GENETICS, BREEDING, AND ANIMAL HEALTH RESEARCH UNIT

1. Lord of the Flies

Scientist: J. W. Keele

Background: Horn flies cause stress to cattle and reduce growth rate, milk production and pregnancy rate. Furthermore, horn flies can spread pathogens such as those causing mastitis between animals. Insecticides in sprays, dusters and fly tags may reduce horn fly numbers but have negative effects on non-target beneficial organisms such as honey bees and dung beetles and the insecticides in these applications lose effectiveness over the course of the summer because horn flies evolve resistance when challenged with insecticide. Seedstock producers (cattle breeders) are interested in selecting for reduced horn fly susceptibility if it enables them to market resistant bulls. Previous research has demonstrated that there are heritable cow genetic effects on horn fly numbers so genetic improvement methods should be effective. The objective of this research proposal is to understand the cow (host) genetic basis underlying horn fly susceptibility.

Project Description: The student will use artificial intelligence to leverage big data on small biting flies. Digital pictures will be taken of flies on cattle on multiple days over the course of the summer. This project provides a unique opportunity to work both indoors and outdoors. About half of the work will be conducted outdoors photographing cattle during mornings; the cool time of day. The student will spend the afternoons indoors in the comfort of air conditioning annotating pictures and running analyses. The cattle in this study have previously been genotyped for functional variants (sequence differences inside of genes). Fly counts and genotypes will be analyzed to estimate the effects of genes. The student will have the opportunity to interact with and learn from geneticists, entomologists and experts in artificial intelligence at USMARC and University of Nebraska at Lincoln. The proposed research is part of a 5-year experiment to phenotype cattle for horn fly burden and investigate the cow genes underlying the number of flies observed on an animal. The selected student will be able to collect data for 8 weeks in peak fly season, augment their data with that collected in the previous 2 years and analyze the results.

Duties and Responsibilities: The student selected for this project will take pictures of horn flies on cattle in pastures. Annotation of the images and artificial intelligence (neural network) will be used to count flies from the images. Fly counts and genotypes will be analyzed to estimate genetic effects specific to chromosomal regions of the cow. Applicants for this position should be interested in and have taken coursework that encompasses biology and genetics. In addition, interest or coursework in entomology, beef production, computer science, photography, or artificial intelligence will be considered as strengths. Applicants should also be willing to learn the basics of entomology, statistical analysis, photography, genetics, and artificial intelligence.

2. Evaluating Microbial Community Variation Associated with Bovine Respiratory Disease Complex in Cattle

Scientist: T. McDaneld

Background: Bovine respiratory disease (BRD) is the most expensive disease in U.S. beef cattle costing the cattle industry over 1 billion dollars annually. Past efforts to reduce the incidence and severity of BRD have been frustrated by complexity of the disease. However, recent advances in genomics (high density genotyping arrays and whole genome sequencing) have improved capabilities for identifying factors (variation in bacterial community) associated with complex diseases such as BRD. The objective of this research proposal is to identify the bacterial populations that may predispose cattle to becoming susceptible to BRD.

Project Description: The student selected for this project will identify bacterial populations present in the U.S. Meat Animal Research Center disease population of cattle by learning and implementing a variety of laboratory methods. The student will accomplish this by first learning and using laboratory techniques that include basic microbiology techniques for working with bacteria, DNA and RNA extraction, polymerase chain reaction (PCR), and basic sequencing protocols. In the first two weeks, the student will become familiar with sequence analysis software by assisting a scientist to evaluate 16S sequence data collected from the 2016 Disease Resistance population last summer. In the remaining six weeks, the student will (1) extract DNA from the nasal samples collected in 2017 from the Disease Resistance population at USMARC, (2) identify bacterial populations present in the nasal samples through initial 16S sequencing of the DNA, (3) identify species of Mycoplasma present in the nasal samples through PCR, and (4) help in collection of lung tissue from abattoir to evaluate variation in the cattle genome associated with BRDC. In cooperation with other scientists at US Meat Animal Research Center, we have developed methods to sequence and analyze DNA sequence from bacterial samples. Therefore, we believe that the intern will be able to complete this proposed project in the eight-week time frame.

Duties and Responsibilities: Applicants for this position should be interested in and have taken coursework that encompasses biology, microbiology, and genetics. Applicants should also be willing to learn laboratory techniques and how to use DNA information to improve cattle genetics.

MEAT SAFETY & QUALITY RESEARCH UNIT

3. Novel approaches to slice shear force

Scientist: S. Shackelford

Background: Meat scientists frequently measure tenderness to evaluate the effect of factors on meat quality. In many cases, this is done via an instrumental measure of tenderness. Slice shear force (SSF) is utilized because it is amenable to high-throughput and is technically simpler than Warner-Bratzler shear force. But, for some muscles, whose fiber angle is inconsistent and hard to discern, protocols for SSF require skill and differ depending on which side of the carcass the muscle came from. This has made it difficult for many labs to utilize SSF for some muscles. Consequentially, *USMARC scientists are testing novel approaches to SSF for some muscles*

Project Description: The student selected for this project will conduct experiments including collection of beef cuts, cutting of steaks, cooking steaks, sampling steaks for SSF, measurement of SSF, and analysis of data. The intern will assist in collection of carcass data, at large-scale commercial beef packing plants, as part of large-scale beef genetic studies. Additionally, the intern will have a variety of opportunities to participate in meat safety and quality research measuring meat color stability and sampling and testing for microbial pathogens.

Duties and Responsibilities: The incumbent will be expected to participate in research endeavors in a variety of environments including livestock production settings, packing plants, abattoirs, sensory laboratories, and research laboratories. The incumbent will be responsible for thorough and accurate collection and analysis of data while adhering to stringent personal, livestock, and food safety protocols.

4. Evaluating the specificity of a multiplex-PCR assay for the detection of highly pathogenic *Salmonella*

Scientist: D. Harhay

Background: *Salmonella enterica* is a leading agent of gastrointestinal illness worldwide, causing an estimated 94 million cases of enteritis and diarrheal disease that result in ~155,000 deaths each year. *Salmonella* are also a diverse group of bacteria with over 1,700 serotypes noted for causing human illness. And while all *Salmonella* are technically regarded as human pathogens because of their ability to invade intestinal epithelial cells and potential for causing systemic illness, only 20 serotypes account for greater than 70% of all illnesses. This suggests that some *Salmonella* may be more pathogenic because they possess a greater repertoire of virulence factors that enable them to cause disease.

Common food sources of *Salmonella* exposure include produce, eggs, poultry, raw milk and dairy products, and beef. Recent outbreaks of disease attributed to multi-drug resistant *Salmonella* have led to increasing pressure on the USDA Food Safety and Inspection Service (FSIS) to declare certain types of *Salmonella* as targets for regulation in ground beef and other meat commodities. Given the potential for future regulation, both FSIS beef producers will need tools allowing for the rapid detection of particular *Salmonella* strains when present

as ground beef or trim contaminants. To address this need, a multiplex polymerase chain reaction (PCR) assay was developed to identify and distinguish more virulent *Salmonella* strains from the less virulent or commensal *Salmonella*.

Project Description: The student selected for this project will evaluate the sensitivity and specificity of this multiplex PCR assay for identifying highly pathogenic *Salmonella* (HPS). To accomplish this the student will first learn laboratory techniques to safely work with pathogenic bacteria. In addition, the student will learn general microbiological techniques including bacterial culture, isolation, and identification, as well as molecular biological techniques such as bacterial DNA extraction, PCR assay methods, and agarose gel electrophoresis.

In the first two weeks, the student will become familiar with the necessary laboratory techniques, media preparation, and chemicals required for the experiments. In the remaining six weeks, the student will isolate bacterial DNA from strains previously isolated, identified and stored as -80°C glycerol stocks. In all, 2000 bacterial isolates will be screened using the PCR assay. Accordingly, this proposed project can be completed in an eight-week time frame.

Duties and Responsibilities: Successful applicants for this position should be interested in food safety microbiology and/or molecular biology, and will have taken coursework that includes biology, and microbiology. Applicants should be willing to learn laboratory techniques including bacterial DNA isolation, PCR methodology, and agarose gel electrophoresis. Applicants should also be willing to learn statistical methods to assess assay performance (i.e. sensitivity and specificity measures).

5. *Effectiveness of Sanitizer D7™ against E. coli O157:H7 and Salmonella Biofilms*

Scientist: R. Wang

Background:

Biofilm formation by *E. coli* O157:H7 and *Salmonella enterica* at meat processing plants poses a potential risk of product contamination. Available studies have shown that many common sanitizers were unable to completely eradicate biofilms by these foodborne pathogens due to the 3-dimensional biofilm structure and the bacterial polymeric extracellular substances. Thus, the meat industry is in urgent need of novel and effective sanitizer products to control and eliminate foodborne pathogen biofilms. D7™ is a sanitizer product consisting of quaternary ammonium compound, hydrogen peroxide, and an accelerator, diacetin. This product can be applied with either a foam or a fog method.

D7 has been tested on *Listeria* and *Pseudomonas* biofilms and showed promising results. Preliminary study conducted at our laboratory suggested that D7 applied with the foam method was effective against *E. coli* O157:H7 and *Salmonella* biofilms as well. However, a comprehensive study evaluating the effectiveness of D7 applied at various concentrations and exposure time periods with either foam or fog method is much needed to provide practical information for the meat industry to use this product appropriately and cost effectively. Therefore, the objective of this study is to evaluate the effectiveness of D7, applied at various combinations of concentration/exposure time/method, in inactivating and removing *E. coli* O157:H7 and *Salmonella* biofilms under meat plant conditions.

Project Description:

The student selected for this project will evaluate the effectiveness of D7 against *E. coli* O157:H7 and *Salmonella* biofilms by learning and implementing a variety of laboratory methods. The student will accomplish this by first learning and using laboratory techniques that include basic microbiology protocols of bacterial biofilm development on contact surface, biofilm treatment with sanitizers, as well as biofilm harvesting, plating and colony enumeration.

During the first week, the student will become familiar with the experiments of biofilm formation on stainless steel chip surface and sanitization using D7 following the manufacture's recommended protocol. In the remaining seven weeks, the student will (1) evaluate the effectiveness of D7 while applied at different concentrations for various exposure time periods to inactivate *E. coli* O157:H7 and *Salmonella* biofilms on stainless steel surface (2) compare the effectiveness of D7 applied with foam or fog method in terms of inactivating biofilms (3) help process samples for scanning electron microscope (SEM) analysis in order to directly visualize biofilm removal after D7 treatment.

In cooperation with other scientists at USMARC and the University of Nebraska Lincoln, we have developed standard protocols to build biofilms on contact surface, quantify biofilm cell density, measure biofilm cell reduction after sanitizer treatments, and observe biofilm removal with SEM analysis. Therefore, we believe that the intern will be able to complete this proposed project in the eight-week time frame.

Duties and Responsibilities:

Applicants for this position should be interested in food safety research and have taken coursework that encompasses biology, microbiology, and bacteriology. Applicants should be willing to learn laboratory techniques and how to use the study results to help the meat industry control biofilm persistence efficiently in order to improve meat safety.

NUTRITION & ENVIRONMENTAL MANAGEMENT RESEARCH UNIT

6. Odor and gas emissions from beef feedlot surfaces

Scientists: M. Spiehs and B. Woodbury

Background: Concentrated animal feeding operations are a source of environmental concerns for livestock producers as well as the rural and urban communities. The organic, inorganic, pathogenic, and pharmaceutical residues in manure are potential sources for contamination of soil, surface and groundwater, and air quality. Many management tools are available to producers to reduce the environmental impact from livestock production, but each tool must be evaluated for effectiveness, economics, and ease of implementation. One management tool available to producers for reducing odor and gas emissions is the application of amendments to the feedlot surface. These amendments change the pH of the feedlot surface, which may eliminate microorganisms and lower emissions of odorous compounds. Lime and alum are two surface amendments that have the ability to change the pH of the feedlot surface and have been shown to lower some gas emissions in lab-scale studies. However, the surface of a beef feedlot is variable, with different nutrient, moisture, and organic content throughout the surface. Additional research needs to be conducted to determine how these surface amendments respond to the spatial variability on a beef feedlot surface.

Project Description: The student selected for this project will assist Drs. Spiehs and Woodbury with lab-scaled experiments to determine the concentration and application frequency of lime that will successfully reduce odor and gas emissions from feedlot surface material. This will involve two 14-day studies. During each study, air samples will be collected twice weekly. This will be the primary research project of the student during the 8-week internship. The student will be trained to operate and calibrate a suite of air sampling equipment used to collect samples for analysis of ammonia, hydrogen sulfide, and greenhouse gases (methane, carbon dioxide, and nitrous oxide). Additionally, the student will be trained to operate a gas chromatography/mass spectrometry (GC/MS) to analyze air samples for odorous volatile organic compounds emitted from the feedlot surface material. Student will be responsible for data collection and will work with Dr. Spiehs on statistical analysis and interpretation of the data. The intern will also work with Dr. Woodbury on a field-scaled research project to evaluate odor and gas emissions due to spatial variability on the feedlot surface of several pens at the USMARC feedlot. Other on-going environmental management research projects which will encompass measurements of antimicrobial resistance/residue measurements from a crop field that received manure as a fertilizer. Due to the nature of the research, the student will spend a fair amount of time in the field, without much exposure to a traditional laboratory setting.

Duties and Responsibilities: Applicants for this position should be interested in and have taken coursework that encompasses biology, microbiology, animal science, and/or engineering. Applicants should also be willing to learn techniques for collecting gas samples and operation of air sampling equipment to improve air quality and nutrient management from concentrated cattle feeding operations.

REPRODUCTION RESEARCH UNIT

7. Develop early detection of sow lameness with electronic monitoring and biomarkers for joint health.

Scientist: G. Rohrer

Background: An audit of the swine industry recently identified sow lameness as a significant welfare issue in commercial swine production. In addition, lameness robs producers of profit via reduced production, higher replacement rates and loss of salvage value when the animal requires euthanization. Therefore, lameness in sows is a serious welfare and economic issue that needs to be addressed to identify real-world solutions for producers. Lameness can be caused by numerous factors, such as: housing, flooring, nutrition, body structure and trauma. Selection of animals with sound body-type is a viable solution to this problem, but availability of herdsmen that can accurately identify these animals is scarce. Thus, additional research and application of objective methods to assess lameness consistently on farms and identify mildly to moderately lame animals are needed to improve pig welfare prior to the condition reaching severe lameness.

Project Description: We plan to develop early detection of sow lameness with electronic monitoring and attempt to identify biomarkers for joint health. The approach will monitor gilts as they walk across a 10-meter pressure-sensitive mat at approximately 5 months of age to identify early phenotypes that can be used to remove animals prior to reaching the breeding herd. These phenotypes will include stride lengths of each foot and weight carried by each foot using the mat and multiple video imaging systems will also be used. Blood samples will be collected to search for biomarkers present in the blood. Analyses will attempt to create phenotypes that are predictive of body structures and joint health, which can be incorporated in modern production systems. These phenotypes may also be useful to improve body structure in future generations via genomic selection. Similar analyses will also be conducted in sows.

Duties and Responsibilities: In the summer of 2019, we will begin to collect data on gilts and sows using the pressure-sensitive mat, video imaging and collect blood for future metabolic analyses. The successful candidate will be expected to go into the USMARC swine facilities and help move animals and collect blood samples. Laboratory work will include processing blood samples, extracting DNA and entering data.