocorticoids and catecholamines in their blood. During a stress event, the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system are activated, leading to secretion of glucocorticoids and catecholamines (Hay et al., 2000).

In cattle a primary glucocorticoid is cortisol, followed by epinephrine (Burdick et al., 2011). Secretion of glucocorticoids is a standard endocrine response to stress. During a stress event, glucocorticoids are secreted and then synthesized by the adrenal cortex as a response to adrenocorticotropic hormone which ultimately stimulates the process of gluconeogenesis to provide energy for a "fight or flight" response. Elevated serum concentrations of glucocorticoids indicate exposure to physical and physiological stressors (Whirledge et al., 2010).

Although glucocorticoids and catecholamines are extremely good indicators of a stress response, it is difficult to effectively collect samples for reliable basal values of their concentrations. These physiological hormones have been difficult to evaluate due to pulsatility and circadian variations of their secretions (Hay et al., 2000). Inconsistencies in the release of these hormones have required samples to be collected very frequently. Continual bloodwork and evaluation of biomarkers is difficult in cattle research, as cattle require more labor to work than other livestock species.

Catecholamines are secreted from adrenal glands; specifically, the adrenal medulla. Norepinephrine is also secreted from sympathetic nerve endings (Mathar et al., 2010; Papadelis et al., 2003). Secretions of catecholamines are a body's reaction to stress, leading to increased heart rate, blood pressure, and breathing rate (Goldstein et al., 2003). Catecholamines include dopamine, epinephrine, and norepinephrine. Each

neurotransmitting hormone carries out functions within a body. Epinephrine, also known as adrenaline, is largely responsible for a body's fight-or-flight response (Goldstein et al., 2003). Secretion of epinephrine during stress allows for increased blood flow to key organs including heart and lungs as well as muscles. Norepinephrine, or noradrenaline, assists epinephrine in responses to stress by increasing heart rate and blood pressure (Goldstein et al., 2003; Mathar et al., 2010). Dopamine regulates motor control, cognitive functions, and brain reward mechanisms (Klein et al., 2018).

Catecholamines are key biological markers for diseases related to blood pressure. One study evaluating increased catecholamine secretion effects on hypertension in mice (Mathar et al., 2010) showed that TRPM4-deficient mice expressed elevated concentrations of plasma epinephrine and urinary excretions of catecholamine metabolites. Another study evaluated effects of dietary copper on lipid and catecholamine metabolism in finishing steers by analyzing blood samples for volatile fatty acids and catecholamine concentrations after steers were administered a dose of norepinephrine at feeding (Engle et al., 2000). These studies are examples of evaluating epinephrine and norepinephrine in animals as biomarkers in blood to determine effects of disease or stress related incidents.

Another biomarker indicative of a stress event occurring in the body is LDH activity. This presence of this enzyme provides evidence of oxidative stress and possible tissue damage as a result (Hooks et al., 2018; Kumar et al., 2018). LDH is contained in most body tissues and is abundant in heart and skeletal muscles. LDH catalyzes the conversion of pyruvic acid into lactic acid as part of carbohydrate metabolism (Brooks et al., 1999; Jovanovic et al., 2010). Concentrations of this enzyme are higher in body

tissues than in blood serum or other tissue fluids (Jovanovic et al., 2010). Location of LDH within a cell is in the cytoplasm. LDH is released into body fluids when cell membranes are disrupted as in the case of tissue damage (Jovanovic et al., 2010). Under normal conditions, healthy animals will have low blood LDH levels. However, under stressful conditions, if the cellular membrane is disrupted LDH will then be released from the cell causing a significant rise in blood concentrations (Jovanovic et al., 2010). Therefore, evaluation of LDH concentrations is a viable means of assessing stress on the body. A study on heat stress in broiler chickens demonstrated that a combination effect of supplementation with genistein and hesperidin reduced LDH levels in breast muscles, indicating a potential reduction in myopathy (Kamboh et al., 2013).

Another neurotransmitter, nitric oxide (NO), is associated with oxidative stress of a body. NO is a signaling molecule synthesized by three enzyme subtypes of nitric oxide synthase (NOS) (Förstermann et al., 2011). Specifically, neuronal NOS has been localized largely in central and peripheral neurons and functions in blood pressure regulation, smooth muscle relaxation, and synaptic plasticity in the central nervous system (Förstermann et al., 2011). Levels of NOS in the brain have been directly related to stress and depression, suggesting NO assists with modulating releases of other neurotransmitters (McLeod et al., 2001). Synthesis of NO has interrelated pathways common to that of production and negative feedback of certain glucocorticoids (McLeod et al., 2001).

Physical Indicators.

Physical indicators of stress can be assessed through numerous noninvasive tests. A straightforward way to determine stress in cattle is through changes in body weight.

Stressed cattle typically exhibit weight loss (shrink) in events like transportation or in high ambient temperatures. There are two types of cattle shrink, excretory and tissue loss. A review of transportation shrink in beef cattle written by Ohio State University defines excretory shrink as a loss of feces or urine and tissue shrink as a loss of fluid from cells which usually occurs when cattle are held off feed and water and subjected to transportation stress (Coffey et al., 2014). The second kind of shrink, tissue loss shrink, has potential to be more important the longer the cattle are transported and subjected to those conditions established previously. Tissue shrink is harder to replace because it is loss of actual tissue weight, not just water weight in the gut and bladder. Urine and feces loss accounts for roughly 10-15% of shrink but this weight can be regained when cattle are back on feed and water (Coffey et al., 2014).

To calculate shrinkage rate, body weight should be recorded prior to and after transportation and used to find the difference. Determined from a study based on factors affecting body weight loss during commercial long-haul transport of cattle, González et al. (2012) found feeder cattle (younger feeder calves) exhibited greater shrinkage rate than fat cattle (older finished calves ready for slaughter) due to longer transport durations. This study also found that cattle loaded in an afternoon and evening experienced greater shrink than those loaded during a night and in mornings because of ambient temperatures during the day (González et al., 2012).

Many factors influence severity of tissue and excretory shrink including type of shrink, weather conditions, cattle health, and prior access to feed/water (Barnes et al., 2017). Limited water and feed access is the primary cause of shrinkage during transportation. Additionally, cattle under distress exhibit decreased feed and water intake,

even when it is available, leading to fill shrink with loss of manure, urine, and rumen fill (Barnes et al., 2017). Heat stress alone causes decreased nutrient uptake in many species. For example, dairy cattle commonly show a 30% decrease in dry matter intake when temperatures are high (Collier et al., 2019). Cattle require a few days or weeks to fully recover excretory and tissue shrink after experiencing weaning and transportation stress. According to a review of managing shrink and weighing conditions in beef cattle, Barnes et al.(2017), cattle experiencing excretory shrink will have a minimal recovery time as they usually gain pre-market weight back after being back on feed and water. Recovery from excretory shrink could be within hours or a day. However, cattle undergoing tissue shrink require days, sometimes weeks for full recovery of pre-market weight because other production and environmental factors are usually taking effect during this time.

Effects of stress can also be evaluated through observing changes in cattle behavior. Cattle temperament and tameness play a major role in their intensity of reaction to a stress-inducing event. Animals accustomed to handling and being around humans are less likely to exhibit stress behaviors than those with no experience being handled (Grandin et al., 1980). Novel methods used to assess cattle temperament include chute scores (Tulloh, 1961) and exit velocity (Vetters et al., 2013). Both tests occur while cattle are in the working facility. Chute scores require cattle to be restrained in a head gate and given a score on how the animal reacts after closure of the head gate (Hoppe et al., 2010). Chute scores are usually given by a single observer using a 5-point scale suggested by Grandin (1993): 1=calm, no movement; 2=restless, shifting; 3=squirming, occasionally shaking of the chute; 4=continuous vigorous movement, and shaking of the chute; 5=rearing, twisting of the body, or violent struggling. Exit velocity can be calculated

using a subjective 4-point scale taken from Lanier and Grandin (2002): 1=walk; 2=trot; 3=run; 4=jumping out of the chute. Another way to evaluate exit velocity has been to measure flight speed after cattle are released from the chute using a timer at a predetermined distance (Vetters et al., 2013). One study showed a relationship between chute scores and exit velocity with growth performance of cattle. To evaluate exit velocity, they used an infrared sensor to measure flight speed over 1.68 m, or 5.5 ft (Bruno et al., 2016).

In conventional operations, cattle handling occurs during routine management practices and is usually associated with stress for cattle. Cattle with limited human interaction typically exhibit more negative behavioral responses during handling, leading to increased risk of injury (Le Neindre et al., 2002). Those animals with excitable temperaments have shown decreased rates of gain in production, decreased immune function, and decreased feed efficiency because of lowered nutrient intake (Bruno et al., 2016; Fell et al., 1999). Use of temperament measurements in breeding programs is suggested to evaluate underlying traits. Heritability of temperament is small to moderate, (Bruno et al., 2016; Burrow et al., 2000; Hoppe et al., 2010). There are differences between breeds for many temperament traits. Hoppe et al., (2010) found that Charolais and Limousin cattle had higher (less desirable) chute scores and exit velocities than did Hereford and German Angus.

Calves exhibit stress-related behaviors during weaning including bawling, pacing the fence-line, and standing at the fence-line (Lynch et al., 2019). Weaning stress behaviors can visually be observed in the field. Drones can be used to monitor livestock health (Merwe et al., 2020). Although predominantly used in agronomic agriculture,

implementation of precision agriculture technologies has paved new ways for drone use in wildlife and livestock management. Improvements in geographic coordinates (geo-tagged images) and heat detection have increased the possibilities for drone monitoring of plant and animal health (Petkovics et al., 2017). Elevated body temperatures in cattle indicate presence of disease or sickness, making temperature screening of animals a viable method of disease detection (Glaster et al., 2011). Use of temperature screening in animals can also detect skin surface temperature, which is important in locating heat stress, as well as showing changes in blood flow patterns to illustrate possible inflammation of injured animals (McManus et al., 2016). Traditional methods of checking temperatures in livestock require close handling and restraint for rectal temperatures, increasing stress load on livestock which leads to decreased performance and immune function (Mihalache et al., 2020). Drones can reduce labor and stress in handling livestock when assessing health and temperature screening through non-invasive practices and could potentially scan entire herds faster than manual processing of animals.

Biological Effects of Stress

Stress adversely affects an animal's immune function, increasing susceptibility to disease. During routine livestock handling, secretion of stress-related hormones, such as glucocorticoids and catecholamines, increases as part of the immune system's reaction to stress events. Cattle with more excitable temperaments exhibit higher concentrations of glucocorticoids and catecholamines, which is associated with poorer immune responses (Burdick et al., 2011). Increased secretions of stress related hormones can inhibit basic physiological functions of a body pertaining to reproductive performance and immune

function (Burdick et al., 2011). Reduced immune response greatly increases risk of disease, leading to lower quality of production and decreased performance of other bodily functions.

Bovine Respiratory Disease

Weaning and transportation stress have a negative impact on cattle immune function, increasing a calf's susceptibility to respiratory illness and disease. BRD is considered a general term referring to upper and lower respiratory disease in cattle (Johnson et al., 2017). BRD is one of the most economically dominant diseases affecting stocker and feedlot cattle in the United States (Theurer et al., 2013). Numerous studies have described BRD as a complex, multifactorial disease with a variety of physical and psychological stressors that can be associated with infectious and non-infectious risk factors (Chirase et al., 2004; Smith et al., 2020; Taylor et al., 2010). Chirase et al., (2004) claimed "BRD complex still represents the main cause of morbidity and mortality of feedlot cattle, with substantial annual economic losses resulting from decreased feed efficiency, and increased therapeutic costs, as well as lower final body weight, average daily gain, carcass weight, and standard USDA grades".

There has been an ongoing economic impact BRD on the beef cattle industry for decades. In the United States, National Animal Health Monitoring System's (NAHMS) systematically surveys feedlot operations regarding overall cattle health and management in order to make a statistical inference on an entire feedlot cattle population in the country. A 1999 NAHMS study showed that roughly 14.4% of beef cattle in a feedlot setting were affected by BRD. By 2011, that had increased to 21.2% of feedlot calves, leading to 45-55% of total feedlot deaths. An estimated $800-$900 million is lost

annually due to cost for treatment and death loss from BRD, putting costs to treat one case of BRD per head at $23.63 (NAHMS Feedlot, 2011). Studies show that with any respiratory disease, producers incur the cost of therapeutic treatment, production losses due to morbidity and mortality, and costs associated with lower production performance (Johnson et al., 2017; Chirase et al., 2004). Calves that recover from BRD still represent a monetary loss to beef producers due to reduced feed efficiency and poor average daily gain during the treatment period compared to healthy, efficient calves.

Beef cattle can become susceptible to BRD by exposure to a multitude of infections and non-infectious stimuli, such as stress, viral infection, or bacterial infection (Johnson et al., 2017). Taylor et al. (2010), identified exposure to transportation stress as the greatest non-infectious risk factor increasing the likelihood of young cattle developing BRD. Several reviews and studies have named transportation as the most probable cause of decreased cattle immune function leading to BRD, hence the layman's term "shipping fever" (Taylor et al., 2010). The event of transporting livestock incorporates different components, each having an effect on incidences of BRD (Taylor et al., 2010). During transport, cattle can experience major changes in ambient temperatures, high stocking densities on the trailer, and limited access to feed and water (Coffey et al., 2014; Galyean et al., 1999; Smith et al., 2020; Theurer et al., 2013). A recent review of management practices to improve animal health and well-being for newly weaned calves established a profile for those calves that were most susceptible to developing BRD. The review indicated that high risk calves were those that have been abruptly weaned, were administered no vaccinations, had not been castrated or dehorned,

had been commingled with other calves, and had moved through an auction market (Wilson et al., 2017).

In 1978, there was a breakthrough in better understanding BRD in feedlots when a study came out evaluating a generation of the pathology of pneumonia, using aerosols of bovine herpesvirus-1 and *Pasteurella haemolytica* (Jericho et al., 1978). This experimental research led to the assumption that BRD could largely be decreased or eliminated when cattle were properly vaccinated (Smith et al., 2020). Further research from Bryant et al., 2008, using *Mannheimia haemolytica* exhibited interesting results when two large scale feedlot operations compared vaccinating with *M. haemolytica* toxoid and non-vaccinated control calves. The study determined that BRD mortality was reduced by 28% (feedlot 1) and 50% (feedlot 2) in calves within treatment groups (Bryant et al., 2008).

Oklahoma State University published an article discussing the epidemiology of BRD, where they explained how efforts have been made to identify the primary bacterial pathogens associated with this disease through necropsy of affected animals. According to their article, BRD is a multifactorial syndrome. The pathogens most implicated are *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis* (Taylor et al., 2010). These known pathogens serve as a basis for using appropriate vaccines on cattle. Multiple vaccines have been used as a preventative against bacterial pathogens previously mentioned including a modified live or a killed virus for bovine viral diarrhea (BVD) types 1 & 2 and bovine respiratory syncytial virus (BRSV) which cover most bacterial agents that lead to contraction of BRD. However, no single vaccine prevents or treats cattle from contracting BRD.

Preventative Measures

There are a few basic animal husbandry practices that are used as preventative measures against calf susceptibility to BRD. One way to reduce risk factors in cattle is to move newly weaned calves through a preconditioning program before they reach feed yards. Reviews of best management practices for newly weaned calves referred to previously indicate that calves at lower risk for BRD will typically be those that originated from a single source, went through a preconditioning program that includes weaning, vaccination, castration/dehorning, and an adaptation to a higher concentrate diet as opposed to nursing and grazing forages (Wilson et al., 2017). When cattle are commingled with other cattle from different farms, they are exposed to different pathogens and may not have the immunity for those new pathogens. This could influence the immune system, causing increased susceptibility to disease

Industrial Hemp

Industrial Hemp is a plant of the species *Cannabis sativa*, with a tetrahydrocannabinol (THC) content of less than 0.35% on a dry weight basis. *C sativa* plants with more than 0.3% THC are classified as marijuana. THC causes intoxication in humans. IH production has increased in recent years, stimulating interest in using it as a feed source for livestock. Traditionally, IH was grown to produce fibers, obtained from stalks and oils extracted from seeds, buds, and unprocessed female flowers (Crini et al., 2020; Kleinhenz et al., 2020a). As production of IH has increased, IH has been grown for many purposes including oil, seed, fiber, and medicine (Small et al., 2002). Hemp fibers are used in production of fabrics, building materials, paper materials, energy, and fuel production. The waste by-product of these processes has been used as bedding for

animals (Crini et al., 2020); Johnson et al., 2018). Hemp oils have a variety of applications including cooking, cosmetics, medicine, personal care, and paints (Crini et al., 2020). Current IH varieties are bred for oil, fiber, or a dual-purpose combination of the two. (Kleinhenz et al., 2020a).

Industrial hemp and marijuana are varieties of plants from the *Cannabis sativa* species. Industrial hemp contains less than 0.3% tetrahydrocannabinol (THC).

Hemp originated in Asia and has been considered one of the oldest domesticated crops. The history of hemp production stretches back at least 4,500 years to China, where the crop was cultivated for fiber, seed, and oil (Allegret et al., 2013; Amaducci et al., 2010; Bouloc et al., 2013; Crini et al., 2020).

In the 1930's, regulation of hemp production began in the U.S. through the Drug Enforcement Agency because of the THC concentrations (Marihuana Tax Act et al., 1937). In 1970, the Controlled Substance Act made all *Cannabis sativa* a Schedule 1 controlled substance. (Controlled Substance Act et al., 1970). Recently, the 2014 Agriculture Improvement Act re-established production and cultivation of industrial hemp only for research purposes, allowing certain research institutions and state departments of agriculture to grow IH through regulated pilot research programs (Johnson et al., 2018; Nepveux et al., 2019). The 2014 Agriculture Improvement Act made further changes, defining industrial hemp as "the plant *Cannabis sativa* L. and any part of such plant, whether grown or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis". The 2018 Agriculture Improvement Act opened even more doors for hemp research and production by removing IH from the Federal List of Controlled Substances, paving a way for

possibilities of investigating IH and IH by-products (IHBP) effects livestock health and performance (Agriculture Improvement Act of 2018; Nepveux et al., 2019).

Alternative Feedstuff for livestock

IH production and processing creates a significant amount of waste by-products. including leaves, fodder, and residual plant fiber. These have a high potential to serve as viable nutrient sources in livestock rations (Kleinhenz et al., 2020a). Since these by-products are fibrous materials, ruminants are the target species for utilization. Little information is available on the effects of feeding industrial hemp by-products to cattle. IH is comparable to other high-fiber by-product commodities. Kansas State University recently evaluated nutrient concentrations, digestibility, and cannabinoid concentrations of different IH plant components and published a complete nutrient analysis (Kleinhenz et al., 2020a). The plants overall had very high fiber content with neutral detergent fiber (NDF) ranging from 28-80% and acid detergent fiber (ADF) ranging from 18-65% dry matter (DM). and determined that in general, plant materials had lower digestibility values. Flowers, leaves, and seed heads are higher in crude protein (CP) and energy content than whole plant and stalk portions (Kleinhenz et al., 2020a).

IH components could serve as a good source of fiber in rations but would be a poor source of energy depending on what portion of a plant was fed (Bailoni et al., 2021; Kleinhenz et al., 2020a). Feeding hemp seed or a variation of that, such as hemp seed cake or meal, provides high crude protein values in a ration for numerous species including ruminants, poultry, horses, pigs and fish (Bailoni et al., 2021; Bouloc et al., 2013). Generally, hemp seed protein values are comparable to soybean and sunflower seeds (Bailoni et al., 2021; National Research Council, 2001). Hemp seed plays a major

role in animal nutrition, with bird and fish feed being top markets (Crini et al., 2020; Farinon et al., 2020).

Cannabinoids

IH varieties grown for oil production contain the greatest amounts of cannabinoids. Cannabinoids derived from IH have been classified as phytocannabinoids, meaning they are plant derived (Kicman et al., 2020). Over 100 phytocannabinoids have been identified, including secondary phytochemical metabolites that all belong to the terpenophenolic compound family, specifically found in *C. sativa* plants (Farinon et al., 2020; Kicman et al., 2020). There are also over 100 terpene compounds found in *C. sativa* (Aizpurua-Olaizola et al., 2016). Cannabinoids and terpenes are responsible for separate effects of the plant, however, together they have acted synergistically together for therapeutic/medicinal purposes (Aizpurua-Olaizola et al., 2016). Two important cannabinoids are THC, the only psychoactive and toxicant compound, and cannabidiol (CBD), a non-psychoactive compound (Atalay et al., 2020; Farinon et al., 2020; Hao et al., 2014; Kicman et al., 2020). Cannabinoids are synthesized and stored in cells within the glandular trichome, which are secretory epidermal cells located in greatest concentrations on the flower of a female plant (Farinon et al., 2020; Sirikantaramas et al., 2005). Leaves and stems have slightly lower concentrations of cannabinoids and they are absent in roots and seeds (EMCDDA et al., 2018; Onofri et al., 2015).

In the cannabinoid biosynthetic pathway Cannabigerolic acid is converted into an acidic form of three key cannabinoids: tetrahydrocannabinol acid, cannabidiolic acid (CBDA), and cannabichromenic acid (Aizpurua-Olaizola et al., 2016; Farinon et al., 2020). This conversion takes place in the cytosol. After conversion, these acidic forms

undergo non-enzymatic carboxylation into their neutral and active forms known as THC, CBD, cannabichromene, and cannabigerol (CBG) (Farinon et al., 2020; Pacifico et al., 2006; Rotherham et al., 2011). The most widely recognized cannabinoids are THC, CBD, cannabichromene, and CBG.

CBD Benefits in Human and Animal Medicine

Numerous studies suggest that CBD oil provides therapeutic benefits in humans and several different animal species (Deabold et al., 2019; Draeger et al., 2020). CBD based nutraceutical use has risen in popularity among cats and dogs to help mitigate disorders like anxiety, seizures, general pain, and cancer (Brutlag et al., 2018; Deabold et al., 2019; Gamble et al., 2018). Evaluation of single-dose pharmacokinetics in dogs from, Gamble et al. (2018), determined that dogs receiving a 2 mg/kg dose of CBD oil exhibited significantly decreased pain and increased activity based on canine brief pain inventory and Hudson activity scores. Increased cannabinoid concentrations and activity have been observed in dogs and cats after CBD treatment. One study found that after treatment of 2 mg of CBD per kg of body weight, time to maximum cannabinoid concentration for dogs was 1.4 h and cats was 2 h (Deabold et al., 2019). This study determined that supplementation of CBD every 12 h for 12 wk was not detrimental to animal complete blood counts (CBC) and serum chemistry (Deabold et al., 2019). Use of CBD to mitigate pain and anxiety related issues has grown in the equine industry. Draeger et al. (2020) found that horses administered a pelletized CBD supplement exhibited calmer behavior when exposed to a novel object test used to measure reactivity to a startle stimulus. They reported peak concentrations of serum CBD at 2 h post treatment (Draeger et al., 2020).

Research findings of CBD therapeutic benefits in humans are similar to those in animals. A review of human studies assessing CBD effects by White et al. (2019) details actions and potential benefits of using CBD products to mitigate symptoms of major disorders. CBD supplementation can reduce seizure occurrence and frequency in epilepsy patients (Stockings et al., 2018). Studies assessing the role of CBD in anxiety cases are prevalent. However, a lack of data and statistical significance poses a threat to validity and reliability of these studies since most of them utilize a small sample size and subjects that do not have a true anxiety disorder (White et al., 2019). Studies regarding CBD effects on anxiety induced by THC suggest that CBD can reduce anxiety promoting effects of a single dose of THC (Martin-Santos et al., 2012; White et al., 2019; Zuardi et al.,1982). CBD administered before a public speaking event has been shown to alleviate speaker anxiety (Bergamaschi et al., 2011; Linares et al., 2017). Evidence suggests that CBD has antipsychotic and anxiolytic factors, which can lower short-term anxiety and stress response (Appiah-Kusi et al., 2020). Supplementation of CBD could potentially reduce individuals' risk of developing or worsening of psychosis. Stress, especially during childhood and early adulthood, is a risk factor for onset of psychosis (Appiah-Kusi et al., 2020; Beards et al., 2013; Pruessner et al., 2017; Winkel et al., 2008).

Several products are available for human use including the only CBD product approved by the US Food and Drug Administration (FDA) called Epidolex® (GW Pharmaceuticals, UK). Other non-approved products containing CBD and constituents are available at local convenience stores, online, and local dispensaries of medical marijuana (White et al., 2019). Epidolex® was approved in 2018 to treat severe cases of epilepsy and has shown major reductions in frequency of seizures (Vandolah et al.,

2019). The issues with non-FDA approved products are lack of cannabinoid consistency and quality. Manufacturer guarantees of cannabinoid concentrations are hard to verify due to lack of standardized testing and verification of procedures (White et al., 2019). As of 2020, none of the CBD products used for animal medicine and therapeutic use were FDA approved. A common characteristic of these products is inconsistency in cannabinoid concentrations (Draeger et al., 2020). Many products carry manufacturer claims of being THC free, when independent laboratory testing shows detectable levels of THC (Draeger et al., 2020; Harrison et al., 2019; White et al., 2019).

IH and CBD use in Cattle

Little information exists regarding cannabinoid pharmacokinetics in cattle. Due to regulatory restrictions on feeding IH to livestock, there have been few studies of cannabinoid bioavailability and disposition of bioactive residue in food animal tissue. Kleinhenz, et al. (2020b), reported serum cannabinoid concentrations in cattle following a single dose of industrial hemp flowers. Mean maximum CBDA concentration occurred at 14 h, with geometric mean half-life 14.1 h. Results showed no changes of serum chemistry (liver and kidney enzymes) after administration of IH flowers when compared to baseline values, meaning a target dosage rate of 5.4 mg CBDA per kg of BW exhibited no adverse effects on cattle health during the study. Acidic cannabinoids like CBDA were readily absorbed through the rumen wall and distributed throughout the body (Kleinhenz et al., 2020b). This suggests that rumen microbes are capable of metabolizing cannabinoids. Further investigation is warranted to understand the overall availability of cannabinoids for absorption in the ruminant.

Summary

Therapeutic benefits of cannabinoid supplementation have been observed in humans (White et al., 2019), dogs (Deabold et al., 2019), and horses (Draeger et al., 2020). These benefits include reduced stress, anxiety, and improved behavior. Cannabinoid supplementation could be an avenue for mitigating the effects of weaning and transportation stress in cattle. Ruminants are well suited for digestion of IH and by-products from hemp oil production. IH and its by-products could provide suitable fiber for cattle diets, reducing hemp oil production waste. Little information currently exists regarding cannabinoid pharmacokinetics in cattle. Regulatory approval of IH products as feedstuffs will require further research on residues in food animal tissues.

Methodology

Management of Subjects

Subjects for the pilot study were used on loan from a faculty member at Murray State University (MSU). Subjects for Phase 1 and Phase 2 were part of the MSU beef cattle herd. General herd management practices were maintained throughout the entirety of each phase regardless of treatment or research procedures. Calf vaccination records and management changes were documented throughout the study. All procedures used for data collection were approved by the Murray State University Institutional Animal Care and Use Committee (Appendix E).

Cannabinoid Supplement

The product used in this study was a liquid cannabinoid supplement (Equilibrium, CannaHorse, Toronto, Canada) marketed for calming anxiety in horses. Specifications for the product are listed in Appendix H. A sample of the product was evaluated for cannabinoid content by the Kansas State University Department of Anatomy and Physiology Laboratory (A&P, Manhattan, KS, USA). Analysis used the LC-MS/MS method detailed in the Pilot Study below.

Pilot Study

Research Design

The pilot study was conducted to confirm dosage and plasma cannabinoid clearance rates for use in subsequent phases. Each of the two cows was administered a single dose of the cannabinoid supplement.

Subject Selection

Two mature angus-cross beef cows from a faculty member of the University were used for the study. Subject 1was 5 years old, weighed 453 kg, and was nursing a calf at the time. Subject 2 was 6 years old, weighed 683 kg, and had lost her calf two weeks prior to the study.

Treatment

Treatment dosages were based on manufacturer recommendations for equines of 0.5 mg CBD per kg of body weight (BW). This dose is comparable to the CBD dosage used in Kansas State University's study on cannabinoid pharmacokinetics in cattle after a single dose of industrial hemp flowers (Kleinhenz et al., 2020b).

Subjects were given a single oral dose of the cannabinoid supplement in the morning of the treatment day. Subject 1 (454 kg) received 2,500 mg (2.5 ml). Subject 2 (683 kg) received 3,800 mg (3.8 ml). Treatments were administered per manufacturer instructions using 3 ml syringes placed inside the subject's mouth between the cheek and tongue for absorption.

Data Collection

Blood samples were obtained via jugular venipuncture prior to treatment (PRE) and at .5, 1, 2, 4, 8, 12, 16, and 24 h post-treatment. Three 10 ml samples were collected (BD Vacutainer EDTA). Baseline blood collection allowed subjects to serve as their own control. Samples were stored in a cooler and transported from the cattle working area to a laboratory where they were centrifuged at 1,876 x g for 10 min (LWS Combo V24 Centrifuge, LW Scientific, Atlanta, GA). 1.5 ml of plasma was pipetted into Eppendorf storage tubes (Eppendorf Flex-Tube 22364111, Eppendorf, Enfield, CT) and stored at -28

ºC. After the 24 h collection, samples were packed with dry ice in a cooler and shipped overnight to Kansas State University Department of Anatomy and Physiology Laboratory (Manhattan, KS, USA).

Cannabinoid analysis was performed using an Acquity H UPLC and a TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford, MA). Chromatographic separation utilized a UPLC column (Eclipse Plus C18, Agilent Technologies, Santa Clara, CA) that was 100 x 2.1 mm, 1.8 µm, heated at 55ºC. Plasma pharmacokinetic parameters were determined for cannabinoid concentration versus time using the Phoenix software platform (Phoenix 8.2, Certara, Inc., Princeton, NJ, USA). The approach taken was non-compartmental based on statistical moment theory. Analysis showed the area under the plasma concentration versus time curve and the area under the first moment of concentration versus time curve when using the linear trapezoidal interpolation method for concentrations and time progression (Kleinhenz et al., 2020b). The pharmacokinetic parameters represent geometric means, medians, and ranges based on log-normal distributions of sample testing. Parameters tested include7-hydroxy cannabidiol (CBD-7 acid), THC acid, cannabidivarinic acid (CBDVA), cannabidivarin (CBDV), CBDA, cannabigerolic acid CBGA, tetrahydrocannabivarin (THCV), CBG, CBD, cannabinol (CBN), 9-THC, 8-THC, canabicyclol (CBL), cannabichromene, THCA-A, CBCA, CBLA, THCP, THC acid-glu, THC-glu, and THC-OH.

Weaning and Transportation Stress Study

Sampling Procedure

Weaning stress (Phase 1) and transportation stress (Phase 2) subjects were chosen from 31 calves in the MSU beef herd. One bull calf was pulled per farm manager request

leaving 30 steers and heifers accessible for study use. Three calves were excluded due to high BW. The 27 remaining calves were sorted by BW, sex, and sire. Calves were given a study identification number (ID) and randomly assigned to the treatment (TRT) or control (CON) groups. Treatment groups were pre-determined that day based on BW, sex, and if they were bred from artificial insemination (AI bull) or from a clean-up bull (Table 1).

Average BW for the group was 289.12 kg. All calves received a 7-way blackleg protection vaccine prior to study, per standard vaccine protocol for weaning calves on the farm. Prior to the start of study, calves were brought into working facilities for collecting body weight (BW), of sex, and application of livestock paint identification numbers on their back for behavior monitoring. Table 1 lists the treatment group, BW, CBD supplement dosage, sex and transportation group for calves in all phases of the study.

Table 1. Treatment group, body weight, CBD supplement dosage, sex, and transportation group for calves in the study

Calf ID	Treatment Group	BW (lb)	CBD supplement dose (µL)	Sex	Transportation Group
1	TRT	650	3,250	Steer	1
2	CON	690	0	Steer	2
3	CON	615	0	Heifer	1
4	TRT	745	3,725	Heifer	1
5	TRT	640	3,200	Steer	2
6	TRT	665	3,325	Heifer	2
7	CON	715	0	Heifer	1
8	TRT	665	3,325	Steer	1
9	CON	625	0	Heifer	2
10	TRT	655	3,275	Steer	1
11	TRT	520	2,600	Heifer	2
12	TRT	525	2,625	Heifer	2
13	CON	575	0	Steer	2
14	TRT	610	3,050	Heifer	2
15	TRT	660	3,300	Steer	2
16	CON	570	0	Heifer	1
17	TRT	610	3,050	Heifer	1
18	TRT	675	3,375	Heifer	2
19	CON	590	0	Steer	2
20	TRT	640	3,200	Heifer	2
21	TRT	715	3,575	Steer	1
22	CON	715	0	Steer	1
23	CON	725	0	Heifer	1
24	CON	550	0	Heifer	2
26	CON	635	0	Steer	2
27	CON	620	0	Heifer	1
30	TRT	610	3,050	Steer	1

Phase 1: Weaning Stress

Research Design

Phase 1 examined the effects of cannabinoid supplementation on weaning stress in calves. TRT group subjects received a dose of the cannabinoid supplement delivering 5 mg CBD per kg BW. CON group subjects received a comparable amount of water. Treatments were administered per manufacturer instructions using a 3 ml syringe placed

inside the subject's mouth between the cheek and tongue for absorption. Doses for each calf can be found in Table 1.

Data Collection: Blood

Pre-treatment blood collections served as baseline values for all calves. At the beginning of the study, calves were weaned from dams and brought up to working chute for processing. In the chute, each calf was restrained with a rope halter for treatment and blood sample collection. Calves received their assigned dose of gel or water as previously described.

Blood samples were obtained via jugular venipuncture at 0, 6, 12, 24, 48, and 72 h following treatment. Blood was collected into 3 10 ml BD Vacutainer® serum tubes (Vacutainer 366668, Becton, Dickson and Company, Franklin Lakes, NJ) and 3 10 ml Vacutainer® EDTA tubes (Vacutainer 366643, Becton, Dickson and Company, Franklin Lakes, NJ). Samples were stored in a cooler, moved to a laboratory from the working area, and centrifuged as detailed earlier in the pilot study. Serum and plasma were separated and pipetted into 1.5 ml Eppendorf storage tubes (Eppendorf Flex-Tube 22364111, Eppendorf, Enfield, CT). Samples were stored at -28ºC until shipped for testing.

One plasma tube from each calf was used for complete blood count (CBC) analysis. The tube was transported on ice in a cooler to Breathitt Veterinary Center (BVC, Hopkinsville, KY). CBC analysis was completed on a Sysmex XT-2000i V hematology analyzer. (Sysmex America, Inc., Mundelein, IL). Samples were prepped and smeared for testing. Then blood samples were given a barcode before running through the

Sysmex system. During testing, samples were aspirated into three sections: white blood cell (WBC), red blood cell (RBC), and platelet count (PCT) differentiations for results using fluorescent flow cytometry. The reagent used was Stromatolyser FFS (Sysmex America, Inc., Mundelein, IL) and the machine was rinsed after every sample with CellPack EPK (Sysmex America, Inc., Mundelein, IL). All CBC samples were processed on the day of collection. Parameters evaluated in CBC include white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils (NEU ABS), lymphocytes (LYMPH ABS), monocytes (MONOS ABS), eosinophils (EOS ABS), and basophils (BASO ABS).

Serum was analyzed for glucose, blood urea nitrogen (BUN), creatinine, sodium, chloride, potassium, CO_2, calcium, magnesium, phosphorus, cholesterol, alkaline phosphatase (ALP), bilirubin (total, direct and indirect), aspartate transferase (AST), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), osmolality, albumin, globulin, albumin:globulin ratio, and anion gap with a Beckman AU480 Chemistry Analyzer (Beckman Coulter, Brea, CA) according to manufacturer's instructions.

Data Collection: Weaning Behavior

After application of treatment, calves were weaned using fence-line separation from their dam (Figure 1). Calves were commingled in large paddocks for behavior observations. Calves were moved as necessary as part of the unit's rotational grazing management system.

Figure 1: Photo illustrating the fence-line weaning practice. Weaned calves (left) are separated from their dams (right) by an electric fence.

A drone (Phantom 4 Advanced Drone, DJI Technology Co., CA) was utilized to record and observe weaning stress behaviors in the calves. All flights were coordinated through the DJI Go 4 app on an electronic tablet (iPad, Apple Inc., Cupertino, CA). Videos were recorded and then transferred using a micro-SD card located on board the drone during flights.

Observation flights were scheduled for 3 times a day (AM, Mid-day, and PM) over 4 days. The flights on Day 0 were limited to a single PM flight due to prolonged processing of subjects that morning. Each flight was roughly 30 minutes of video depending on battery life of the drone.

Prior to analysis, drone footage was edited to have a length of approximately 14 to 16 minutes per flight time. All footage reserved for analysis included the majority of, if

not the entire, herd throughout the video. Video editing was done through the Lightworks program software (LKWS Software Ltd, UK).

The edited videos were observed for the following behaviors: bawling, pacing the fence-line, and standing at fence-line. For each calf, a minute-by-minute count of the expression of each of the three behaviors was conducted. If a particular behavior was observed during a one-minute time period, that calf was recorded as expressing that behavior. For each calf, individual behavior was not counted more than once in a 1-minute time span. Using this procedure, each behavior could have been observed in an individual calf a maximum of 15 times per video session.

Phase 2: Transportation Stress

Research Design

Phase 2 evaluated the effects of cannabinoid supplementation on transportation stress in calves. Phase 2 used the same design, treatment groups and dosage rates as Phase 1. Differences in stress biomarkers in the blood and calf temperament resulting from transportation were examined following treatment.

Sampling Procedure

The TRT and CON groups of calves chosen for Phase 1 were used in Phase 2. Calves were randomly assigned to one of two transportation groups before Phase 2 began and are listed in Table 1.

Data Collection: Temperament Scores

Seven days after the first treatment of Phase 1, calves were brought up to a working chute for processing. Calf temperament was evaluated through chute scores and exit velocities. Assessment of subject temperament was conducted using two methods.

The first method used a chute test (modified from Tulloh et al., 1961). Videos (Canon EOS Rebel T6, Canon U.S.A., Inc. Melville, NY) were taken of subjects walking into a chute system and given a score of 1-5 while they were restrained in the head gate, immediately after closure of head gate. The rubric for temperament is presented in Table 2. A score of 1 shows calm temperament, no movement whereas a 5 represents rearing, twisting of the body, or violent struggling (Grandin et al., 1993). Chute scores were observed before and after treatment and transportation. The second method evaluated subject exit velocity using two sets of laser timers (FarmTek, Inc. Wylie, TX). One set was placed in front of the head gate, a second set was placed 1.83 m out from the first set going away from the chute. Subjects tripped both sets of timers themselves but both timers had to manually be stopped. Exit velocities were calculated by dividing the distance (1.8 m) by the difference in time between sensor 1 and sensor 2.

Table 2. Rubric for evaluating calf temperament during chute score test (Tulloh et al., 1961; Grandin et al., 1993)

Score	Description
1	Calm, no movement
2	Restless, shifting
3	Squirming, occasionally shaking of the chute
4	Continuous vigorous movement and shaking of the chute
5	Rearing, twisting of the body, or violent struggling

After chute scores were obtained BW was recorded and the calves were restrained with a rope halter. Blood samples were collected via jugular venipuncture as described in Phase 1 pre-treatment and at the conclusion of transportation. CBD supplement or water were administered to each calf as described in Phase 1. Once treatment was administered, the calf was released and exit velocity was recorded in meters per second. Velocity was evaluated using two sets of laser timers (FarmTek, Inc. Wylie, TX) with calves being

timed from the point of exit at the chute until they reached the second set of timers spaced 1.83 m away (Figure 2).

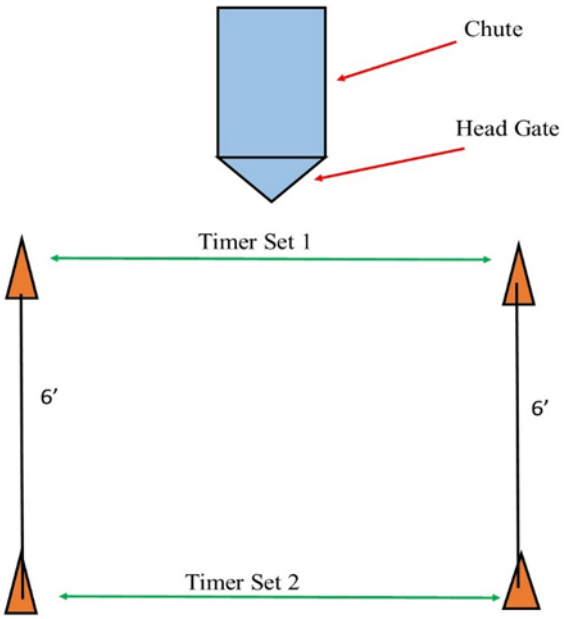

Figure 2. Diagram of layout for chute score and exit velocity determination. The chute scores were obtained after the calf entered the chute and the head gate was closed. The orange triangles indicate the positions of the laser timer emitters and receivers. Exit velocity was calculated from the times from the two sets of laser times set at a distance of 1.83 m.

After processing, the pre-assigned groups of calves were loaded onto one of two trailers. Drivers took the trailers out for 6 h. Departure times for the two loads were staggered by 1 h 45 min to allow for post-travel processing time, so no calves were standing on the trailer for longer than 30 min at the end of transportation. Upon arrival, blood samples were collected and BW, chute score and exit velocity recorded for all calves. Calves were then placed back into their paddock for the remainder of the study.

Blood analysis was completed using the same procedures as in Phase 1.

Validity and Reliability of Instrument

To reduce variability, one evaluator determined the chute scores of all calves. Additionally, one evaluator scored all behaviors from the drone videos.

Data Analysis

Statistical analysis for all phases was performed through MIXED Procedure of SAS (SAS, Cary, NC, USA, version 9.4). For Phase 1, results were determined under effect of treatment and time for CBC data. All results are reported as least squared means. Significance for all results is defined as $P \leq 0.05$ and tendencies defined as $P > 0.05$ and $P \leq 0.09$. The experimental unit was a calf, with each calf serving as its own control. Dependent variables were WBC, RBC, HGB, HTC, MCV, MCH, MCHC, PLT, NEU ABS, LYMPH ABS, MONOS ABS, EOS ABS, and BASOABS and were analyzed as repeated measures. Compound symmetry generated the lowest Akaike information criteria in all statistical analyses and considered the covariance structure for all repeated measures analyses.

Phase 1 serum chemistry results were determined under effect of treatment and time. Statistical analysis was the same as CBC results. Experimental unit was calf and dependent variables were glucose, BUN, creatinine, chloride, sodium, potassium, CO_2 bicarbonate, calcium, magnesium, phosphorus, cholesterol, alkaline phosphate, bilirubin, AST, GGT, CPK, osmolality, total protein, albumin, globulin, A G ratio, and anion gap.

Proc Mixed analysis was used for evaluations of weaning stress behaviors gathered from drone video observations. Analysis followed three behaviors indicative of weaning stress including bawling, pacing fence-line, and standing at fence-line.

All results for Phase 2 were analyzed through the Mixed Procedure of SAS (SAS, Cary, NC, USA). CBC results were examined under the effect of treatment and time including equivalent experimental unit and dependent variables to results of Phase 1. Serum chemistry analysis also followed that of phase 1 procedures. Further results were examined under effect of treatment on chute scores and exit velocity. Experimental unit was calf, with each one serving as their own control. Dependent variables were noted as pre-chute scores, post chute scores, pre-exit velocity, and post exit velocity.

Variables

Calves were evaluated based on effects from treatment and reaction to weaning and transportation stress. The independent variable included dosage concentration of water or cannabinoid supplement. Dosage rates for calves were determined on a per pound basis. The dependent variables included changes in weaning stress behavior, chute scores, exit velocity, shrinkage rate, serum cannabinoid concentration, fluctuations in complete blood count (CBC) or serum chemistry levels, and changes in lactate dehydrogenase concentrations. Each variable was evaluated, and a statistical analysis performed to determine any significance between each variable. The relationship between treatment and levels of stress were observed in this study.

Results

Pilot study

Analysis of the CBD supplement showed concentrations of CBD at 43,944 µg/ml, cannabichromene at 2,700 µg/ml, CBG at 1,279 µg/ml, CBDV at 407 µg/ml, and CBN at 3,900 µg/ml. Trace amounts of 9-THC were detected, however, they were below the lower limit of quantification (1.0 ng/ml).

Plasma from both cows contained quantifiable levels of CBD and CBD-7 acid. 6.1 ng/ml CBD was detected at 24 h post treatment in Animal 1. 5.8 ng/ml CBD was detected at 1 h post treatment in Animal 2. The CBD metabolite CBD-7 acid was detected as early as 8 h and steadily increased in both animals up to 24 h post-treatment (Figure 3).

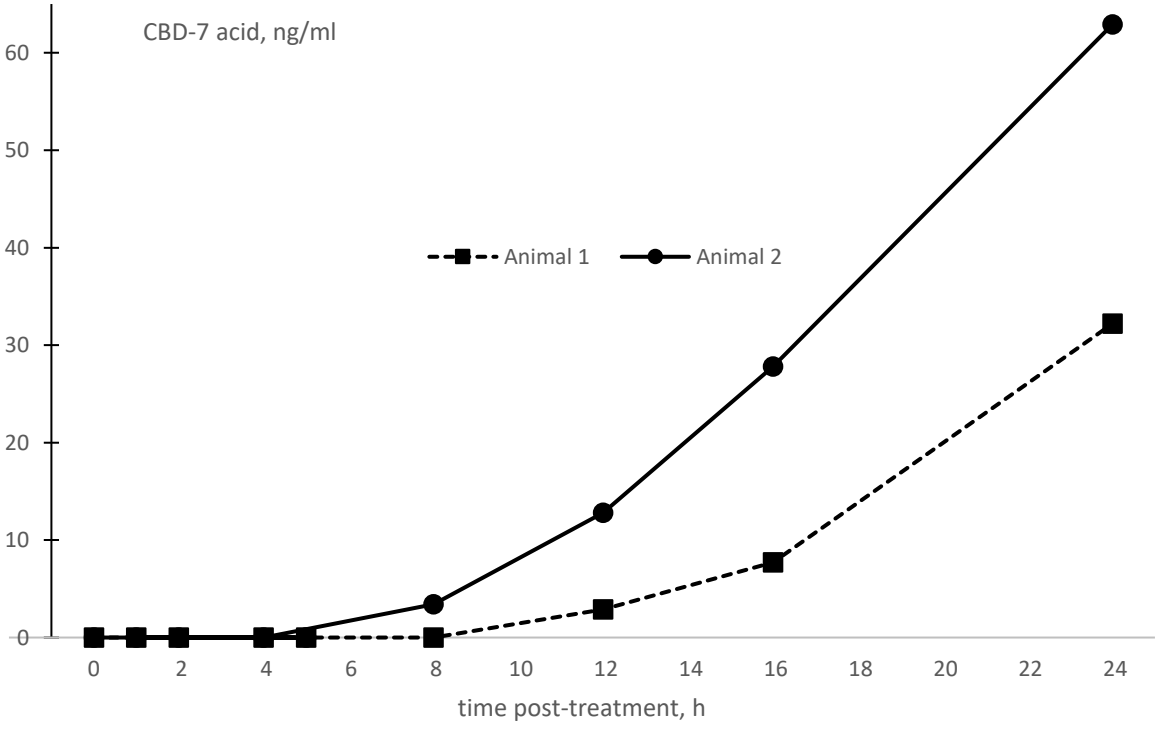

Figure 3. Serum 7-Hydroxycannabidiol (CDB-7 acid) concentrations (ng/ml) of two cattle administered a cannabinoid supplement up to 24 h post-treatment.

Phase 1

Complete blood count parameters across treatments and sampling hour are presented in Table 3. MONO ABS were significantly lower (P<0.0001) in TRT calves (0.70 $\times 10^3/\mu L$) than in control (0.92 $\times 10^3/\mu L$). Interactions between treatment and hour were observed for WBC (Figure 3), RBC (Figure 4), HTC (Figure 5) and EOS ABS (Figure 6). CON calves tended to be higher in LYMPH ABS (7.25 $\times 10^3/\mu L$) than the TRT calves (5.50 $\times 10^3/\mu L$) (P=0.0695).

Serum blood chemistry parameters across treatments and sampling hour are presented in Table 4. Statistical significance was found for CPK and bilirubin based on effect of treatment. Effect of hour was observed for all parameters.

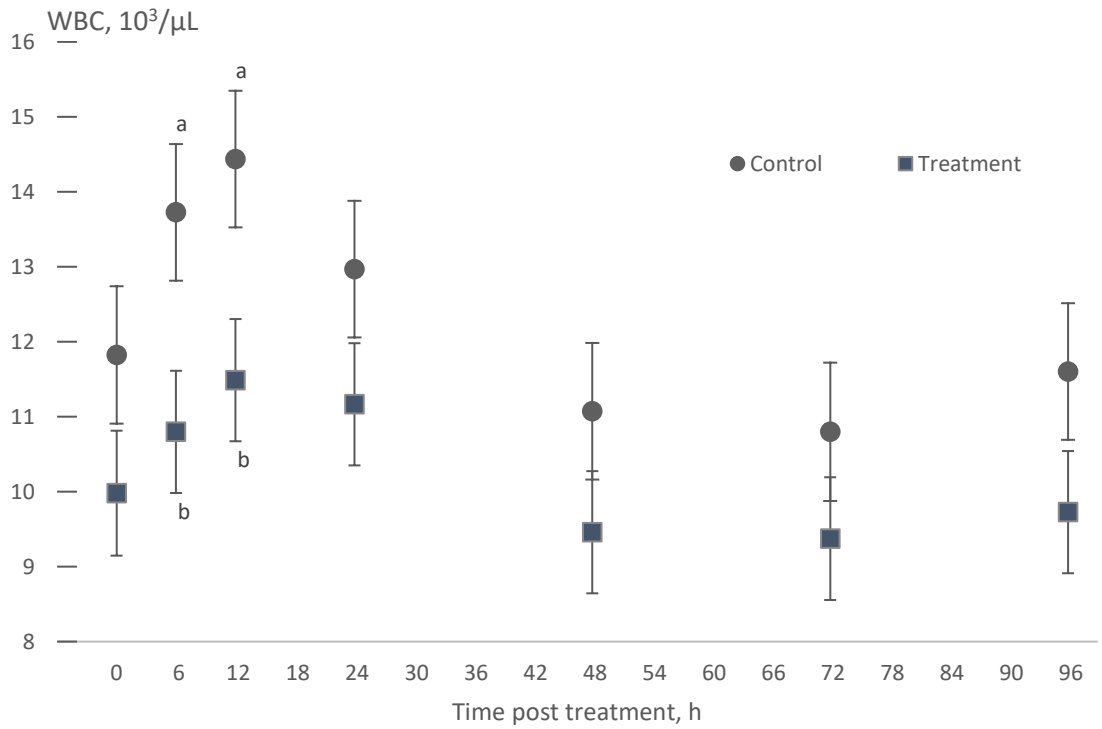

Figure 3. Interaction of CBD supplement and collection time on the white blood cell count of weaned calves up to 96 hours post-treatment. Values within the same hour with unlike superscripts differ ($P < 0.05$).

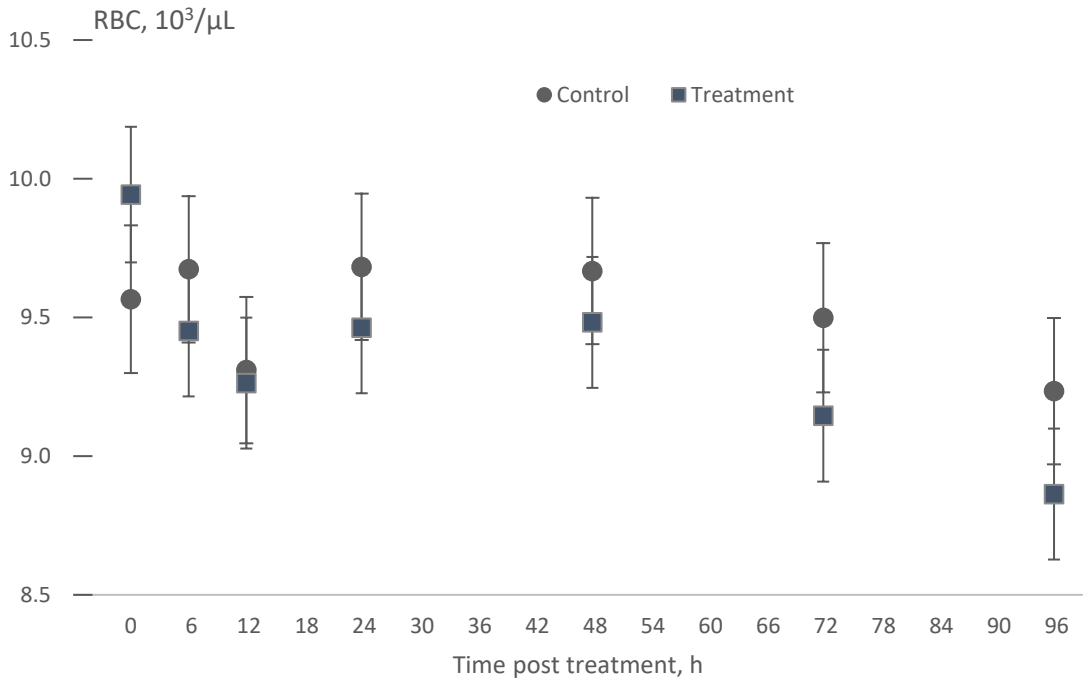

Figure 4. Interaction of CBD supplement and collection time on the red blood cell count of weaned calves up to 96 hours post-treatment. Values within the same hour were not different ($P > 0.05$).

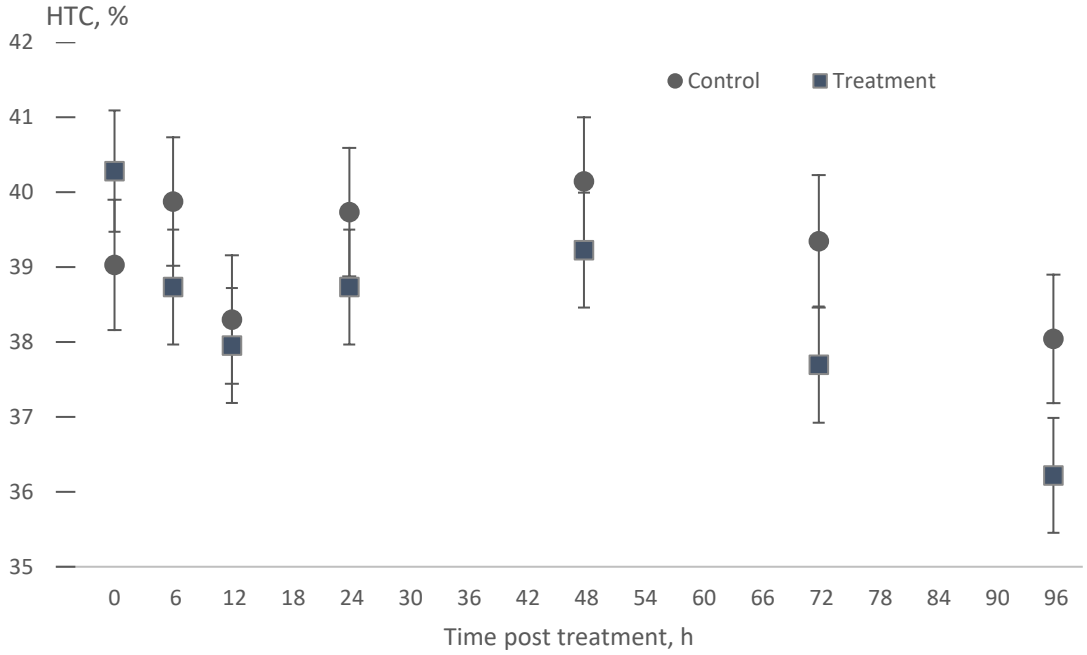

Figure 5. Interaction of CBD supplement and collection time on the hematocrit (%) of

weaned calves up to 96 hours post-treatment. Values within the same hour were not different ($P > 0.05$).

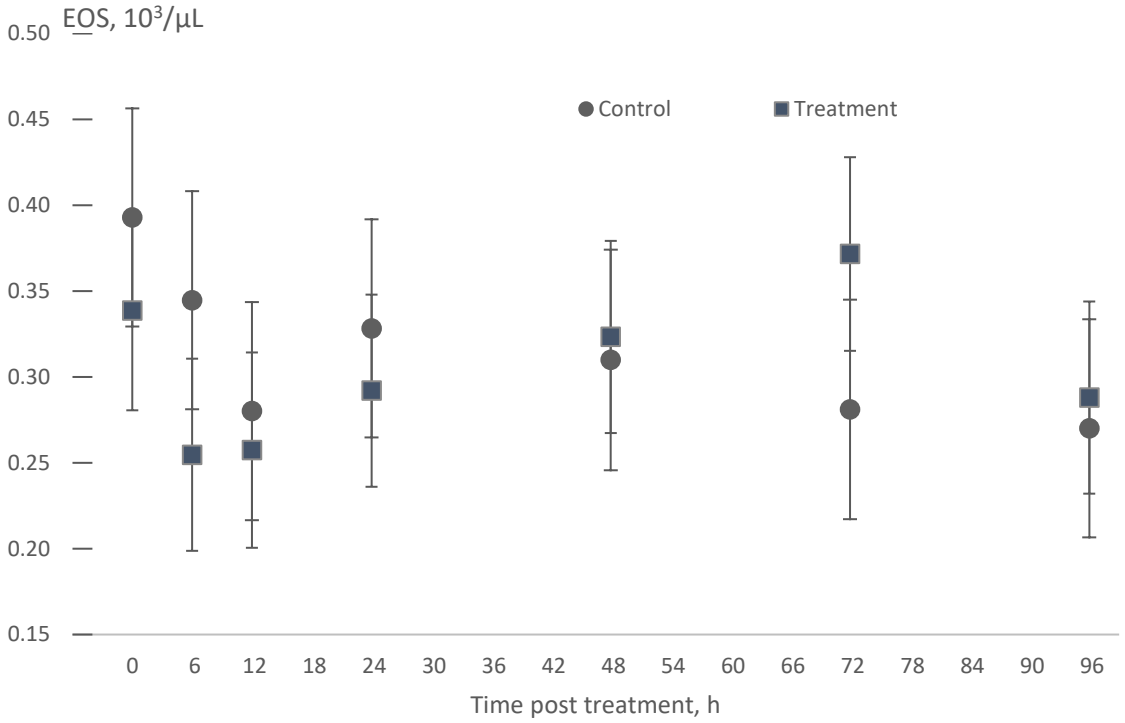

Figure 6. Interaction of CBD supplement and collection time on the eosinophil count of weaned calves up to 96 hours post-treatment. Values within the same hour were not different ($P > 0.05$).

Table 3. Least square mean values of the complete blood count parameters based on the treatment received and sampling hour

Parameter[1]	Reference Value[2]	Treatment status[3]			Sampling hour[4]							
		CON	TRT	P value[5]	0	6	12	24	48	72	96	P value[5]
[6]WBC, $10^3/\mu L$	4.0 – 12.0	12.35	10.28	0.09	10.91^{c}	12.26^{ab}	12.96^{a}	12.07^{b}	10.27^{d}	10.09^{d}	10.67^{cd}	<.0001
[7]RBC, $10^3/\mu L$	5.0 – 10.0	9.52	9.37	0.67	9.75^{a}	9.56^{b}	9.29^{cd}	9.57^{b}	9.58^{ab}	9.32^{c}	9.05^{d}	<.0001
HGB, g/dL	8.0 – 15.0	12.48	12.14	0.27	12.73^{a}	12.50^{a}	12.10^{b}	12.48^{a}	12.45^{a}	12.13^{b}	11.78^{c}	<.0001
[8]HCT, %	24 – 26	39.21	38.41	0.43	39.66^{a}	39.31^{ab}	38.13^{c}	39.23^{ab}	39.68^{a}	38.52^{bc}	37.13^{d}	<.0001
MCV, fL	40.0 – 60.0	41.38	41.30	0.96	40.92^{d}	41.33^{c}	41.31^{c}	41.26^{c}	41.69^{a}	41.57^{b}	41.32^{c}	<.0001
MCH, pg	11 – 17	13.16	13.05	0.79	13.11	13.13	13.10	13.11	13.06	13.10	13.12	0.24
MCHC, g/dL	30 – 36	31.85	31.65	0.62	32.11^{a}	31.86^{b}	31.76^{b}	31.83^{b}	31.38^{d}	31.54^{c}	31.79^{b}	<.0001
PLT, $10^3/\mu L$	100 - 800	442.66	443.18	0.99	463.79^{a}	367.48^{c}	422.39^{b}	465.19^{a}	456.89^{a}	451.22^{ab}	473.49^{a}	<.0001
NEU ABS, $10^3/\mu L$	0.6 – 4.8	3.62	3.71	0.75	3.07^{c}	5.25^{a}	5.07^{a}	3.88^{b}	2.77^{cd}	2.68^{d}	2.92^{cd}	<.0001
LYMPH ABS, $10^3/\mu L$	2.5 – 7.5	7.25	5.50	0.06	6.49^{ab}	5.60^{c}	6.51^{ab}	6.75^{a}	6.46^{ab}	6.23^{b}	6.58^{a}	<.0001
MONO ABS, $10^3/\mu L$	0.02 – 0.84	0.92	0.70	0.01	0.77^{c}	0.80^{bc}	0.90^{a}	0.92^{a}	0.70^{d}	0.82^{b}	0.75^{cd}	<.0001
[9]EOS ABS, $10^3/\mu L$	0 – 2.4	0.32	0.31	0.88	0.37^{a}	0.30^{bc}	0.27^{c}	0.31^{bc}	0.32^{abc}	0.33^{ab}	0.28^{bc}	0.02
BASO ABS, $10^3/\mu L$	0 – 0.2	0.10	0.09	0.28	0.11^{a}	0.09^{b}	0.07^{c}	0.09^{b}	0.10^{ab}	0.09^{b}	0.11^{a}	<.0001

[1]Complete blood count parameters include white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT), and white blood cell differential. White blood cell differential parameters included absolute (ABS) and percentage values for neutrophils (NEU), lymphocytes (LYMPH), monocytes (MONO) eosinophils (EOS), and basophils (BASO). All were assessed using a hematology analyzer.
[2]Breathitt Veterinary Center laboratory reference ranges.
[3]One group was treated with a cannabinoid supplement (TRT) and the other as a control (CON).
[4]Sampling hour refers to the time post-treatment in hours when the blood sample was obtained.
[5]Significance was considered at $P < 0.05$.
[abcd]Means within a row with different superscripts differ ($P < 0.05$).
[6]for WBC, there was a treatment × sampling hour interaction ($P<0.05$) that has been presented in Figure 3.
[7]for RBC, there was a treatment × sampling hour interaction ($P<0.05$) that has been presented in Figure 4.
[8]for HCT, there was a treatment × sampling hour interaction ($P<0.05$) that has been presented in Figure 5.
[9]for EOS ABS, there was a treatment × sampling hour interaction ($P<0.05$) that has been presented in Figure 6.

Table 4. Least square mean values of the serum chemistry panel parameters based on the treatment received and sampling hour

Parameter[1]	Reference Value[2]	Treatment status[3]			Sampling hour[4]				
		CON	TRT	P value[5]	0	6	12	24	P Value[5]
Glucose, mg/dL	50 – 110	76.65	78.80	0.41	82.81^{a}	85.14^{a}	70.29^{b}	72.62^{b}	<.0001
BUN, mg/dL	6 – 27	12.21	12.03	0.76	9.61^{c}	9.89^{c}	13.08^{b}	15.89^{a}	<.0001
Creatinine, mg/dL	0.45 – 1.8	1.45	1.49	0.67	1.44^{b}	1.45^{b}	1.53^{a}	1.46^{b}	<.0001
Sodium, mmol/L	132-152	142.29	142.30	0.99	139.96^{c}	141.30^{b}	144.37^{a}	143.56^{a}	<.0001
Chloride, mmol/L	97-111	101.06	101.98	0.16	101.39^{b}	102.36^{a}	101.28^{b}	101.06^{b}	0.04
Potassium, mmol/L	3.9-5.8	4.225	4.198	0.76	4.276^{ab}	4.150^{bc}	4.139^{c}	4.276^{a}	0.03
CO_2, mmol/L	23-29	26.66	25.94	0.30	25.09^{b}	25.17^{b}	27.11^{a}	27.82^{a}	<.0001
Calcium, mg/dL	9.2-12.2	9.83	9.82	0.88	9.81^{ab}	9.82^{ab}	9.73^{b}	9.94^{a}	0.03
Magnesium, mg/dL	1.7-2.3	1.96	1.92	0.52	1.95^{a}	1.95^{a}	1.89^{b}	1.98^{a}	<.0001
Phosphorus, mg/dL	5-8.5	7.23	7.20	0.88	7.32^{ab}	6.87^{c}	7.47^{a}	7.20^{b}	<.0001
Cholesterol, mg/dL	78-142	177.88	160.12	0.33	169.91^{b}	172.95^{a}	167.62^{bc}	165.51^{c}	<.0001
ALP, IU/L	0-110	131.13	122.23	0.63	129.16^{a}	128.83^{a}	122.02^{b}	126.72^{ab}	0.02
Total bilirubin, mg/dL	0-0.8	0.33	0.28	0.05	0.27^{c}	0.33^{a}	0.30^{b}	0.32^{ab}	<.0001
AST, IU/L	75-135	60.29	56.40	0.48	49.99^{d}	57.14^{c}	61.14^{b}	65.12^{a}	<.0001
GGT, IU/L	7-50	13.85	13.18	0.31	13.26	13.60	13.45	13.78	0.09
CPK, IU/L	100-515	488.79	350.48	0.30	235.03^{b}	461.17^{a}	517.36^{a}	464.99^{a}	0.01
Osmolality, mOsm/L	273-293	280.12	282.36	0.28	273.33^{c}	280.02^{b}	285.99^{a}	285.60^{ab}	<.0001
Total protein, g/dL	6.5-8.9	6.52	6.50	0.78	6.40^{b}	6.59^{a}	6.46^{b}	6.59^{a}	<.0001
Albumin, g/dL	2.5-3.5	3.78	3.76	0.73	3.70^{c}	3.80^{a}	3.75^{b}	3.82^{a}	<.0001
Globulin, gms%	3-4.2	2.75	2.74	0.89	2.70^{c}	2.79^{a}	2.71^{bc}	2.76^{ab}	0.01
Albumin:globulin ratio	0.6-1.4	1.38	1.38	0.96	1.38	1.37	1.39	1.39	0.24
Anion gap	-	18.74	18.58	0.75	17.64^{c}	17.93^{c}	20.11^{a}	18.96^{b}	<.0001

[1]Blood serum parameters include glucose, blood urea nitrogen (BUN), creatinine, sodium, chloride, potassium, CO_2, calcium, magnesium, phosphorus, cholesterol, alkaline phosphatase (ALP), bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), osmolality, total protein, albumin, globulin, albumin:globulin ratio, and anion gap. All were assessed using a Beckman AU480 chemistry analyzer.
[2]Breathitt Veterinary Center laboratory reference ranges.
[3]One group was treated with a cannabinoid supplement (TRT) and the other as a control (CON).
[4]Sampling hour refers to the time post-treatment in hours when the blood sample was obtained.
[5]Significance was considered at $P < 0.05$.

Weaning Stress Behaviors

Statistical significance was not observed for bawling, pacing, or standing at fence-line on treatment (Table 5). Effect of hour on weaning behaviors was observed.

Table 5. Mean counts of weaning behavior observations based on effect of time post weaning represented as average amount of behavior exhibited during a 15-minute span

	Time Post Weaning[2]								
Parameter[1]	10 h	24 h	30 h	36 h	48 h	72 h	78 h	84 h	P value
Bawling	3.29	5.11	1.44	2.01	1.66	0.48	0.03	0	0.94
Pacing	1.55	1.95	0.03	0.21	0.55	0	0	0	0.37
Standing	4.69	6.35	1.13	3.43	2.91	0.83	0.02	2.57	0.57

[1]Weaning behaviors include bawling, pacing, and standing at the fence-line.
[2]Behaviors were evaluated by drone footage up to 84 hours post weaning.

Phase 2

CBC parameter averages were within reference ranges. No two-way interactions were found for any variables. However, effects of treatment (Table 6) were observed for WBC, Lymph, Mono, and Baso. Effects of hour were also observed for MCHC, Lymph, EOS, and Baso.

Differences were observed for glucose and bilirubin levels based on effect of treatment. A tendency was observed for CPK (P=0.06). Summary of enzyme concentrations based on treatment are listed in Table 7.

Table 6. Least square mean values of the complete blood count parameters based on the treatment received and sampling hour

Parameter[1]	Reference Value[2]	Treatment status[3]			Sampling time[3]		
		CON	TRT	P value[5]	Pre	Post	P Value[5]
WBC, $10^3/\mu L$	4.0 – 12.0	12.15	9.85	0.03	10.88	11.12	0.29
RBC, $10^3/\mu L$	5.0 – 10.0	9.20	9.08	0.71	9.14	9.15	0.93
HGB, g/dL	8.0 – 15.0	12.05	11.78	0.44	11.91	11.91	0.97
HCT, %	24 – 26	52.33	37.16	0.25	37.70	51.79	0.29
MCV, fL	40.0 – 60.0	40.81	40.83	0.99	40.62	41.02	0.34
MCH, pg	11 – 17	13.13	13.03	0.79	13.08	13.07	0.87
MCHC, g/dL	30 – 36	32.23	31.99	0.54	32.29	31.93	0.03
PLT, $10^3/\mu L$	100 - 800	466.57	436.90	0.57	470.43	433.04	0.09
NEU ABS, $10^3/\mu L$	0.6 – 4.8	18.68	3.71	0.25	16.26	6.13	0.43
LYMPH ABS, $10^3/\mu L$	2.5 – 7.5	6.13	5.10	0.03	6.32	4.91	<.0001
MONO ABS, $10^3/\mu L$	0.02 – 0.84	0.79	0.59	0.01	0.70	0.68	0.27
EOS ABS, $10^3/\mu L$	0 – 2.4	0.23	0.20	0.41	0.29	0.14	0.01
BASO ABS, $10^3/\mu L$	0 – 0.2	0.07	0.06	0.04	0.07	0.06	0.03

[1]Complete blood count parameters include white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT), and white blood cell differential. White blood cell differential parameters included absolute (ABS) and percentage values for neutrophils (NEU), lymphocytes (LYMPH), monocytes (MONO) eosinophils (EOS), and basophils (BASO). All were assessed using a hematology analyzer.
[2]Breathitt Veterinary Center laboratory reference ranges.
[3]One group was treated with a cannabinoid supplement (TRT) and the other as a control (CON).
[4]Pre indicates sample taken before transportation. Post indicates sample taken after transportation.
[5]Significance was considered at $P < 0.05$.

Table 7. Least square mean values of the serum chemistry panel parameters based on the treatment received and sampling hour

Parameter[1]	Reference Value[2]	Treatment status[3]			Sampling hour[4]		
		CON	TRT	P value[5]	Pre	Post	P Value[5]
Glucose, mg/dL	50 – 110	58.86	62.40	0.04	51.95	69.32	<.0001
BUN, mg/dL	6 – 27	13.42	13.03	0.69	13.65	12.80	<.0001
Creatinine, mg/dL	0.45 – 1.8	1.60	1.74	0.30	1.62	1.72	0.0002
Sodium, mmol/L	132-152	137.75	137.70	0.97	136.85	138.60	0.25
Chloride, mmol/L	97-111	102.33	103.17	0.43	101.06	104.44	0.01
Potassium, mmol/L	3.9-5.8	4.36	4.29	0.36	4.38	4.27	0.14
CO_2, mmol/L	23-29	22.72	22.34	0.54	23.24	21.82	<.0001
Calcium, mg/dL	9.2-12.2	9.29	9.40	0.36	9.28	9.40	0.35
Magnesium, mg/dL	1.7-2.3	2.18	2.14	0.46	2.14	2.18	0.18
Phosphorus, mg/dL	5-8.5	6.57	6.28	0.29	6.88	5.96	<.0001
Cholesterol, mg/dL	78-142	121.13	105.57	0.20	111.01	115.68	0.01
ALP, IU/L	0-110	88.42	75.33	0.33	84.80	78.95	0.01
Total bilirubin, mg/dL	0-0.8	0.35	0.33	0.25	0.32	0.37	0.0007
AST, IU/L	75-135	64.83	49.90	0.14	56.24	58.50	0.20
GGT, IU/L	7-50	12.75	11.90	0.23	12.46	12.20	0.50
CPK, IU/L	100-515	325.08	135.85	0.06	208.37	252.56	0.46
Osmolality, mOsm/L	273-293	273.28	273.25	0.99	271.32	275.21	0.18
Total protein, g/dL	6.5-8.9	6.50	6.48	0.83	6.33	6.65	0.004
Albumin, g/dL	2.5-3.5	3.80	3.80	1.0	3.71	3.89	0.002
Globulin, gms%	3-4.2	2.70	2.68	0.78	2.61	2.76	0.01
Albumin:globulin ratio	0.6-1.4	1.42	1.43	0.75	1.43	1.42	0.42
Anion gap	-	17.06	16.93	0.21	16.93	16.62	0.39

[1]Blood serum parameters include glucose, blood urea nitrogen (BUN), creatinine, sodium, chloride, potassium, CO_2, calcium, magnesium, phosphorus, cholesterol, alkaline phosphatase (ALP), bilirubin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), osmolality, total protein, albumin, globulin, albumin:globulin ratio, and anion gap. All were assessed using a Beckman AU480 chemistry analyzer.
[2]Breathitt Veterinary Center laboratory reference ranges.
[3]One group was treated with a cannabinoid supplement (TRT) and the other as a control (CON).
[4]Pre indicates sample taken before transportation. Post indicates sample taken after transportation.
[5]Significance was considered at $P < 0.05$.

Chute Scores

Chute scores were assigned to calves based on temperament when entering the chute system before and after transportation. The highest pre chute score given was 4 to a treatment calf. The highest post chute score given was 2 for two control calves. Most scores given to both groups were of calm (1) or restless (2) temperament. No severe ratings were given. Summary of chute scores can be found in Table 8.

Table 8. Effect of cannabinoid gel supplementation on chute scores[1] of weaned calves before and after six h of transportation

Observation time	Treatment	Control	P value	Standard Error
Pre transportation	1.63	1.54	0.78	0.32
Post transportation	1.13	1.21	0.54	0.12

[1]Chute scores based on a rubric as follows: 1= calm, no movement, 2=restless, shifting, 3=squirming, occasionally shaking of the chute, 4= continuous vigorous movement and shaking of the chute, and 5= rearing, twisting of the body, or violent struggling

Exit Velocity

Exit velocity was determined using two sets of timers. Times were taken for calves exiting the chute. No severe times were recorded. Statistical significance was not observed for treatment or hour on exit velocities (Table 9).

Table 9. Effect of cannabinoid gel supplementation on exit velocity (m/s) observations of weaned calves before and after six h of transportation.

Parameter	Treatment	Control	P value	Standard Error
Pre transportation	1.99	1.43	0.08	0.31
Post transportation	1.39	1.23	0.54	0.25

Shrinkage Rate

Body weights were taken prior to and post treatment and transportation of calves for shrinkage rates. Weights were also recorded 30, 60 and 90-days post study and are recorded in Table 10. Fluctuations in calf weights from 30 days to 90 days is typical in average production settings.

Table 10. Summary of body weight (BW) observations through shrinkage rate and average growth of calves over time

Parameter	Treatment	Control	P value	Standard Error
Pre BW, lb	639.00	630.00	0.73	25.34
Post BW, lb	606.33	600.00	0.80	24.63
Shrink, %[1]	-5.15	-4.73	0.53	0.65
30d BW, lb	660.67	656.25	0.87	25.94
60d BW, lb	712.67	714.17	0.96	27.29
ADG 60d[2], lb/d	1.23	1.41	0.20	0.13
90d BW	699.00	694.17	0.85	24.58
ADG 90d[3], lb/d	0.67	0.71	0.64	0.09

[1]Shrink percent of body weight was calculated during transportation.
[2,3]Average daily gain was calculated for all calves at 60 and 90 days post treatment and transportation.

Discussion

Pilot study

Kansas State's A&P laboratory was able to detect presence of CBD well above 0.1 LLOQ in two beef cows after treatment. The gel product contained high levels of CBD (43,944 μg/ml); however, CBD was only detected in two samples at different times. CBD appeared in plasma concentrations at hours 1 (cow-2) and 24 (cow-1) respectively. An interesting note was that the metabolite CBD7-acid was detected as early as 8 hours post treatment and was on an upward trend at the end of our 24-hour sampling. This demonstrates evidence of CBD entering the body and being metabolized rather quickly in cattle. Manufacturer recommendations explain gel product should be administered into the mouth and absorbed orally for better bioavailability and faster delivery (Equilibrium, CannaHorse, Lexington, KY). This product was formulated for equine specifically, with recommended dosage rates on a per pound basis. While precautions were taken to ensure maximum absorption of product in both cows, a possible explanation is that the product was not equally ingested in both animals. Oral absorption has not been a typical method of pharmaceutical delivery in cattle. Products have commonly been administered through top dress of feed, drenching, or bolus (Kleinhenz et al., 2020b) form to ensure effective delivery. One cow was noted exhibiting increased mouth movements (i.e., licking, smacking, eating) after treatment administration, whereas the other cow had little to no mouth movements. This could be a potential explanation for CBD amounts showing up later in cow-2 than cow-1 because of delayed or slower rate of absorption of product due to level of mouth activity post treatment. Limited research was available on administering industrial hemp or cannabinoids to cattle. As a result, there were no identified and proven cattle dosage rates for products. Although the product contained high levels of CBD, is

likely that the dosage rate adopted from CannaHorse and Kleinhenz et al., 2020b for this bovine study was too low for plasma detection from a pharmacokinetic standpoint. Results from this pilot study validate that CBD from the gel were available for absorption and were metabolizable in cattle. This provides justification for future cannabinoid trials, looking further into effective dosage rates and rates of clearance from the body.

Phase 1

Although average complete blood count concentrations remained within respective reference ranges, changes occurred with interactions between treatment and hour for white blood cell, red blood cell, hematocrit, and eosinophil count in calves. Weaning events applied significant levels of stress on calves, leading to increased risk of susceptibility to disease, specifically respiratory disease. Stress events trigger an immune response, usually leading to increased counts of white blood cells, neutrophils and eosinophils (Jaime et al., 2021). This study indicated an interaction for white blood cells, showing a possible increase in WBC count in response to weaning stress for control calves. Significance of hematocrit levels in these calves could indicate that an increase in HTC would potentially lead to dehydration, which would be associated with water loss. During weaning, calves experience stress from separation from their mothers and are only concerned with finding them. Calves typically exhibit decreased feed and water intake during the first day or so of weaning, depending on calf temperament and stress level. HTC measures the proportion of red blood cells within the body. RBC carries oxygen throughout the body, with a potential of too few or too many RBC being a sign of disease or infection. Numerous studies document a relationship between weaning stress

and development of BRD in calves (Lynch et al., 2019; Price et al., 2003; Taylor et al., 2010; Weary et al., 2008; Wilson et al., 2017). This study observed treatment differences for monocyte counts (P=0.0042) in calves. Monocyte concentrations were greater in CON calves (0.92 $\times 10^3/\mu L$) versus TRT calves (0.70 $\times 10^3/\mu L$). Monocytes can be defined as the immune system's first line of defense, where lymphocytes are the body's second line of defense. Lymphocyte concentrations (P=0.06) tended to be greater in CON (7.25 $\times 10^3/\mu L$) versus TRT calves (5.49 $\times 10^3/\mu L$) for phase 1. This data suggest that CON calves were experiencing a more significant immune response during the weaning process than that of calves on treatment. Treatment exhibited a suppressed immune response in calves administered supplementation This implies that cannabinoid treatment potentially reduces the effects of stress if the product is administered at the time of weaning. Dosage rates were adjusted slightly from the pilot study for phase 1 administration to calves. Doses were still on a per pound basis but were doubled based on CBD concentrations found from the pilot study data. Calves received a dose of 0.005ml/lb.

Serum chemistry concentrations remained within standard ranges for healthy cattle. However, changes occurred with creatine phosphokinase (CPK) and bilirubin enzyme levels based on effect of treatment. High levels of CPK indicate stress or injury to the heart or muscles of an individual (Anderson et al., 1976). Increased levels of CPK also occur with exercise. This provides a possible explanation for an effect of treatment on CPK, where it is possible that certain calves exhibiting weaning stress behaviors, like pacing the fence-line, would have increased CPK concentrations due to increased body movement and stress. Bilirubin has commonly been used as a biomarker of liver status in

cattle, where high levels are related to negative energy balance after a stress event. This can be caused by prolonged periods of fasting, reiterating a possibility that stressed cattle experiencing decreased feed and water intake will show signs of stress when looking at liver and kidney enzyme panels. Changes observed from serum chemistry in Phase 1 were not representative of immediate danger to calves and all levels were not concerning at this dosage rate.

Weaning Stress Behaviors

Weaning stress behaviors observed via drone footage for calves included bawling, pacing, and standing at fence-line. Although statistical significance was observed in treatment and hour effects on CBC data, no change in behavior or temperament was observed. Calf behavior has been highly dependent on temperament and environment. Calves were weaned using fence-line weaning methods, which has been used by producers to mitigate stress on calves and dams. There was an effect of hour on weaning stress behaviors, which was expected to occur. This means that as time passed, calves presented a calmer disposition which is to be expected during a weaning process. Calves experience higher levels of stress within the first day or two of weaning and will begin to calm after that time. Although dosage rates were doubled from the pilot study, it is possibly that doses were too low to influence observable changes in behavior. This also paves the way for future research on stress and anxiety reducing factors of CBD and pursuing cannabinoid effects on cattle behavior.

Phase 2

Complete blood count levels remained within reference ranges and no two-way interactions were observed between treatment and hour during analysis. Changes were observed with effect of treatment on white blood cell, lymphocyte, monocyte, and basophil counts. Congruent with results from Phase 1, lymphocyte and monocyte concentrations were similar in effects based on treatment. CON calves tended to have higher levels than TRT calves, alluding to a possibility that those calves experienced slightly higher levels of stress, thus increasing counts for those parameters as a response to the stress of transportation. The effect of treatment on these parameters could mean that CBD supplementation could influence biomarkers and a stress response with cattle during transportation. Basophils are a type of white blood cell that are responsible for inflammatory responses and can produce compounds that coordinate immune responses. When animals have experienced stress, counts of white blood cells could be affected based on the type of immune response activated. Effects of hour were observed for MCHC, Lymphocyte, eosinophil, and basophil counts. Eosinophil analysis ultimately measures the type of white blood cell in the body. Eosinophils become active when infection or disease is present. Differences between eosinophil values have been attributed to physical and emotional stress that coincides with high levels of epinephrine and cortisol. Often when cattle are stressed, their immune systems exhibit decreased function, making it more difficult to fight off infection or disease. This causes an increase in the body's line of defenses with different white blood cells present and other parameters involved during a response to stress stimuli.

Though serum chemistry concentrations returned in safe, standard ranges, differences were observed for glucose and bilirubin levels based on the effect of treatment among calves. Serum glucose levels measure blood sugar levels in the body. High levels of glucose are likely to occur during stress events, like transportation, whereas low levels will be detected when an animal does not eat. Stressed cattle usually experience reduced feed intake, so depending on a stress event and any effect on a calf, glucose levels could show high or low. Bilirubin has commonly been a biomarker of liver status in cattle and typically has a negative effect on cattle during long-distance transportation. This indicates that a difference of treatment among groups could influence glucose and bilirubin values in calves. During Phase 2, serum chemistry analysis also found a tendency for CPK values for effect of treatment. CPK levels have been known to increase with length of travel during cattle transport (Jaime et al., 2021).

Chute Scores

Chute scores were evaluated and are used to determine cattle temperament. If cattle received a high chute score, that meant they were more likely to experience higher levels of stress than other calves in the same stress event. Lower chute scores indicated naturally calmer calves. This study reported no significant difference in chute scores recorded pre and post transportation and no difference between treatment groups. Like weaning stress behaviors, it is likely that dosage rates of the gel supplement were too low to produce an observable change in temperament.

Exit Velocity

Exit velocity was another method used to evaluate cattle temperament. Exit velocity quantified a calf's time coming out of the chute. Faster exit times indicated a

more excitable temperament, and those calves were more likely to experience higher levels of stress. Calves with a calmer disposition exhibited slower exit times. Fast times meant they were running out of the chute, whereas slower times indicate a calf is walking or moving slower out of the chute. This study indicated no difference or significance of exit velocity times with effect of hour or treatment. All calves were relatively calm or docile in disposition.

Shrinkage Rate

Shrinkage rate was calculated pre and post transportation, with no major fluctuations in calf weights. It is expected for cattle to exhibit some amount of shrink during transportation. This study resulted in an average shrink rate of 31.48 lbs per calf. Transportation of calves was also conducted during summer so heat stress could have also played a role in shrinkage rates. Body weights were taken at 30, 60, and 90 days post treatment. There was no significance with respect to effect of treatment, however, the effect of day was observed. Fluctuations in calf weights over the 90-day period has been typical and was expected in this study. This weight change was typical in an average production setting and has been highly variable depending on the environment and nutritional programs set up for cattle. During cattle growth, at some point body weight can easily be maintained, lost, or gained. This depends on how hard calves have been pushed on grain or grazing forages. Cattle in this study were fed daily, for a target gain of 1.5-2 pounds a day. They also had free choice of forage while they were rotationally grazed. Some calves continued to experience gains up to that 90 weight whereas some calves lost weight from the 60 to 90 weights. This has been common throughout cattle production operations.

Summary

CBD and CBD-7 acid were readily available for absorption in cattle and were being metabolized rather quickly after administration. This shows basic characteristics to other pharmacokinetic studies on bovine. However, there was still limited information regarding residual effects of feeding IH and administration of cannabinoids available in cattle. The results of this study provided a framework to future research regarding dosage rates and clearance rates over time of cannabinoid concentrations. Current dosage rates available in cattle were not adequate for consistent results or blood analysis detection that would help determine main effects of IH and cannabinoids. CBD products in general have not been consistent with cannabinoid concentrations due to unregulated methods of extraction and processing of industrial hemp especially in livestock species. This study strongly supports the need for further investigation on the effects of treatment on physically observed changes in behavior during a stress response. Several effects were observed on stress biomarkers within blood analysis, suggesting that cannabinoid supplementation could potentially elicit a stress response in cattle. However, no changes in behavior were observed during this study. This was likely due to dosage rates and not administering enough product available to see a change. Future research into CBD mitigation of stress in cattle will likely need to utilize higher dosage rates to be able to detect an observable change in behaviors. Most products for CBD use in animals have been formulated for small animals like cats and dogs. Products used in horses were a closer match to dosing per pound. However, horses and cattle have different digestive systems that could play a role in how effective digestion or absorption is for cattle. Finally, this study was one of few research studies focusing on effects of IH and

cannabinoids in cattle. Little to no information is available, making the possibilities of future research on this endless.

Appendix A: Definition of Terms

BRD-Bovine Respiratory Disease, also known as shipping fever, is the most common and costly disease affecting the cattle industry covering upper and lower respiratory tract disease.

BW- body weight

Cannabinoid- any group of closely related compounds which include cannabinol and the active constituents of cannabis.

Cannabis sativa- Hemp

CBC- Complete blood count

CBC- Cannabichromene

CBD- Cannabidiol

CBG- Cannabigerol

Chute Score (CS)- a 5-point scoring system used to determine temperament of cattle. Scoring a 1 means the calf is calm with no movement. Scoring a 5 means the calf is rearing or twisting or violently struggling within the chute (Grandin et al., 1993; modified from Tulloh et al., 1961).

Exit Velocity (EV)- Use of a timer to show the speed at which a calf leaves the chute. An indicator of physiological stress resulting from an animal's encounter with humans (Burrow et al., 1988; Curley et al., 2006).

IH- Industrial hemp

IHBP- Industrial hemp by-products

LDH- Lactate dehydrogenase; an enzyme found in most tissues throughout the body and is responsible for the conversion of pyruvate into lactic acid (Jovanovic et al., 2010).

THC- Tetrahydrocannabinol

Weaning- process of gradually separating a calf from their source of milk. (Separation of calf and cow)

Appendix B: Limitations and Assumptions

The following limitations create restraints on the research:

1) The sample size of calves was limited to the population located at the MSU Beef Unit. The study included 2 cows for the pilot and 27 calves for Phase 1 and Phase 2.
2) Observation of weaning stress through drone footage attempted to provide standardized behavior evaluation. However, this can be subject to human error due to subjectivity of the individual recording the behaviors.
3) Although chute scores have been proposed as a viable method to measure temperament of cattle, this can also be subject to human error depending on the consistency of the observer.
4) All phases of the study were conducted on farm and not in a controlled environment of a laboratory setting.

The research was conducted under the following assumptions:

1) Cannabinoid supplement used in the study were being administered appropriately, making sure each calf received a full dose for effectiveness.
2) Blood samples were handled and stored properly.
3) Subjective evaluations including weaning stress behavior, chute scores, and exit velocity were completed consistently with honest opinions. The same individual recorded data for each evaluation to ensure validity.
4) All animals were handled under the same conditions to ensure the same amount of stress was put for consistent and effective results.

Appendix C: Budget

The total budget, excluding the value of donated product, was $17,167.97. The cost breakdown for each project component is provided below. Estimated cost of donated product was included. Supply calculations include materials for blood sampling and general organization.

Description	Cost
Laboratory Testing Services	
Cannabinoid Testing	$10,670.00
CBC blood analysis	1,890.00
SC blood analysis	1,575.00
LDH blood analysis	2,160.00
Blood Collection Supplies	
10ml vacutainer blood tubes	555.11
18-gauge vacutainer needles	26.70
1.5ml microcentrifuge tubes	141.16
Other	
Transportation-fuel	150.00
Sample shipping	317.34
Product-cannabinoid gel	Donated

Appendix D: Time Schedule

The Pilot study was completed in 24 hours. This period allotted time for cattle processing, treatment administration, blood collection, and to return cattle to the pasture. Phase 1 was the most intensive of the three studies, transpiring over a 5-day period. Phase 2 was scheduled for approximately 12 hrs. Three months after Phase 2 completion was taken for records of calf weights and data analysis.

Appendix E: IACUC Approval

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 modification 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

Kristi Stockdale
IACUC Coordinator

cc:
IACUC File

Appendix F: Specifications for the CannaHorse Equilibrium Product

Unit size: 3.5 ml

CBD per unit: 250mg

Dosage: 1 unit CannaHorse per 453 kg body weight (.5 mg CBD per kg body weight)

Active Ingredients: CBD, CBC, CBG, linalool, beta-caryophyllene

Inactive Ingredients: water, organic coconut oil, quillaia extract, citric acid, mixed tocopherols, potassium sorbate, sodium benzoate, gum acacia

Free of THC

References

Agricultural Act of 2014, 7 U.S.C. § 5940 (2014)

Agricultural Improvement Act of 2018. Public Law 115-334-Dec. 20, 2018. Accessed December 31, 2020. https://www.congress.gov/115/plaws/publ334/PLAW-115publ334.pdf.

Aizpurua-Olaizola, O., U. Soydaner, E. Ozturk, D. Schibano, Y. Simsir, P. Navarro, N. Extebarria, and A. Usobiaga. 2016. Evaluation of the cannabinoid and terpene content during the growth of *Cannabis Sativa* plants from different chemotypes. J. Nat Prod. 79. 324-331. Doi:10.1021/acs.jnatprod.5b00949.

Alam, M., Hasanuzzaman, M. Hassan, T. Rakib, E. Hosssain, H. Rashid, A. Sayeed, L.B. Philips, and A. Hoque. 2018. Assessment of transport stress on cattle travelling a long distance (≈648 km), from Jessore (Indian border) to Chittagong, Bangladesh. *Veterinary Record Open.* 2018;5:e000248. doi:10.1136/vetreco-2017-00248.

Allegret, S., P. Bouloc, L. Arnaud. 2013. The history of hemp. Hemp- industrial production and uses. CAB International, Wallingford.

Amaducci, S., H. J. Gusovious. 2010. Hemp- cultivation, extraction and processing. Industrial applications of natural fibres: structure, properties and technical applications. Pp. 109-135. https://doi.org/10.1002/9780470660324.ch5

Anderson, P. H., S. Berrett, and D. S. Patterson. 1976. The significance of elevated plasma creatine phosphokinase activity in muscle disease of cattle. J. of Comparative Pathology. Vol. 86. P. 531-538. https://doi.org/10.1016/0021-9975(76)90062-1

Appiah-Kusi, E., N. Petros, R. Wilson, M. Colizzi, M. G. Bossong, L. Valmaggia, V. Mondelli, P. McGuire, and S. Bhattacharyya. 2020. Effects of short-term cannabidiol treatment on response to social stress in subjects at clinical high risk of developing psychosis. Pyschopharmacology. (2020) 237:1121-1130. https://doi.org/10.1007/s00213-019-05442-6.

Atalay, S., I. Dobrryznska, A. Gegotek, and E. Skrzydlewska. 2020. Cannabidiol protects keratinocyte cell membranes following exposure to UVB and hydrogen peroxide. Redox Biol. 36 (2020). 101603. https://doi.org/10.1016/j.redox.2020.101613

Barnes, K., S. Smith, D. Lalman. 2017. Managing Shrink and Weighing Conditions in Beef Cattle. Oklahoma Cooperative Extension Service. ANSI-3257.

Bailoni, L., Bacchin, E., Trocino, A., & Arango, S. (2021). Hemp (*Cannabis sativa* L.) Seed and Co-Products Inclusion in Diets for Dairy Ruminants: A Review. *Animals : an open access journal from MDPI*, *11*(3), 856. https://doi.org/10.3390/ani11030856

Beards, S., C. G. Anderson, S. Borges, M. E. Dewey, H. L. Fisher, and C. Morgan. 2013. Life events and psychosis: a review and meta-analysis. Schizophrenia Bulletin vol. 39 no. 4 pp. 740-747. Doi: 10.1093/schbul/sbt065.

Bergamaschi, M., R. Queiroz, M. Chagas, D. Oliveira, B. Martinis, F. Kapczinski, J. Quevedo, R. Roesiers, N. Schroder, A. Nardi, R. Martin-santos, J. Hallak, A. Zuardi, and J. Crippa. 2011. Cannabindiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. Neuropsychopharmacology. 36, 1219-1226.

Bouloc, P. 2013. Hemp: industrial production and uses. CABI. Oxfordshire, p.312. ISBN 978-1-845-93793-5

Brooks, G. A., H. Dubouchaud, M. Brown, J. P. Sicurello, and C. E. Butz. 1999. Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. Proc. Natl. Acad. Sci. USA. Vol. 96. Pp1129-1134.

Bruno, K. A., E. S. Vanzant, and K. R. Mcleod. 2016. Relationships of a novel objective chute score and exit velocity with growth performance of receiving cattle. J Anim Sci. 2016.94:4819-4831. doi:10.2527/jas2016-0438.

Brutlag, A., and H. Hommerding. 2018. Toxicology of marijuana, synthetic cannabinoids, and cannabidiol in dogs and cats. Vet. Clin. Small Anim.48 1087-1102. https://doi.org/10.1016/j.cvsm.201807.008.

Bryant, T. C., J. R. Nichols, J. R. Adams, T. D. Farmer, and D. G. miles. 2008. Effect of Tilmicosin Alone or in Combination with Mannheima haemolytica Toxoid Administered at Initial Feedlot Processing on Morbidity and Mortality of High Risk Calves. The Bovine Practitioner. Vol. 42, NO. 1.

Burdick, N. C., R. D. Randel, J. A. Caroll, and T. H. Welsh Jr. 2011. Interactions between Temperament, Stress, and Immune Function in Cattle. International Journal of Zoology. doi:10.1155/2011/373197.

Burrow, H., G. Seifort, and N. Corbet. 1988. A new technique for measuring temperament in cattle. Proc. Aust. Soc. Anim. Prod. 17:154-157.

Chirase, N., L. Greene, C. Purdy, R. Loan, B. Auvermann, D. Parker, E. Walborg Jr, D. Stevenson, Y. Xu, and J. Klaunig. 2003. Effect of Transport Stress on Respiratory Disease, Serum Antioxidant Status, and Serum Concentrations of Lipid Peroxidation Biomarkers in Beef Cattle. Am. J. Vet. Res. 2004;65:860-864.

Coffey, K. P., W. K. Coblentz, J. B. Humphry, and F. K. Brazle. (2014). Transportation shrink in Beef Cattle. Transportation Shrink in Beef Cattle | Ohio BEEF Cattle Letter (osu.edu)

Collier, R. J., L. H. Hall, L. H. Baumgard, R. B. Zimbelman, Y. Xiao. 2019. Feed and Water Intake of Heat Stressed Cattle. Univ. of Arizona. Intermountain Feed Co. Iowa State Univ. Dairy Nutrition Services, Univ. of Florida.

Controlled Substance Act of 1970, 21 U.S.C. § 801 (1970)

Crini, G., E. Lichtfouse, G. Chanet, and N. Morin-Crini. 2020. Applications of hemp in textiles, paper industry, insulation and building materials, horticulture, animal nutrition, food and beverages, nutraceuticals, cosmetics, and hygiene, medicine,

agrochemistry, energy production, and environment: a review. Environmental Chemistry Letters. 18:1451-1476. Hhttps://doi.org/10.1007/s10311-020-01029-2.

Curley, K. O., J. C. Paschal, T. H. Welsh Jr, and R. D. Randel. 2006. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103. doi:10.2527/jas.2006-055

Damtew A., Y. Erega, H. Ebrahim, S. Tsegaye, and D. Mysigie. 2018. The Effect of Long Distance Transportation Stress on Cattle: a Review. Biomed. J. Sci.&Tech. Res. 3(3). BJSTR.MS.ID.000908. DOI: 10.26717/BJSTR.03.000908.

Deabold, K. A., W. S. Schwark, L. Wolf, and J. J. Wakshlag. 2019. Single Dose Pharmacokinetics and Preliminary Safety Assessment with use of CBD-Rich Hemp Nutraceutical in Healthy Dogs and Cats. MDPI. J.Anim. 9 832. doi: 10.3390/ani9100832.

Dhabhar, F.S., B.S. McEwen. 2001. Bidirection Effects of Stress and Glucocorticoid Hormones on Immune Function: Possible Explanations for Paradoxical Observations. Psychneuroimmunology. Third Edition, Academic Press, San Diego. Pp.301-338.b.

Dhabahr, F. S. 2018. The short-term stress response- Mother nature's mechanism for enhancing protection and performances under conditions of threat, challenge, and opportunity. Frontiers in Neuroendocrinology, 49, 175-192. Doi:10.1016/j.yfrne.2018.03.004.

Draeger, A., L. K. Hoffman, P. R. Godwin, A. J. Davis, and C. A. Porr. 2020. Pharmacokinetics of a single feeding of pelleted cannabidiol in horses. Steeplechase, Murray State Digital Commons, 4:2:1-12 https://digitalcommons.murraystate.edu/cgi/viewcontent.cgi?article=1067&context=steeplechase

Engle, T. E., J. W. Spears, L. Xi, and F. W. Edens. 2000. Dietary copper effects on lipid metabolism and circulating catecholamine concentrations in finishing steers.J. Anim. Sci. 78:2737-2744.

Enriquez, D. M. J. Hötzel, and R. Ungerfield. 2011. Minimising the stress of weaning beef calves: a review. Acta Veterinaria Scandinavica. 53:28. http://www.actavetscand.com/content/53/1/28

European Monitoring Centre for Drugs and Drug Addiction. 2018. Cannabis legislation in Europe: An Overview. Doi: 10.2810/930744.

Farinon, B., R. Molinari, L. Costantini, and N. Merendino. 2020. The seed of industrial hemp (*Cannabis sativa* L.): Nutritional quality and potential functionality for human health and nutrition. Nutrients 12, 1935;doi:10.3390/nu12071935.

Fell, L.R. Colditz, I.G., Walker, K.H., and Watson, D.L. 1999. Associations between temperament, performance, and immune function in cattle entering a commercial feedlot. Aust. J. Exper. Agric. 39:795-802.

Förstermann, U., W. C. Sessa. 2011. Nitric oxide synthases: regulation and function. Eur. Heart J. 33(7):829-37, 837a-837d. doi:10.1093/eurheartj/ehr304.

Gamble, L. J., J. Boesch, C. Frye, W. Schwark, S. Mann, L. Wolfe, H. Brown, E. Berthelsen, and J. Wakshlag. 2018. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. Front. Vet. Sci. 5:165. Doi: 10.3389/fvets.2018.00165.

Gaylean, M. L., L. J. Perino, and G. C. Duff. 1999. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. 77:1120-1134.

Glaster, J. K. Elbert, S. Gubbins, J. Bashiruddin, D.J. Paton. 2011. Normal Variations in Thermal Radiated Temperature in Cattle: Implications for Foot and Mouth Disease Detection.

González, L, A., K. S. Schwartzkopf-Genswein, M. Bryan, R. Silasi, F. Brown. 2012. J. Anim. Sci. 90(10):3630-9. Doi:10.2527/jas.2011-4786.

Goldstein, D. S. 2003. Catecholamines and Stress. Endocrine Regulations. Vol. 37. 69-80.

Grandin, T. 1980. The Effect of Stress on Livestock and Meat Quality Prior to and During Slaughter. International Journal for the Study of Animal Problems, 1(5), 313-337.

Grandin, T. 1992. Behavioral agitation during handling of cattle is persistent over time. Appl. Anim. Behav. Sci. 36:1-9. doi:10.1016/0168-1591(93)90094-6

Hao, E., P. Mukhopadhyay, Z. Cao, K. Erdelyi, E. Holovac, L. Liuadet, W. Lee, G. Hasko, R. Mechoulam, and P. Pacher. 2015. Molecular Med. 21:38-45.

Hay, M., M.C. Meunier-Salun, F. Brulaud, M. Monnier, and P. Mormede. 2000. Assessment of hypothalamic-pituitary-adrenal axis and sympathetic nervous system activity in pregnant sows through the measurement of glucocorticoids and catecholamines in urine. J. Anim. Sci. 78:420-428.

Hooks, C. A., N. R. Denney, M. R. Hovey, J. F. Miller, B. M. Papajeski, J. C. Robertson, E. A. Backes, C. A Porr, A. J. Davis. 2018. Differences in Fecal Egg Count (FEC), Performance, Blood Parameters, and Lactate Dehydrogenase Activity of Stocker Calves Treated with Various Anthelmintics. J. Anim. Sci. Vol. 96, Suppl. S1.

Hoppe, S., H. R. Brandt, S. Konig, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. J. Anim. Sci. 88:1982-1989. Doi:10.2527/jas.2008-1557.

Jericho, K. W. F., and E. V. Langford. 1978. Pneumonia in calves produced with aerosols of bovine herpesvirus 1 and Pasteurella haemoltyica. Animal Pathology Division. Vol. 42.

Johnson, K., D. L. Pendell. 2017. Market Impacts of Reducing Prevalence of Bovine Respiratory Disease in United States Beef Cattle Feedlots. Front. Vet. Sci., 4:189. doi:10.3389/vets.2017.00189.

Jovanovic, P., L. Zoric, I. Stefanovic, B. Dzunic, J. Djordjevic-Jocic, M. Radenkovic, M. Jovanovic. 2010. Lactate Dehydrogenase and Oxidative Stress Activity in Primary Open-Angle Glaucoma Aqueous Humour. B. J. B. Med. Sci.10 (1):83-88.

Kamboh, A. A., S. Q. Hang, M. Bakhetgul, and W-Y Zhu. 2013. Effects of genistein and hesperidin on biomarkers of heat stress in broilers under persistent summer stress. Poultry Sci. 92:2411-2418.http://dx.doi.org/10.3382/ps.2012-02960.

Kicman, A., and M. Toczek. 2020. The effects of cannabidiol, a non-intoxicating compound of cannabis, on the cardiovascular system in health and disease. Int. J. Mol. Sci. 21, 6740; doi: 10.3390/ijms21186740.

Klein, M. O., D. S. Battagello, A. R. Cardoso, D. N. Houser, J. C. Bittencourt, and R. G. Correa. 2018. Dopamine: Functions, Signaling, and Association with Neurological Diseases. Cellular and Molecular Neurobiology. 39:31-59. https://doi.org/10.1007/s10571-018-0632-3.

Kleinhenz, M., G. Magnin, S. T. Ensley, J. J. Griffin, J. Goeser, E. Lynch, and J. F. Coetzee. 2020a.Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. App. Anim. Sci. 36:489-494. https://doi.org/10.15232/aas.2020-02018

Kleinhenz, M., G. Magnin, Z. Lin, J. Grifn, K. Kleinhenz, S. Montgomery, A. Curtis, M. Martin, and J. Coetzee. 2020 Plasma Concentrations of Eleven Cannabinoids in Cattle Following Oral Administration of Industrial Hemp (*Cannabis sativa*). Sci. Rep. 10:12753. https://doi.org/10.1038/s41598-020-69768-4 (b)

Kumar, P., A. Nagarajan, P. D. Uchil. 2018. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb Protoc. 1;2018(6). Doi10.1101/pdb.prot095497.

Lanier, J. L., T. Grandin. 2002. The relationship between Bos Taurus feedlot cattle temperament and foreleg bone measurements. Western Section, American Society of Animal Science, Vol. 53:97-98.

Le, A., C. R. Cooper, A. M. Gouw, R. Dinavahi, A. Maitra, L. M. Deck, R. E. Royer, D. L. Vander Jagt, G. L. Semenza, C. V. Dang. 2010. Inhibition of Lactate Dehydrogenase A Induces Oxidative Stress and Inhibits Tumor Progression. PNAS. 107 (5) 2037-2042 https://doi.org/10.1073/pnas.0914433107

Linares, I., A. Zuardi, L. Pereira, R. Queiroz. R. Mechoulam, F. S. Guimaraes, and J. Crippa. 2019. Cannabidiol presents an inverted U-shaped dose-response curve in a simulated public speaking test. Brazilian Journal of Psychiatry. 41(1):9-14.

Lynch, E., M. McGee, B. Earley. 2019. Weaning Management of Beef Calves with Implications for Animal Health and Welfare. J. Appl. Anim. Research, 47:1, 167-

175, doi: 10:1080/09712119.2019.1598425. https://doi.org/10.1080/09712119.2019.1594825

Mackay, J. R. D., S. P. Turner, J. Hyslop, J. M. Deag, and M. J. Haskell. 2013. Short-term temperament test in beef cattle related to long-term measures of behavior recorded in the home pen. J. Anim. Sci. 91:4917-4924. doi:10.2527/jas.2021-5473

Marihuana Tax Act of 1937 Pub. L.. 75-238 , 50 Stat 551.

Mathar, I., F. Schweda, M. Freichel. 2010. Increased catecholamine secretion contributes to hypertension in TRPM4-deficient mice. J. Clin. Invest. 120(9):3267-3279. https://doi.org/10.1172/JCI41384.

McEwen, BS. 1998. Protective and Damaging Effects of Stress Mediators. N. Engl. J. Med. 338 171-179.

McLeod, T. M., A. L. Lopez-Figueroa, M. O. lopez-Figeuroa. 2001. Psychopharmacol. Bull. 35(1):24-41.

McManus, C., C.B. Tanure, V. Peripolli, L. Seisax, V. Fischer, A.M. Gabbi, S.R.O. Menegassi. 2016. Infrared Thermography in Animal Production: an overview.

Merwe, D. V. D., D. R. Burchfield, T. D. Witt, K. P. Price, and A. Sharda. 2020. Chapter One- Drones in Agriculture. Advances in Agronomy. Vol. 162, p.1-30. https://doi.org./10.1016/bs.agron.2020.03.001.

Mihalache, D. B., N. A. Vangehele, A. A. Petre, A. Matache. 2020. The Use of Drones in Modern Agriculture. Annals Univ. of Craiova-Agriculture, Montanology, Cadastre Series. Vol. L/2020.

Moisá, S. J., S. S. Aly, T. W. Lehenbauer, W. J. Love, P. V. Rossitto, A. L. Van Eenennaam, S. C. Trombetta, E. M. Bortoluzzi, and L. E. Hulbert. 2019. Association of plasma haptoglobin concentration and other biomarkers with bovine respiratory disease status in pre-weaned dairy calves. Journal of Veterinary Diagnostic Investigation. Vol. 31(1) 40-46. Doi:10.1177/1040638718807242.

Neindre,L P., Grignard, L., Trillat, G., Boissy, A., Menissier, F., Sapa, J. and Boivin, X. 2002. Docile Limousin cows are not poor mothers. Proceedings of the seventh world congress on genetics applied to livestock production19–23 August, 2002Montpelliercomm. 14–13

Nepveux, M. 2018 Farm Bill Provides A Path Forward For Industrial Hemp. American Farm Bureau Federation. Accessed 03/17/2021. https://www.fb.org/market-intel/2018-farm-bill-provides-a-path-forward-for-industrial-hemp

Pacifico, D., F. Miselli, M. Micheler, A. Carboni, P. Ranalli, and G. Mandolino. 2018. Genetics and marker-assisted selection of the chemotype in *Cannabis sativa* L. Molecular breeding. 17: 257-268. Doi: 10.1007/s11032-005-5681-x.

Papadelis, C., C. Kourtidou-Papideli, E. Vlachogiannis, P. Skepastianos, P. Bamidis, N. Maglaveras, and K. Pappas. 2003. Effects of mental workload and caffeine on

catecholamines and blood pressure compared to performance variations. Brain Cogn. 51(1):143-54. Doi:10.1016/s0278-2626(02)00530-4.

Petkovics, I., D. Petokovic, and A. Petkovics. 2017. IoT Devices vs. Drones for Data Collection in Agriculture. DAAAM International Scientific Book. Ch. 6, pp.063-080, B. Katalinic. (Ed), Doi:10.2507/daaam.scibook.2017.06.

Price, E. O., J. E. Harris, R. E. Borgwadt, M. L. Sween, and J. M. Connor. 2003. Fenceline contact of beef calves with their dams at weaning reduces the negative effects of separation on behavior and growth rate. J. Anim. Sci.81:116-121.

Pruessner, M., Cullen, A., Aas, M., & Walker, E. F. (2017). The neural diathesis-stress model of schizophrenia revisited An update on recent findings considering illness stage and neurobiological and methodological complexities. Neuroscience and biobehavioral reviews. 73. 191-218. Doi: 10.1016/j.neubiorev.2016.12.013

Rotherham, D., S. A. Harbison. 2011. Differentiation of drug and non-drug *Cannabis* using a single nucleotide polymorphism (SNP) assay. J. Forsiint. 0379-0738. Doi:10.1016/j.forsciint.2010.10.006.

Sirikantaramas, S., F. Taura, Y. Tanaka, Y. Ishikawa, S. Morimoto, and Y. Shoyama. 2005. Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. Plant Cell Physiol. 46(9): 1578-1582. Doi: 10.1093/pcp/pci166.

Smith, R. A., D. L. Step, A. R. Woolums. 2020. Bovine Respiratory Disease: Looking back and seeing forward , what do we see? Vet. Clin. Food. Anim. 36 (2020). P239-251. https://doi.org/10.1016/j.cvfa.2020.03.009.

Smith, K., L. Kleynhans, R. M. Warren, W. J. Goosen, and M. A. Miller. 2021. Cell-Mediated Imunnological Biomarkers and their Diagnostic Application in Livestock and Wildlife Infected with *Mycobacterium bovis*. Front. Immunol. 12:609635. doi:10.3389/fimmu.630605.

Strimbu, K., J. A. Travel. 2010. What are Biomarkers? Curr Opin HIV Aids. 5(6):463-466.doi.10:1097/COH.0b013e32833ed177.

Stockings, E., D. Zagic, G. Campbell, M. Weier, W. D. Hall, S. Nielsen, G. K. Herkes, M. Farrell, and L. Degenhardt. 2018. Evidence for cannabis and cannabinoids for epilepsy: a systemic review of controlled and observational evidence. J. Neurol. Neurosurg. Psychiatry. 89:741-753.

Suverly N., B. Bartlett. 2005. Fence-line weaning: A Marketing Tool for your Calves. Michigan State University Extension Bulletin. E-2929.

Taylor, J. D., R. W. Fulton, T. W. Lehenbauer, D. L. Strep, and A. W. Confer. 2010. The Epidemiology of Bovine Respiratory Disease: What is the Evidence for Predisposing Factors? Can. Vet. J. doi:51:1095-1102.

Theurer, M. E., B. J. White, D. E. Anderson, M. D. Miesner, D. A. Mosier, J. F. Coetzee, D. E. Amrine. 2013. Effect of Transportation During Periods of High Ambient

Temperature on Physiologic and Behavioral Indices of Beef Heifers. Am. J. vet. Res., 74:481-490.

Tulloh, N. M. 1961. Behavior of cattle in yards. II. A study of temperament. Jour. Animal Behavior. Doi:10.1016/0003-3472(61)90046-x.

USDA-APHIS-VS. 2013. National Animal Monitoring System Beef Feedlot Study 2011, Part IV Health and Health Management on U.S. Feedlots with a Capacity of 1,000 or More Head. Accessed December 31, 2020. http://www.aphis.usda.gov/nahms

Vandolah, H., B. Bauer, K. Mauck. 2019. Clinicians guide to cannabidiol and hemp oils. Mayo Clin. Proc. 94(9):1840-1851. https://doi.org.10.1016/j.mayocp.2019.01.003.

Vetters, M., Engle, T., Ahola, J., Grandin, T. 2014. Comparison of Flight Speed and Exit Score as Measurements of Temperament in Beef Cattle. J. Anim. Sci. 91:374-381.

Weary, D. M., J. Jasper, M. Hötzel. 2008. Understanding Weaning Distress. J. Appl. Anim. doi: 10:1016/japplanim.2007.03.025.

Whirledge, S., J. A. Cidlowski. 2010. Glucocorticoids, Stress, and Fertility. Minerva Endocrinol. 35(2):109-125.

White, C. M. 2019. A Review of Human Studies Assessing Cannabidiol's (CBD) Therapeutic Actions and Potential. The Journal of Clinical Pharmacology. (59)7 923-934. doi: 10.1002/jcph. 1387.

Wilson, B. K., C. J. Richards, D. L. Step, C. R. Krehbiel. 2017. Beef Species Symposium: *Best management practices for newly weaned calves for improved health and well-being.* J. Anim. Sci., 95:2170-2182. doi:10.2527/jas2016.1006

Winkel, R. V., N. C. Stefanis, and I. M. Germeys. 2008. Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophrenia Bulletin vol. 34 no. 6 pp. 1095-1105. Doi:10.1093/schbul/sbn101.

World Health Organization. 2011. Biomarkers and Human Biomonitoring. Children's Health and the Environment. WHO training package for Health Sector. www.who.int/ceh

Zuardi, A. W., I. Shirikawa, E. Finkelfarb, and I. G. Karniol. 1982. Action of cannabidiol and on the anxiety and other effects produced by delta-9 THC in normal subjects. Psychopharmacology. 76. 245-250.