



2019 Hemp in Animal Feed Research Proposals
Compiled for distribution by the Hemp Feed Coalition

Colorado State University

1. Dose response of hemp seed meal on nutrient use and final health in finishing wether lambs. \$137,425 - **Proposal Funded by IND Hemp**, anticipated start in 30 days.....P3

Kansas State

2. Effects of Hemp Meal Supplementation on Cow and Heifer Performance, Timed Artificial Insemination, Overall Pregnancy, and on Subsequent Calf Performance. Cost: \$62,370P3

3. Effects of increasing hemp seed meal in newly weaned pig diets on growth, feed intake and feed efficiency. Cost: \$90,744.....P5

4. Tissue Kinetics of Cannabinoids in Swine following feeding of Hemp Seed Cake
Cost: \$60,344.....P9

5. Digestibility of Hemp Seed Cake in the Mature Equine. Cost: \$51,618.....P11

6. Lincoln Memorial University-College of Veterinary Medicine (Tennessee)

Pharmacokinetics (PK) of cannabidiol in horses (Subject to approval) Cost: \$78,810.....P13

7. Murray State University

Cost for all three: \$25,600.....P15

Hemp Fed Poultry Study (Broilers and Layers)- hemp seed as a replacement for soybean meal for broilers and for layers to increase Omega 3 fatty acids in egg yolks. Cost: \$9000

Swine Study- swine will be fed varying levels of hemp seed meal to replace their normal soy bean and corn protein provisions. Cost \$9,500

Equine Study -This study will look at feeding hemp seed meal to horses for a maintenance and performance diet. Cost: 7,100

Oregon State University; Department of Animal and Rangeland Sciences,

8. Effect of hemp seed supplement to dairy animal on animal health, performance and safety of milk and meat for human consumption. Cost: \$149,600.....P17

9. Evaluation of hemp seed cake supplementation in pasture-based lamb finishing systems: Effect on the cannabinoid residuals, animal health, behavior, and meat quality.
Cost \$93,380.....P19

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- 10. Southern Illinois University -Edwardsville, National Corn to Ethanol Research Center**
A Fast Screening Method for Potential Residual THC in Hemp Seed. \$79,000.....P20
- 11. College of Agriculture, Forestry and Natural Resource Management University of Hawaii at Hilo**
Feeding Hemp and DDGS to Tilapia. Cost: \$113,000.....P23
- 12. Iowa State University**
Improving Egg Quality by Feeding Industrial Hemp Seed and DDGS to Layers.
Cost: \$108,000.....P25

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Dose response of hemp seed meal on nutrient use and final health in finishing wether lambs.

Project Principal Investigator:

Dr. Shawn Archibeque,
Assistant Professor, Department of Animal Sciences, Colorado State University.
Phone: (970)-491-6649. Email: shawn.archibeque@colostate.edu

Project Co-PIs:

Dr. Terry Engle,
Professor, Department of Animal Sciences, Colorado State University.
Phone: (970)-491-3597. Email: terry.engle@colostate.edu

Dr. John Wagner,
Professor, Department of Animal Sciences, Colorado State University. Phone: (970)-491-6923.
Email: john.wagner@colostate.edu

Dr. Jasmine Dillon,
Assistant Professor, Department of Animal Sciences, Colorado State University, Phone: (970)
372-7966. Email: jasmine.dillon@colostate.edu

Objectives

Goals and Specific Aims. While the importance of dietary nutrient concentrations on the balance of nutrients in multiple animal models is well understood, there is very limited or no data in the literature assessing the impact of feeding hemp seed meal to animals with respect to nutrient use and excretion as well as the economic value of these products, impacts on metabolism, final composition of animals, and the altered manure profile which could serve as a viable fertilizer for future biofuel crops. Hemp seed meal is defined as the residual seed cake after mechanically extracting the oil from hemp seed. Therefore, the overall objective of the research proposed herein is to provide data on the interactions between the myriad changes in nutrient provisions associated with feeding hemp seed meal on nutrient use, and potential animal impacts. The specific objectives are:

- A. To determine the potential influence of dietary inclusion of hemp seed meal on nutrient utilization and excretion.
- B. To determine the potential influence of dietary inclusion of hemp extract on blood metabolites and final tissue histology.

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Project Timeline

Project Timeline	
Date	Action
January 2019	Acquire test diets and do quality control analysis
February 2019	Acquire lambs and initiate feeding protocols
April 2019	Begin balance studies
July 2019	Finish all animal studies
October 2019	Finish all laboratory analysis
November 2019	Complete data analysis
January 2020	Complete final write up
May 2020	Submit final report and submit for publication

Budget

The budget and budget explanation reflect the anticipated personnel, travel, and materials and supplies requirements. Dr. Archibeque (Associate Professor of Ruminant Nutrition) will function as the PI on this project and Drs. Engle (Professor of Ruminant Nutrition), Wagner (Professor of Ruminant Nutrition), and Dillon (Assistant Professor of Animal Sciences) will function as the co-PI's on this project. Dr. Archibeque will mentor the CSU Graduate Student who will be working on this project. All year 2 costs include a 4% inflation.

Personnel Salaries	\$62,814
Fringe Benefits at CSU	\$8,132
Materials and Supplies	\$8,100
In-State Tuition	\$21,245
“Other” Analytical fees	\$20,150
Publication Cost	\$1,030
Travel Cost	\$1,030
<u>Total F&A Costs (15% MTDC):</u>	<u>\$14,502</u>
Total Project Cost:	\$137,425

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Effects of Hemp Meal Supplementation on Cow and Heifer Performance, Timed Artificial Insemination, Overall Pregnancy, and on Subsequent Calf Performance.

Objectives

- Measure the effects of pre-partum hemp meal supplementation on response to estrus synchronization and timed artificial insemination (AI) by cows and 1st-calf heifers.
- Measure the effects of pre-partum hemp meal supplementation on overall pregnancy rates, pregnancy distribution, and subsequent calf performance.

Hypotheses

- Pre-partum supplementation with hemp meal will produce a comparable number of AI pregnancies and natural-service pregnancies as standard winter protein supplementation programs used in Kansas.
- Cow and calf performance will be comparable between prepartum hemp meal supplementation and standard winter protein supplementation programs used in Kansas.

Background / Abstract

- Protein supplementation is an essential part of beef cow wintering systems that rely on low-quality warm season forages.
- Gestating beef cows and heifers supplied with an average of 1.5 lbs of supplemental CP per day generally triple their intakes of low-quality warm season forage and increase dietary TDN approximately 30% when compared to non-supplemented animals (KSU data).
- Soaring feed costs dictate that beef producers investigate alternatives to soy-based supplements for the purposes of supporting beef cows during the winter months.

Approach and Methods

Study Site. The study will be conducted on 5 pastures (approximately 300 acres each) located at the Kansas State University Commercial Cow-Calf Unit (CCU).

Cattle. Four pastures will be stocked with approximately 60 mature cows and one pasture will be stocked with 55 to 65 bred heifers. All animals will be of British breeding, moderate frame size, and moderate body condition.

Supplements. All supplements will supply cattle with similar total amounts of ruminally-degradable protein, metabolizable protein, and net energy per day. Specific treatments will be:

- Approximately 4.1lbs of hemp meal per animal per day (approximately 37% CP as-fed)
- 3.2 lbs of soybean meal per animal per day (48% CP as-fed)

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Data Collection. Cows and heifers in each pasture will be randomly assigned to receive 1 of the 2 supplements for 150 days before the estimated start of the calving season. Animals will be gathered 3x per week and sorted into their respective feeding groups. Supplements will then be pen-fed in bunks. Feed delivery at each feeding episode will be prorated to achieve an average intake of 1.5 lbs of supplemental CP/cow/day. Individual body weights and condition

scores will be collected immediately before the feeding period commences and at calving. Supplementation will be discontinued as individual cows calve.

Approximately 45 days after the conclusion of the calving season, animals will be estrus-synchronized using the 5-day Co-Sync CIDR protocol. Immediately thereafter, animals will be subject to a single round of timed AI. Two weeks after timed AI, cows and heifers will be bull exposed for an additional 30 days.

Response to timed AI and natural-service breeding will be monitored. Cow and calf performance will also be measured during the subsequent summer grazing season by comparing pre-breeding body weights and body condition scores with weaning body weights and body condition scores.

Proposed Budget

To conduct this trial as described, we propose a donation of 50 tons of hemp meal, including transportation costs of same to Manhattan, KS. Additional funds requested are as follows:

Hemp Meal (50 tons)	Donated
Soybean Meal (50 tons, estimated market price = \$504/ton delivered)	\$ 25,200
Chemical analysis	\$ 630
Labor	\$ 31,500
Publication, travel	\$ 5,040
Total	\$ 62,370

Timeline

The trial will be initiated on or about December 1, 2020 and be completed by the end of October 2021. A full report will be available by the end of November 2021. Progress reports will be completed as requested by cooperators.

Contact Information

KC Olson
W.M. & F.A. Lewis Professor
Animal Sciences and Industry
Kansas State University

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Manhattan, KS 66506
785-532-1254 – Phone
785-532-5481 – Fax
kcolson@ksu.edu

Effects of increasing hemp seed meal in newly weaned pig diets on growth, feed intake and feed efficiency

Office of the VP for Research
PreAward Services
Kansas State University
1601 Vattier Street, 2 Fairchild Hall
Manhattan, KS 66506-1103
785-532-6804

Principal Investigator

Jason Woodworth, PhD; Research Professor; Kansas State University, 251 Weber Hall, Manhattan, KS 66506. jwoodworth@ksu.edu. 785-532-1157. www.KSUswine.org

Co-Investigators:

Dr. Mike Tokach, PhD University Distinguished Professor
Dr. Joel DeRouchey, PhD Professor, Swine Nutrition
Dr. Bob Goodband, PhD Professor, Swine Nutrition
Dr. Steve Dritz, DVM, PhD Professor, Swine Management

Timeline: Study preparation will begin immediately after notification of funding is made with anticipated start date prior to 6 months of award notification assuming hemp seed meal availability is granted. The feeding study will last for 5 weeks, not including data and statistical analysis and creation of final report. The final report will be delivered to the sponsor prior to 90 days from the start of the feeding study.

Deliverables: The sponsor will receive a final report that includes an overall abstract, as well as sections that cover an introduction, procedures, results & discussion, and final conclusion as well as all data tables. Once the sponsor has reviewed the final report, project results will be disseminated through popular press magazines (National Hog Farmer, PORK), abstracts at national or regional professional meetings, and submitted for publishing in an appropriate scientific journal such as the Journal of Animal Science. Results also will be shared at producer meetings such as the KSU Swine Day and published online at the KSU swine research and extension site at ksuswine.org.

Budget: *This budget assumes hemp seed meal does not have regulatory approval to be fed to animals destined for the human food chain, and therefore they must be*

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ethanized and discarded. The total fixed cost of the experiment which includes pig use, feed cost, labor, sacrificed pig reimbursement, laboratory analysis and associated university research fees is \$90,744.

If hemp seed meal can be fed to pigs destined for human consumption as noted by FDA-CVM/AAFCO or if a withdrawal period prior to slaughter can be granted that is less than 90 days, then pigs will not need to be euthanized. In this case the total fixed cost of the experiment which includes pig use, feed cost, labor, laboratory analysis and associated university research fees is \$39,368.

Abstract

One of the most stressful times in a pig's life is at weaning. Piglets are transferred from their mother sows to a brand new barn and environment, placed in pens with other piglets, and expected to simultaneously transition from a diet of sow's milk to solid feed. This creates an opportunity for improvement because managing newly weaned pigs to maximize feed intake and subsequently growth leads to better health, optimum performance, and lower mortality, but represents one of the biggest challenges for the US swine industry today. Additionally, newly weaned pig diets are formulated to contain specialized and highly digestible ingredients in order to promote feed intake and growth, but this strategy also creates high diet costs. Alternative ingredients that can promote better performance or maintain performance at lower diet costs are continuously sought by swine producers.

Hemp seed meal is a by-product of the hemp oil industry and is growing in availability. While little data exists that demonstrates its value in diets fed to pigs, the nutritional composition suggests that it could be an interesting component of newly weaned pig diets. Consequently, the objective of this proposed trial is to determine the impact of feeding diets containing 0, 2, 4, 6, 8, or 10% hemp seed meal on weaned pig average daily gain, feed intake, and feed efficiency. The hemp seed meal will be included in Phase 1 and 2 diets fed from weaning until approximately 25 lb. A common diet will be fed for the subsequent 2 weeks to determine if any carryover effects are observed. Therefore, the entire study will last approximately 35 days. Piglet weights and feed disappearance will be recorded weekly to determine performance parameters. In addition, mortality and other health assessments will be recorded as an indication of dietary treatment on overall health and wellbeing. A total of 300 pigs will be used with 5 pigs per pen and 10 replications per treatment to allow for appropriate statistical power to conduct this experiment.

The proposed study design is one that is routinely used to measure the impact of new ingredients in weaned pig formulations. The Kansas State University Applied Swine Nutrition Team (Drs. Woodworth, Tokach, DeRouchey, Goodband, and Dritz) will oversee the research with the help of Graduate Research Assistants which creates a unique educational experience with faculty oversight. Each year this team conducts over 50

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swine-related research trials utilizing over 55,000 pigs which illustrates the industry-level respect and acceptance for the research they conduct.

Tissue Kinetics of Cannabinoids in Swine following feeding of Hemp Seed Cake

Office of the VP for Research

Kansas State University
102 Anderson Hall
Manhattan, KS 66506
785-532-5110
research@ksu.edu

Principal Investigators

Michael Kleinhenz, DVM, PhD

Dept. of Clinical Sciences
109 Coles Hall
1620 Denison Ave.
Manhattan, KS 66506
785-532-7500
mkleinhe@vet.k-state.edu

Johann Coetzee BVSc, Cert CHP, PhD,
DACVCP, DACAW, DECAWSEL

Dept. of Anatomy & Physiology
226 Coles Hall
1620 Denison Ave
Manhattan, KS 66506
785-532-5666
jcoetzee@vet.k-state.edu

Other Investigators: Jason Woodworth, PhD, Dept. of Animal Sciences & Industry
Zhoumeng Lin, PhD, DABT, Dept. of Anatomy & Physiology
Geraldine Magnin, DABT, Dept. of Anatomy & Physiology

Abstract

Hemp seed cake is an abundant by-product derived from the production of hemp oil. Hemp seed cake has a desirable nutritional profile making it an attractive ingredient in feeds for swine and other livestock. In order to meet regulatory requirements, understanding of the kinetics of hemp oil and cannabinoid tissue deposition are needed. This study will determine the tissue kinetics of cannabinoids in swine fed hemp seed cake using methods described in FDA Guidance for Industry #207. Sixteen (8 male and 8 female) animals weighing between 40 to 80 kg will be enrolled. The animals will be hand-fed a at a rate of 3-4% of BW that contains 10% hemp seed cake for 14 days to ensure reach steady-state levels of cannabinoids are reached and dose accumulation of cannabinoids can be ruled-out. Blood will be obtained via jugular venipuncture prior to each morning feeding and 12 hours after the morning feeding for the first 5 days and then daily for days 6 to 14. A total of 4 animals (2 male and 2 female) will be

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ethanized using AVMA approved methods and tissue samples obtained at predetermined time points. Proposed time points for sampling are days 14, 15, 17, and 19. Tissues to be collected at each predetermined time point include muscle, liver, kidney, and skin with subcutaneous fat per U.S. FDA Guidance for Industry #207. Tissue samples will be analyzed for cannabinoid and cannabinoid metabolites using the liquid chromatography coupled with mass spectrometry (UPLC-MS/MS) methods within the Analytical Laboratory at Kansas State University College of Veterinary Medicine. Tissue cannabinoid concentrations data will be analyzed by Dr. Zhoumeng Lin, Regional Director for the Food Animal Residue Avoidance Databank (FARAD) at Kansas State University using the tolerance limit method described in FDA Guidance for Industry #3.

Timeline

Animal acclimation: 7 days
 Feeding period: 5 days
 Tissue collection period: 5 days
 Plasma and Tissue analysis: 45 days
 Data analysis: 15 days
Total study duration: 77 days

Deliverables

Sponsor will receive raw data from plasma and tissue concentrations of cannabinoids determined by liquid chromatography coupled with mass spectrometry (UPLC-MS/MS). A final report with results analyzed will be prepared and submitted.

Budget

Animal Costs	\$3200
Housing per diem	\$650
Diets	\$260
Consumables	\$1000
Feed Analysis	\$550
Tissue Cannabinoid Analysis	\$7040
Plasma Cannabinoid analysis	\$22,800
Statistical and Residue Analysis	\$1500
Travel	\$1500
Labor	\$1200
Total Direct Costs	\$39,700
Overhead 52%	\$20,644
Total Project Costs	\$60,344

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Digestibility of Hemp Seed Cake in the Mature Equine

PreAward Services

Kansas State University
2 Fairchild Hall
Manhattan, KS 66506
785-532-6804
research@ksu.edu

Principal Investigator

Teresa Douthit, PhD
Dept. of Animal Sciences & Industry
129 Weber Hall
1424 Claflin Road
Manhattan, KS 66506
785-532-1268
douthit@ksu.edu

Other Investigators

James Lattimer, PhD; Animal Sciences & Industry, College of Agriculture
Michael Kleinhenz, DVM, PhD; Clinical Sciences, College of Veterinary Medicine
Hans Coetzee, BVSc, PhD; Anatomy & Physiology, College of Veterinary Medicine

Abstract

For every ton of hempseed oil produced, there are about 4 tons of seed cake remaining for which hemp producers need to find an outlet. Hemp seed cake has a favorable amino acid profile and might be a suitable protein source for inclusion in equine feeds. This study is designed to compare hemp seed cake to soybean meal with respect to cecal fermentation parameters and impact on apparent total tract nutrient digestion in horses. Eight mature cecally cannulated Quarter Horses will be used in a crossover design in which horses will consume a diet for 28 d consisting of 1.5% BW (as-fed) smooth bromegrass hay and 0.5% BW (as-fed) pelleted concentrate containing either soybean meal or hemp seed cake (from a non-CBD variety) at levels sufficient to bring crude protein in the concentrate to approximately 15%. Samples of cecal digesta will be obtained 0, 2, 4, 6, 8, 10, and 12 h following the morning meal on d 28 of each period and analyzed for pH and concentrations of volatile fatty acids (VFA). The use of cannulated (vs. intact) horses provides the unique opportunity to gain insight into how this product impacts microbial fermentation in the live animal. By evaluating pH and VFA production in the cecum, we can better ascertain how the product might be metabolized by the horse, as well as assessing how it might impact animal health. Fecal samples will be collected and pooled from d 25 to d 27 of each period and analyzed for apparent total tract digestibilities of DM, NDF, ADF, CP, ether extract (EE), and GE using acid-detergent insoluble ash as an internal marker. Weekly urine samples will be collected for cannabinoid analysis. Additionally, 10 ml of whole blood will be collected biweekly for cannabinoid and liver enzyme testing. Together, this information will allow us to determine whether using hemp seed cake in lieu of soybean meal in the equine ration will alter fermentation parameters in the cecum or impact the digestibility of typical feeds, thus providing a reasonable assessment of the potential for hemp seed cake as a protein source in concentrate feeds designed for horses. Additionally, collection of blood and urine will allow for testing of horses known to be fed hemp seed cake to determine toxicological and regulatory implications.

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Timeline

Project preparation: 30 days
 Two 28-d feeding and sampling periods: 56 days
 Laboratory and data analyses: 45 days
Total: 131 days

Deliverables

Sponsor will receive output from feed analyses, raw cecal pH and VFA data, raw data obtained by proximate analyses of feces, results of blood and urine testing, as well as a summary (including statistical analysis) of results.

Budget

Animal & Facility Usage	\$9,534
Diets	\$2,426
Cecal Analysis	\$2,435
Fecal Analysis	\$1,338
Toxicology	\$16,921
Labor	\$14,404
Travel	\$3,040
Statistical Analysis	\$1,520
Total Project	\$51,618

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Pharmacokinetics (PK) of cannabidiol in horses (Subject to ILACUC approval)

Principle Investigators: Dr. William W. Muir and Dr. Stacy Anderson Lincoln Memorial University, Harrogate, Tennessee, and Dr. Richard Sams, Lexington, Kentucky.

Lincoln Memorial University-
College of Veterinary Medicine
6965 Cumberland Gap Parkway
Harrogate, TN 37752
vetmed.LMUnet.edu 423-869-7150

Abstract

We propose to administer cannabidiol (CBD) intravenously and orally to horses in a randomized cross-over study design with each horse receiving CBD by both routes of administration. Blood and urine samples will be collected from all horses after each dose. Plasma and urine concentrations of CBD will be determined by validated tandem liquid chromatography-mass spectrometry (LC-MSⁿ) methods. CBD concentrations in plasma and urine will be analyzed to estimate pharmacokinetic parameters and detection times. Horses (twelve, non-exercised, Thoroughbred mares and geldings of racing age) will be administered a purified CBD product (*i.e.*, “isolate”) IV at a dose of 100 mg of CBD in ethanol and orally in a commercial feed at the same dose rate. The doses will be administered in a randomized cross-over design with a minimum of two weeks between doses.

The CBD isolate and the commercial feed will be obtained from commercial sources for horses. The CBD content of the test articles will be assayed by LC-MSⁿ before use for assessment of potency and purity. Doses administered will be based on assayed potency.

Blood samples for determination of CBD will be collected immediately before (time 0) the IV dose and at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 24, 48, and 72 hours after dosing. Blood samples will also be collected for determination of CBD immediately before the oral dose (time 0) and at 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 24, 48, and 72 hours after dosing.

Urine samples for determination of CBD and its metabolites will be collected volumetrically from 0-2 and 2-4 hours and as point samples before and at 12, 24, 48, 72, 96, and 120 hours after the IV and oral doses.

All animal studies will be conducted at the East Tennessee Clinical Research, Inc. (Rockwood, Tennessee) according to protocols provided by Professor William Muir.

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Blood and urine samples will be analyzed by validated LC-MSⁿ methods for CBD (in blood and urine) and its metabolites (urine). The CBD methods will be based on those reported by Palazzoli *et al.* (2018)* and will be validated and demonstrated to be fit-for-purpose for determining CBD and its metabolites. All sample analyses will be performed under the direction of Dr. Richard Sams at KCA Laboratories, Nicholasville, Kentucky.

Plasma and urine concentrations of CBD will be plotted as a function of time after dose administration. Pharmacokinetic parameters such as area under the plasma concentration versus time curve, total clearance, apparent volume of distribution, and half-life will be estimated for CBD after IV administration. The bioavailability, elimination half-life, and absorption rate will be determined from oral administration data. Pharmacokinetic and statistical analyses will be performed by Dr. Richard Sams.

*Palazzoli, F., Citti, C., Licata, M., Vilella A., Manca, L., Zoli, M., Vandelli, M.A., Forni, F., and Cannazza, G. Development of a simple and sensitive liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) method for the determination of cannabidiol (CBD), Δ⁹-tetrahydrocannabinol (THC) and its metabolites in rat whole blood after oral administration of a single high dose of CBD. *Journal of Pharmaceutical and Biomedical Analysis* 2018, **150**: 25-32.

Timeline and Deliverables

- a. Institutional Laboratory Approval: 1 month
- b. Section 1: Phase 1 – Collect blood and urine samples from horses after IV and oral administration of CBD; 4 months
- c. Section 2: Analyze blood and urine samples collected in Phase 1 by validated LC-MSⁿ methods to identify and quantify CBD and its metabolites; 3 months
- d. Section 3: Compile the resulting blood and urine sample data from Phase 2 for CBD and its metabolites; 2 months
- e. Section 4: Review the resulting blood and urine data from Phase 2 for the IV and oral administration of CBD and prepare a manuscript for publication; 2 months

1 year

Project Cost and Budget: (it is helpful to include if the project can be funded and completed in sections)

a. Horses, facilities & equipment, supplies, Laboratory sample preparation, Labor, Professional and administrative fees:	\$20,000.00
b. Sample analysis:	\$44,400.00
c. Data analysis:	\$3,500.00
d. Final report and publication costs	\$1,500.00
Subtotal	\$69,400.00
e. Overhead (15%)	\$10,410.00
Total	\$78,810.00

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Hemp Fed Poultry Study (Broilers and Layers), Swine Study, and Equine Study

Contact- Brian Parr Murray State University 212 Oakley Applied Science Murray KY 42071
bparr@murraystate.edu

Expertise Provided by:

Dr. Thomas Powell- Meat Scientist

Dr. Debbie Reed- Director of Breathitt Veterinary Diagnostic Lab

Mr. Matt Shultz- Swine Specialist

Dr. Shea Porr- Equine Scientist

Dr. Bikram Subedi- Analytical Chemist

1. Abstract

Poultry- PI- Dr. Brian Parr

This study will be a continuation of research started two years ago where hemp seed was used as a replacement for soybean meal as a protein source for broilers as well as a supplement for layers to increase Omega 3 fatty acids in egg yolks. This round will involve the use of hemp cake as opposed to the complete hemp seed used in the past 2 rounds of the study. Results indicate that the seed is a viable protein source for poultry, is palatable, and does significantly increase Omega 3 fatty acids in egg yolks. Meat analysis will also be conducted to determine color, texture, and chemical impacts of the hemp feed.

Swine- PI- Mr. Matthew Shultz

This study will be a continuation of the swine feeding study initiated this summer. During this study, swine will be fed varying levels of hemp seed meal to replace their normal soy bean and corn protein provisions. This study will examine growth, feed conversion and to determine maximum level of hemp addition to swine diets. Meat analysis will also be conducted to determine color, texture, and chemical impacts of the hemp feed.

Equine- PI- Dr. Shea Porr

This study will look at feeding hemp seed meal to horses for a maintenance and performance diet.

2. Timeline and Deliverables

Poultry

This study can be completed in 8 weeks from the funding date. This is the growth cycle for the broilers and will provide sufficient amount of eggs from the layers for analysis. Deliverables will include all growth, feed conversion, and palatability data from the broilers as well as a comprehensive report. Additionally, all analyses from the chemists will be provided concerning

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the transfer of Omega 3 and other compounds to the egg yolk as well as a comprehensive report from the layer data.

Swine

This study can be completed in 16 weeks from the funding date. Deliverables will include all growth, feed conversion, and palatability data from the swine as well as a comprehensive report.

Equine

This study can be completed in 16 weeks from the funding date. Deliverables will include all weight and palatability data from the horses as well as a comprehensive report.

3. Project Cost and Budget

Poultry

Purchase of broilers and layers- \$500.00
Feed for mixing with hemp cake- \$2,500.00
Student labor to carry out study- \$3,000.00
Faculty overload salary for project- \$3,000.00
Poultry Total- \$9,000.00

Swine

Feed for mixing with hemp cake- \$3,500.00
Student labor to carry out study- \$3,000.00
Faculty overload salary for project- \$3,000.00
Swine Total- \$9,500.00

Equine

Feed for mixing with hemp cake- \$1,100.00
Student labor to carry out study- \$3,000.00
Faculty overload salary for project- \$3,000.00
Equine Total- \$7,100.00

Total Requested for all 3 species- \$25,600.00

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Effect of hemp seed supplement to dairy animal on animal health, performance and safety of milk and meat for human consumption

Lead project Director (PD): Massimo Bionaz, Associate Professor, Department of Animal and Rangeland Sciences, Oregon State University; massimobionaz@gmail.com

Co-PD: Serkan Ates, Assistant Professor, Department of Animal and Rangeland Sciences, Oregon State University; serkan.ates@oregonstate.edu

Title: *Effect of hemp seed supplement to dairy animal on animal health, performance and safety of milk and meat for human consumption*

The growing industry of hemp in the US can provide several low-cost byproducts that can be fed to livestock. Studies conducted in Europe indicated that hempseed cake can be safely used in ruminant rations as a protein supplement. Due to the low amount of tetrahydrocannabinol (THC), it is safe to use spent hemp seed for livestock, including dairy cows and sheep. The relatively high amount of cannabidiol (CBD) might also improve the immune system in cows, as observed in non-ruminant species. There is a scarcity of information on the effect of hempseed cake on the performance and health of dairy animals. One study, carried out in Sweden observed that providing 140 g/kg DM of hempseed cake increased milk production without any detrimental effects to the animals; however, higher doses decreased milk fat production and decreased N efficiency. Fat is the most important economic component in milk. None of the available studies assessed the effect of feeding hemp seed to the health of dairy animals. The hemp is a relatively new crop in the US. Byproducts from hemp are not yet approved by the FDA to be used to feed livestock that will enter the food chain, including dairy animals, due to lack of data on accumulation of THC in milk and meat. Before byproducts of this plant can be used with livestock, a detail analysis of residuals of THC (and CBD) should be performed, so to provide data to the FDA for evaluation and, eventually, approval for use of hemp byproducts with livestock.

There is a need to assess the effect of spent hemp seed on health and performance of dairy animals using detailed physiological parameters and to determine the transfer of THC and CBD into milk and meat.

Using lactating sheep, we will determine the effect of feeding spent hemp seed to milk yield and quality, health, and residuals in milk and meat

Sheep was chosen for this study as a model animal to minimize cost. Fourteen lactating sheep will be fed hay and will be individually supplemented with 300 g/day of a mixture of soybean meal and barley (control) or hempseed cake (treatment). Sheep will be nursed by 2 lambs and milked once a week where milk yield and milk components will be assessed. THC and CBD will be measured in milk using HPLC. Weight of the animals will be measured weekly and blood collected for analysis of metabolic-, inflammatory-, and oxidative status-related parameters. At

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the end of the study, sheep receiving hempseed cake and their lambs will be euthanized and THC and CBD will be measured in muscle.

We expect from the present study to determine the concentration of residuals of THC and CBD in milk of animals fed with spent hemp seed to inform the FDA for the process of legalization of feeding hemp byproducts to livestock. We expect to obtain preliminary data on the effect of spent hemp seed on milk quality, animal performance, and animal health to inform the dairy producers.

Budget Item	Cost
Salary, Benefits, and Tuition	
Summer salary for Dr. Bionaz (1 month salary + benefits)	15,000
Graduate student salary (2 years)	63,000
Graduate student tuitions (2 years)	40,000
Services:	
Animals use and care (\$4/day x 20 animals x 28 days)	2,300
Blood analysis (\$50/sample x 100 samples + shipping)	6,000
Milk component analysis (\$1.5/sample x 100 samples + shipping)	200
Euthanasia+landfill (10 sheep+20 lambseuthanized at the end of the trial)	4,000
Supplies:	
Sheep (10 sheep will be purchased euthanized at the end of the trial)	2,500
Lambs (20 lambs will be purchased euthanized at the end of the trial)	2,000
Blood collection and processing	500
Cannabinoids analysis with HPLC (\$80/sample x 120 samples)	9,600
Other consumables (tubes, syringes, needles, pipette tips, etc....)	1,500
Publication in a peer-reviewed journal	3,000
Overhead (5% via OSU Agriculture Research Foundation)	900
Total	149,600

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Proposal title: Evaluation of hemp seed cake supplementation in pasture-based lamb finishing systems: Effect on the cannabinoid residuals, animal health, behavior, and meat quality

Principal Investigators: Serkan Ates, Assist. Professor Forage/Livestock systems, Department of Animal and Rangeland Sciences, Oregon State University

Phone number: 541-737-5060

E-mail address: serkan.ates@oregonstate.edu

Co-PI: Assoc. Prof. Massimo Bionaz

Collaborator: Prof. Shelby Filley

Abstract: In pasture based lamb finishing systems, the growth of lambs is closely associated with the quantity and quality of herbage available to lambs. However, pasture growth and herbage quality are highly variable and are often not on a par with the increasing nutritional demands of growing lambs, particularly in intensively grazed temperate pastures. Supplementary use of feed concentrates or alfalfa hay is often practiced to offset the reduction of pasture value towards summer and potentially increase the efficiency through sustaining high lamb growth rates. Increased intake of digestible energy and protein towards summer through supplemental grain promotes high lamb growth rates, enabling farmers to bring their lambs to target slaughter weights earlier. However, the increased lamb growth rates often come with higher production costs due to the high price of grain. Therefore, it is imperative for farmers to seek cost effective alternative grains to finish their lambs. The hemp seed-derived byproducts can be that alternative. The rapidly growing hemp industry is producing high amounts of seed-derived byproducts of high nutritive value that can be used in livestock feeding systems with a cost that is inferior to grains.

Studies conducted in Europe have revealed that hempseed cake is safe for inclusion in livestock feed (EFSA, 2011). The most critical aspect of feeding any hemp byproducts is the potential for cannabinoid residuals in meat, particularly the psychoactive tetrahydrocannabinol (THC). Data from Europe have indicated the presence of unacceptable levels of THC in animals fed fresh hemp, but acceptable levels when using byproducts such as hempseed cake. Despite this it is not yet legal to feed hemp byproducts to livestock used for the production of meat. The major limitation is the lack of data on cannabinoids residuals in meat to initiate the process of legalization by the FDA. Therefore, there is a critical need to assess the levels of THC residuals in meat to initiate the approval process by the FDA to feed hemp byproducts to livestock. In this study, we will investigate the supplementation of pasture finished lambs with hemp seed cake instead of soy meal. **The objective of this study is to determine the effect on health, meat quality, and cannabinoids residuals in finishing lambs fed hemp seed cake.** Specifically, we will assess the bio-economic efficiency of hemp seed cake supplementation on THC (and other cannabinoids) residuals in meat cuts, along with evaluating growth performance, grazing behavior, carcass traits, and meat quality.

Results of this study can be postulated to the grass based cattle-finishing systems.

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Duration of study: 12 months from May 2020 to April 2021.

Timeline and Deliverables

By the completion of this study, we expect to have generated data regarding the effect of replacing soy meal with hemp seed cake on performance and meat quality of finishing lamb. Our project will generate fundamental data for the process of the legalization of hemp byproducts to be used with livestock for the production of meat and will provide data to the beef industry about the safety of using spent hemp seed with livestock.

	2020			2021			
Set-up of the experiment on pastures		X					
Conducting the trial			X	X			
Meat quality studies				X			
Data processing and dissemination of the information					X	X	

BUDGET – list cost for each item

Salaries

Graduate Salary: \$63,000 for 2 years (tuition will be waived)
 Summer salary for Dr. Ates (1 month 13,000 salary+ benefits)

Services:

Blood collection and analysis \$3,500
 HPLC analysis \$3,000
 Carcass and meat quality \$6,100
 Feed analyses \$360
 Landfill of carcasses* \$720
 Fatty acid analysis \$1,350

Supplies - includes equipment with an individual piece value less than \$5000, i.e. at \$1000 each are stilled considered to be supplies:

Supplemental feed: \$500
 Lambs* (\$150/head) \$1,350

Equipment -:

Feeders for supplemental feed: \$500
 TOTAL: \$93,380

*Only the lambs that have to be euthanized and landfilled will cost money to the project. This will be only 9 grazing lambs that will be supplemented with hempseed cake. We already have established pastures and fence material to carry out the grazing experiment.

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A Fast Screening Method for Potential Residual THC in Hemp Seed

Yan Zhang, yzhang@ethanolresearch.com, 618-659-6737
National Corn to Ethanol Research
Center Southern Illinois University
Edwardsville

Currently, industrial hemp seed is not an approved ingredient for commercial feed, even though hemp seed was approved by FDA as a human food ingredient with GRAS status (Generally Recognized as Safe). Hemp seeds contain highly digestible protein, are an excellent source of essential fatty acids with a high (3:1) ratio of omega-3-linolenic acid and omega-6- linoleic acid, are enriched in antioxidants, and have other nutraceutical benefits, all of which would make hemp seed a desirable component of animal feed. Before it can be widely adopted, however, a simple and accurate method for measuring trace concentrations of δ -9- tetrahydrocannabinol (THC) in hemp seed is needed. THC is the psychoactive component of marijuana, and although industrial hemp produces only low amounts of this compound, it is important to be able to verify that the seeds have not been contaminated with marijuana seeds or that THC has not been concentrated on the industrial hemp seeds because THC and seeds are both produced by the flowers.

Cannabinoid quantitation is very commonly performed via high-performance liquid chromatography (HPLC) using ultraviolet (UV) detectors or mass spectrometry. NCERC hosts a state-of-the-art analytical testing lab sponsored by Shimadzu, a leading analytical instrument company that has developed and validated methods for measuring THC and other cannabinoids in cannabis and related products. The objective of the proposed research is to develop a fast and economical testing method that can be used for high throughput monitoring of industrial hemp seeds. The testing method would involve extraction of cannabinoids from the surface of the hemp seed, concentration of the extractant, and analysis of the concentrated samples using a near-infrared spectrometer (NIR). Accurate concentrations for THC and other target cannabinoids will be provided by developing a quantitative model that correlates the NIR absorbance data with concentrations that will be measured using HPLC/MS/MS for a large number of independent samples. The target detection limit of the screening method is 0.03%. The NIR calibration model will be developed by NCERC in coordination with an NIR vendor. The labor needed to run the NIR test is expected to be less than 1 hour per sample, and the cost of consumables is expected to be less than \$10 per sample. NIR is commonly used by commercial animal feed testing labs, and with proper calibrations and methods, it can also be used to measure the concentrations of many macronutrients in hemp seed, such as protein and oil.

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Timeline and Deliverables

This is a nine-month project

Timeline Deliverables

Month 1 Collect hemp seed samples from various field in North America, target at 100 independent samples

Month 2 – 3 Test a few choices of extractants, and select the best based on accuracy and cost

Month 4 – 7 Collect extractant samples from the hemp seed samples, and run LC/MS/MS Run NIR spectrum on the dried residual samples from the extractant

Month 8-9 Build the THC calibration curve

Final report

Budget for this project \$79,000 which include project management, hemp seed collection and extraction, and tested by LC/MS/MS and NIR, building NIR calibration and final report.

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Feeding Hemp and DDGS to Tilapia

Yan Zhang, yzhang@ethanolresearch.com, 618-659-6737
National Corn to Ethanol Research Center, Southern Illinois University Edwardsville
and

Armando Garcia-Ortega, agarciao@hawaii.edu; (808) 932-7031

Adrian Barnes, barnesmi@hawaii.edu; (808) 981-4506

Kevin Hopkins, hopkins@hawaii.edu, (808) 932-7037 College of Agriculture,
Forestry and Natural Resource Management University of Hawaii at Hilo

Aquaculture is the fastest food producing sector, comprises almost half of the total fish supply, and is essential to meet growing protein demand from future populations. Currently, aquaculture faces many challenges, and one of the solutions is to replace fish and animal-based fish meal with plant-based products. The distiller's dried grains with soluble (DDGS), a co-product from corn to ethanol processing, is a viable ingredient for fishmeal for its competitive price and large production, high protein and low fiber content from modified processing, and enriched vitamins and phytochemicals from yeast fermentation.

In a promising development that creates new opportunities for agriculture, the 2018 Farm Bill introduced industrial hemp seed as another potential plant based feed source. Currently, industrial hemp is not an approved ingredient for commercial feed, even though hemp seed was approved by FDA as a human food ingredient with GRAS status (Generally Recognized as Safe). Hemp seeds have inherent advantages for aquaculture fish meal. They contain highly digestible protein, are a desirable source of essential fatty acids with perfect 3:1 ratio of omega-3-linolenic acid and omega-6-linoleic acid, and have enriched antioxidants in addition to other nutraceutical benefits. In addition, the nutritional profile of hemp seed is highly complementary to DDGS feed when mixed together. Hemp seed is enriched with arginine in its protein and has high levels of linolenic acid compared with DDGS oil, which can further improve the fish meal quality.

To expedite the usage of DDGS and hemp seed as a common aquafeed ingredient in the U.S., and to maximize the usage of the high quality plant feed, we plan to conduct two preliminary fish trials that will determine the maximum level of inclusion of the best combination of DDGS and hemp feed based on the complementary nutritional profile. The proposed study will investigate six and five different percentage mixes of DDGS and hemp seed respectively for nutritional quality and growth impacts on one fish species: hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). The results from

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both trials will provide information on the optimal combination of DDGS and hemp seed meal dietary inclusion to achieve maximum fish growth.

All the compositional testing on feed material and fish quality will be performed at NCERC of SIUE. The screening test of THC and CBD in the hemp feed and fish meat will be performed at NCERC also.

Timeline and Deliverables This is a one year project

Timeline

First Quarter

Deliverables

Feed characterization which include proximate analysis and nutritional analysis on the control diet and experimental diets.

THC and CBD screening on hemp feed

Second quarter

Fish trial #1: fish weight gain and survival, feed utilization and nutrient retention.

Third quarter

Fish trial #2: fish weight gain and survival, feed utilization and nutrient retention.

Fourth quarter

Fish study data summary

Nutritional testing and THC and CBD screening of fish meat
Final report

Budget for this project \$113,000 which includes project management, chemical characterization of feed and fish, fish trial, evaluation of fish growth/ nutrient retention, and final report.

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Improving Egg Quality by Feeding Industrial Hemp Seed and DDGS to Layers

Yan Zhang, yzhang@ethanolresearch.com, 618-659-6737
National Corn to Ethanol Research Center, Southern Illinois University, Edwardsville
and
Dong Ahn, duahn@iastate.edu, Phone: 515-294-6595, Iowa State University

In 2016, we published a study on the impact of including distiller's dried grains with solubles (DDGS), a co-product from corn to ethanol production, in the poultry diet on the nutritional qualities of egg, and we found out that the eggs from the layers fed with DDGS had enhanced levels of vitamin E and xanthophylls, which are great antioxidants. In a promising development that creates new opportunities for agriculture, the 2018 Farm Bill introduced industrial hemp seed as another potential plant-based feed source. Currently, industrial hemp is not an approved ingredient for commercial feed, even though hemp seed was approved by FDA as a human food ingredient with GRAS status (Generally Recognized as Safe). Hemp seeds have inherent advantages to be used in poultry feed because they contain highly digestible proteins with a high level of arginine, desirable source of essential fatty acids with perfect 3:1 ratio of omega-3 -linolenic acid and omega-6 linoleic acid, and rich in antioxidants and other nutraceuticals. The nutritional profile of hemp seeds is highly complementary to that of the DDGS.

To expedite the usage of hemp seed and DDGS as common poultry feed ingredients in the U.S., we plan to conduct a preliminary poultry trial. We will formulate the poultry diet with a few combinations of DDGS and hemp seeds (total of five diets including the control) using hens from 21 to 41 wk of age, and evaluate the feed impact on egg production, feed consumption and mortality, and a wide range of the internal quality as well as the nutritional quality of eggs produced. The internal quality parameters to be measured include shell strength, yolk color, Haugh unit and albumin height of egg, and the nutritional and functional quality parameters include amino acid, fatty acid profile, antioxidant capacity, and minerals like Fe, Ca, P, K and Se.

While the egg production and egg internal quality will be evaluated at the Iowa State University, representative eggs from each experimental diet will be collected

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during the last week of the trial and shipped to the National-to-Ethanol Research Center for the chemical analyses.

Timeline and Deliverables

This is a one year project

Timeline

Month 1 - 2

Deliverables

Feed characterization which include proximate analysis and nutritional analysis on the control diet and experimental diets

THC and CBD screening of eggs

Month 3 - 8

Poultry trial: daily, weekly, monthly egg production evaluation, egg quality measurement, body weight and feed intake of layers and mortality

Month 9-12

Poultry trial data summary

Nutritional testing and THC and CBD screening of eggs

Feed formulation, preparation

Final report

Budget for this project

\$108,000 which include project management, chemical characterization of feed, and egg, the poultry trial, evaluation of egg production and quality, and final report

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