NUTRITION & ENVIRONMENTAL MANAGEMENT RESEARCH UNIT

Measuring Air Quality in Cattle Facilities

Scientist: M.Spiehs/B. Woodbury, Ph.D.

Background: Questions have been raised concerning the impact on the environment by concentrated animal feeding operations. These questions are a source of environmental concerns, and are creating unease between livestock producers and the rural and urban community's dependent upon livestock production. Microbial degradation of livestock waste can generate gases and odors that impact air quality. Previous research from USMARC scientists has evaluated mitigation strategies to improve air quality in lab-scaled settings. Additional research in a commercial setting is needed to validate findings. Recently, the Nutrition and Environmental Management Research Unit (NEMRU) obtained a state-of-the-art instrument trailer which will be used as a mobile air quality laboratory. The mobile lab will contain instrumentation to measure regulated odorous gases (ammonia and hydrogen sulfide) and greenhouse gas (carbon dioxide, nitrous oxide, and methane) concentrations from the feedlot surface, and allow scientists to measure gases and odors in a commercial situation. The effective measure of these gases in commercial settings is highly dependant on the design and layout of the equipment housed within the trailer. Additionally, measurement efficacy is dependent on the design of the instrumentation at the point of collection. These designs are critical in obtaining accurate information on production and environmental activity of these emissions.

Project Description: The overall objectives of the project will include learning the basics of air emission collection and assist in design and construction of a mobile air emission laboratory to effectively sample emissions from feedlot surfaces. The specific research objective will be to develop an understanding of the spatial and temporal variability of emissions across the pen surface.

Duties and Responsibilities: The Intern will be responsible for assisting Drs. Woodbury and Spiehs and their technicians with the design and installation of emission instruments in the mobile air quality trailer, design and programming of automated controlling systems for data collection, development of sampling protocols and methods, and field testing instruments and data collection systems.

Expression of genes involved in steer feed efficiency

Scientist: A. Lindholm-Perry, Ph.D.

Background: The U.S. Meat Animal Research Center Nutrition Research Unit is seeking a 2015 summer intern to conduct laboratory work in support of its feed efficiency program. Feed constitutes about 65 to 75% of the cost of beef cattle production; however, less than 20% of the nutrients consumed are converted to useful products. The incomplete and inefficient utilization of nutrients has an adverse effect on efficiency of production. Research has been performed to determine the genetic factors that lead to variation in efficiency in beef production and genes that have an effect on feed intake, growth and feed efficiency have been identified. Currently, research is being conducted to evaluate the functional roles of those genes in cattle. Tissue samples from several organs expected to have involvement

with feed efficiency have been collected from steers with extreme feed efficiency phenotypes.

Project Description: These tissues will be used for whole transcriptome RNA analysis. The purpose of this study is to characterize the expression of genes involved in feed intake in several types of steer tissue samples. This research project is being coordinated by Dr. Lindholm-Perry.

Duties and Responsibilities: The successful candidate will be trained to extract RNA from several types of tissue and analyze these samples for whole transcriptome analysis using RNA-Seq and microarray technology. In addition, the intern will be responsible for creating a data set that will allow for statistical analysis. Differences in RNA between efficient and inefficient steers will be used to identify metabolic processes that contribute toward variation in feed efficiency.

GENETICS, BREEDING, AND ANIMAL HEALTH RESEARCH UNIT

Evaluating Microbial and Viral Respiratory Community Variation Associated With Bovine Respiratory Disease Complex in Cattle

Scientist: T. G. McDaneld, Ph.D.

Bovine respiratory disease complex (BRDC) 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 BRDC 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 BRDC. The objective of this research proposal is to identify the bacterial and viral species that may predispose cattle to becoming susceptible to BRDC.

The student selected for this project will identify bacterial and viral species 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 and viral pathogens, 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 2012 Disease Resistance population during the summer of 2014. In the remaining six weeks, the student will (1) extract DNA from the nasal samples collected in 2011 from the Disease Resistance population at USMARC; (2) identify bacterial species present in the nasal samples through initial 16S sequencing of the DNA; (3) extract RNA from the nasal samples collected in 2011 from the Disease Resistance population at USMARC; and (4) identify viral pathogen profiles through next-generation sequencing. In cooperation with other scientists at US Meat Animal Research Center, we have developed methods to sequence and analyze DNA and RNA sequence from nasal swab samples. Therefore, we believe that the intern will be able to complete this proposed project in the eight-week time frame.

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 and RNA information to improve cattle genetics.

Host Immune Response associated with Bovine Respiratory Disease Complex (BRDC)

Scientist: C. G. Chitko-McKown, Ph.D.

RESEARCH PROBLEM
Infectious respiratory diseases of ruminant are a serious health and economic problem for U.S. agriculture. In cattle alone, bovine respiratory disease complex (BRDC) costs the feedlot industry approximately 1 billion dollars annually. The level of resistance or susceptibility an animal has to one of the bacterial or viral pathogens of BRDC can be at least partly linked to the competence of its innate immune response – that arm of immunity that is not dependent upon vaccination or prior exposure to infectious insult in order to respond. Pentraxin-related protein 3 (PTX3) acts as an acute phase protein in response to

infection and inflammation, and blood levels are often associated with outcome and/or severity of disease. In mice, PTX3 has a non-redundant role in antifungal immunity with certain haplotypes increasing the risk of aspergillosis. Additionally, PTX3 plays a role in innate resistance to viral infections such as cytomegalovirus and influenza virus, as well as to chronic lung infections caused by *P. aeruginosa* and *K. pneumonia*. We were the first group to identify PTX3 expression in bovine macrophages after treatment with *E. coli* O157:H7 LPS, and its role in bovine mastitis has been studied by researchers in Australia. At this time, the role of PTX3 in BRDC is unknown.

Our laboratory studies the innate immune response in food animals. We are currently analyzing plasma samples obtained from a population of cattle that were purchased at sale barns throughout the southeast and transferred to a feeding facility in Kansas. Blood was obtained from these animals over the course of a month allowing us to monitor the health in these animals and identify animals presenting signs of BRDC. We hypothesize that concentrations of PTX3 in plasma will be associated with health status, and that animals suffering from BRDC will have higher levels PTX3 in their plasma. The student selected for this project will have the opportunity to learn immunological techniques ranging from blood collection to immune cell isolation, tissue culture, immunological assay procedures, as well as molecular techniques such as RNA isolation and RT-PCR assay development and performance. The student will also have the opportunity to interact with other laboratories studying the genetic diversity of pathogens and of the host.

MEAT SAFETY & QUALITY RESEARCH UNIT

Phenotyping Shiga toxin-containing *Escherichia coli* O157 strains using Biolog Phenotyping MicroArrays

Scientist: J. L. Bono, Ph.D.

Background: Shiga toxin-producing *Escherichia coli* O157 (STEC O157) are genetically diverse bacteria that vary in their ability to cause human disease. Currently, STEC O157 genomes are being sequenced at an advanced rate due to next-generation sequencing platforms. This has provided valuable information on the genetic composition of STEC O157, and the propensity of different strains to cause disease. However, very little is known on how genetic variation impacts STEC O157 physiology and pathogenicity. This project will use Biolog phenotyping microarrys to correlate STEC O157 utilization of different nutrients with genetic variation and human pathogenicity.

Project Description: Biolog phenotype microarrays will be run on ten STEC O157 strains from two major groups, those that rarely cause disease in humans and those that do. The phenotype microarrays will represent different carbon and nitrogen sources that the bacteria may or may not be able to utilize. These results will determine if metabolic strain variation between isolates of STEC O157 correlates with human disease.

Duties and Responsibilities: The intern will be responsible for preparing bacterial strains for microarray plates, running the plates on the Biolog machine, and analyzing results.

Developing a high-throughput, accurate measure of degradation of proteins important to improved tenderness during aging meat

Scientist: T. L. Wheeler, Ph.D.

Background: It is well established that meat tenderness is a very important trait affecting consumers' eating satisfaction with meat. Previous work in our lab has demonstrated variation in tenderness among animals, among muscles within an animal, and among and within steaks within a muscle. Even a steak from a muscle and carcass with above average tenderness can have variation among different parts of the steak that can cause consumer dissatisfaction with the overall eating experience. Much of the variation in tenderness can be explained by either the amount of protein degradation during meat aging or the amount a muscle shortens during rigor mortis development. We have studied protein degradation during aging using Western blotting procedures for the myofibrillar protein desmin. Desmin degradation is strongly related to tenderness, but the Western blotting procedure is long and tedious and limits the number of samples that can be processed in a timely manner. Furthermore, there is some evidence that other proteins besides desmin may have different degradation patterns and may provide additional information about tenderness of a muscle.

Project Description: Steaks will be obtained from 25 each of tough and tender loin muscles that have been aged for 14 days postmortem. These steaks will be cooked to medium degree of doneness on a conveyorized belt grill. Slice shear force will be measured for each steak. The sheared slices will be saved and used to measure postmortem protein degradation. Two approaches will be used. First standard western blotting for degradation of the structural protein desmin will be conducted. In addition, we will compare a new

approach that involves structural protein degradation products for the protein titin that are soluble in the sarcoplasmic fraction of the muscle and can be measured with a high throughput slot-blot approach using the same antibodies used for Western blotting for titin. This approach may provide a faster measure that will be related to tenderness at the same level as desmin or perhaps has a stronger relationship to tenderness if the measure is more repeatable and accurate than Western blotting.

Duties and Responsibilities: The intern will be responsible for preparing the cooked muscle samples, running the Western blot and slot blot assays on muscle extracts, and comparing the results relative to measures of meat tenderness.

REPRODUCTION RESEARCH UNIT

Evaluating the effects of neonatal environment on developmental programming of the ovary in the female pig

Scientist: C.A. Lents, Ph.D.

Background/description: Lifetime productivity of swine is limited by the number of pigs a sow produces. The number of pigs that a sow can carry during gestation and that are born alive is a function of the development of her reproductive tissues. During critical periods of development, external factors can alter an animal's phenotype through changes in gene expression, which can permanently alter the animal's long-term production potential. There is an increasing body of evidence that a females long-term development can be programmed by events that occur in the first weeks of life. Previous studies indicate that that female pigs (gilts) nursed in small litters give birth, when they become mothers, to more piglets than gilts that were nursed in large litters. Other studies show that gilts raised in small litters have greater ovulation rate when they reach puberty. Our hypothesis is that development of the ovary is affected by the size of the litter in which gilts are nursed. To test this hypothesis, we conducted a study in which gilts were raised in either small or large litters. The ovaries were collected from these gilts at 4 critical time points during development; weaning, 60, 100, and 140 d of age.

Duties/responsibilities: The student selected for this project will quantify genes expressed in the ovary during these developmental time points. The student will learn how to extract RNA and perform quantitative real-time RT-PCR, and report the findings of these analyses near the end of the summer.

Effect of glucosamine supplementation on litter size in a commercial setting

Scientist: J.L. Vallet, Ph.D.

Background: Low birth weight is a primary contributor to poor preweaning survival. Piglet birth weights and placental weights are correlated, indicating that impaired placental function is cause of low birth weight. Previous results indicate that along with placental size, which is limited by uterine space, placental structural differences also contribute to placental function. Factors that encourage improved placental development may also improve both litter size and piglet birth weights. Preliminary evidence suggests that glucosamine supplementation may encourage placental development leading to improved placental function and increased litter size. In this experiment, we will test the effect of glucosamine supplementation on litter size in a commercial farm in Diller Nebraska. This experiment is supported by the Nebraska Pork Producers Council.

Project description: 240 pregnant sows from parities 2 through 7 will be randomly allotted to receive either 10 mg glucose or 10 mg glucosamine as a top dress on their feed from day 85 of gestation until farrowing. At 115 days of gestation, all sows will receive estrumate to control the timing of farrowing. At farrowing, the number of piglets born, born alive, stillborn and the number of mummified fetuses will be recorded and each individual piglet will be weighed. Any crossfostering will be recorded. At weaning each individual piglet will be weighed again. Litter size and weaning data will be analyzed for the effect of treatment.

Duties/responsibilities: We plan to farrow sows for this experiment in June such that birth and weaning weights will be collected in June and July. The student will be primarily responsible for helping to collect litter and weaning information at the Diller farm along with USMARC personnel and Diller farm staff. Travel funds will be provided so that the student can stay in the Diller area during farrowing and weaning. Student will participate in analysis of the data and will summarize the findings during a presentation at the end of the summer. In addition to this project, the student will help with other ongoing projects and procedures including computer assisted histological morphometry, electrophoresis and mass spectrometry analysis, immunohistochemistry, surgery and blood sampling of piglets.