

Research Proposal Instructions:

- **Complete the following sections**, either by utilizing the fillable form or by providing your answers on a separate page(s). *Handwritten submissions are strongly discouraged.*
- **If answering on a separate document**, please label each section (A-F).
- **Be concise:** Your research proposal should be as concise as possible; **not to exceed 2 pages total.**
- **For more information on how to write a research proposal**, please see the Technical Bulletin on How to Conduct Research on Your Farm or Ranch by visiting <https://www.sare.org/Learning-Center/Bulletins/How-to-Conduct-Research-on-Your-Farm-or-Ranch>.

SECTION A: Identify your research question and objective, including a statement of the type of research to be conducted and its purpose.

Objective: Determine the fate of industrial hemp feed to cattle and if there is any therapeutic benefit.

Specific Aim 1: To develop and validate sensitive, accurate and robust analytical methods to quantify cannabinoid concentrations in hemp plants, hemp extracts, hemp meal and in the blood, milk and edible tissues of animals exposed to hemp.

Specific Aim 2: Characterize the plasma pharmacokinetics (PK) and milk and edible tissue residue concentrations of cannabinoids and their metabolites in cattle after oral administration of hemp products.

Specific Aim 3: Characterized hemp plant materials will be fed to animals subjected to an induced lameness model to assess the analgesic effects of CBD using a pressure mat to measure changes in gait.

Prospective clinical trial to determine cannabinoid levels in plasma and edible tissue. This data will be used to determine a dose of cannabinoids to cattle to further study potential benefits such as analgesia and enhances production (i.e. weight gains). This project will be an end user of industrial hemp grown on KSRE Research Plots under the Dr. Jason Griffin. Dr. Griffin has a KDA permit (KDA-0621466839) to grow industrial hemp.

SECTION B: Identify your experimental design.

Aim 1: Laboratory bench-top study to develop analytical methods to quantify cannabinoid levels.

Aim 2: Standard pharmacokinetic study with a minimum of 12 animals in each treatment.

Aim 3: Prospective clinical trial for with 8-12 animals per treatment group.

SECTION C: Explain what will be measured and what data will be collected.

Plasma will be obtained from blood samples taken at predetermined time points to determine cannabinoid blood concentrations over time. Blood cannabinoid concentrations will be determined using the analytical methods developed in aim 1. Once the pharmacokinetics of cannabinoids are described; a study to determine tissue concentrations of cannabinoids in edible tissue (muscle, liver, kidney, fat) will be conducted using methods described by the US Food and Drug Administration. Tissue concentration data will be used to establish an appropriate withdrawal period post hemp consumption in cattle. To measure the analgesic properties of cannabinoids derived from industrial hemp in cattle with induced lameness; previously documented measures of pain will be take. These measures include infrared thermography of the affected foot and the contralateral foot; mechanical nociception threshold testing of the affected foot and the contralateral foot; visual lameness scores; and pressure mat gait analysis.

SECTION D: Explain how the project will be implemented, including location and size of your anticipated research areas (in acres or square feet), duration of your research operations and variety of industrial hemp that will be used in your research.

Industrial hemp produced on KSRE research plots managed by Dr. Jason Griffith will be transported to the College of Veterinary Medicine at Kansas State University. Potential varieties to be used in this research include: Otto II: Franklin, Wojko, Helena, CFX-1, Futura 75, and Finola. Varieties with higher CBD content will be preferred for animal studies over those with lower CBD content. Once on the Vet Med campus, the IH will be tested for cannabinoid concentrations in the analytical lab. The analytical lab is located within the Veterinary Diagnostic Lab at KSU and is an 184,648 sq. ft. lab. Once the cannabinoid concentrations are determined, the IH will be fed to a group of 24 Holsteins to determine cannabinoid levels in the plasma. The animals will be housed in research facilities at KSU that is a 60,335 sq. ft. facility supported by the Institutional Animal Care and Use Committee. All animal procedures will be approved by the IACUC at KSU. To determine the cannabinoid levels in edible tissues, the final study will use methods described in Food and Drug Administration Guidance for Industry #207 "Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker residue depletion studies to establish product withdrawal periods."

To determine the analgesic properties of cannabinoids after ingestion of industrial hemp; an experimental lameness model will be used. To induce lameness amphotericin B will be injected into the lateral distal interphalangeal joint of one of the rear limbs. Once lameness has been established around 6 hours, the study treatments will be administered. Outcome measures will be collected at predetermined time points; and include pressure mat gait analysis, mechanical nociception threshold testing, infrared thermography, and visual lameness scores. It is anticipated each phase should last 14 days.

SECTION E: Explain how research data will be collected, recorded and analyzed.

All research will be recorded using methods consistent with FDA Good Laboratory Practices for clinical trials. Plasma samples will be obtained by venipuncture and placed in tubes with sodium heparin. Sample will be centrifuged and plasma harvested for analysis at a later date. Plasma cannabinoid concentration will be determined using ultra-high pressure liquid chromatography coupled with mass spectrometry (UPLC-MS). Each calf will have a unique identification number and samples will be correlated to the calf ID and time point of collection. Tissues for cannabinoid residue analysis will be collected; and the sample weight, tissue type, and interval after last exposure to IH and/or cannabinoids will be recorded.

SECTION F: Explain how the data will be interpreted and how conclusions will be drawn, including anticipated results.

Plasma cannabinoid concentrations will be modeled using noncompartmental pharmacokinetic methods. Tissue samples will be obtained at specific time points, with a minimum of 3 animals per time point to develop a regression line to determine a withdrawal period for cannabinoids in edible tissues. It is anticipated that cannabinoids will be detectable in plasma and tissues of cattle after consumption of IH. This work will serve to guide dosing guidelines and provide the framework needed for future studies. For Aim 3: data will be tested using statistical methods comparing treated and control groups.

2019 Research Report for Michael D. Kleinhenz, Industrial Hemp License KDA-0302873296

Summary of research conducted:

To date little research has been conducted as approvals for animal use (IACUC), hemp varieties, and research cattle have only become available in October 2019. An analytical method to measure cannabinoids in oil has been developed. Adaptation of the method to plant material is underway and should be complete December 2019. This method will allow a more robust measure of cannabinoids being administered to cattle.

In addition to determining the cannabinoid concentration of plant material, we have started work on determining the nutritional value and digestibility of industrial hemp plants. This data is important as we look to IH being included into diets since there is a significant amount of the plant not used in production. Nutrient values are being measured by wet chemistry methods and include: energy content, protein, minerals, and lipids. Fiber digestibility is being measure by in vitro methods. Complete results of this work are not available with the exception of the by-product of seed harvesting. Those results are below. It is too early to make inferences on the results.

Nutrient Content of Hemp seed cleanings:

Results: Dry Matter 92.88

Crude Protein 21.03

Avail. Crude Protein 18.68

ADICP 2.35

NDICP 3.67

ADICP %CP 11.19

ADF 17.97

aNDF 27.86

Calcium 5.67

Phosphorus 0.37

Magnesium 0.51

Potassium 1.86

Sulfur 0.23

Fat (EE) 4.62

Ash 23.39

Lignin 16.85

Starch 1.40

NDF Digest.: Traditional=Goering & Van Soest Method, Standardized=Combs-Goeser Method

NDFD 30, %NDF 46.60

uNDF30 14.88

uNDF30om 11.35

Calculations

NFC 26.77

NRC 2001 Energy calculations Dairy

TDN 1X 46.95

NEL 3x, Mcal/lb 0.491

NEG, Mcal/lb 0.120

NEM, Mcal/lb 0.364