NUTRITION & ENVIRONMENTAL MANAGEMENT RESEARCH UNIT

Investigation of the functionality of SLIT2 as an inhibitor or primordial follicle activation in a bovine ovarian cortical culture system

Scientist: R. Cushman, Ph.D.

Background: Reducing caloric intake is a proven method to increase lifespan and alter the ovarian reserve in mammals. Rats raised on 65% of maintenance during the peri-pubertal period have an increased number of primordial follicles in their ovaries. In studies conducted at USMARC, heifers developed on reduced caloric intake after weaning have an increased number of primordial follicles in their ovaries at breeding, indicating that the mechanisms controlling initiation of follicle growth (activation) have been altered by reducing intake. These same heifers have increased SLIT2 mRNA in their ovaries, and SLIT2 has been reported to influence primordial follicle development in sheep. Therefore, the current study is designed to determine if SLIT2 protein will inhibit primordial activation in a bovine ovarian cortical culture, because if it does, this could explain the mechanisms that lead to an increase in primordial follicle number in the ovaries of heifers developed on reduced caloric intake.

Project Description: Bovine ovaries (n = 6) will be collected at Gibbon Pack and transported to the center in transport medium. Ovarian cortex will be dissected and pieces of ovarian cortex (~1 mm3) will be placed in ovarian cortical culture without any SLIT2 (Control) for 2 days to allow for activation of the primordial follicles (n = 4 pieces/ovary). Additional pieces will cultured with 5, 50, or 500 ng/mL of SLIT2 (n = 4 pieces/dose) to determine if SLIT2 has the ability to inhibit primordial follicle activation at any of these doses. At the end of the cultures, cortical pieces will be fixed in neutral buffered formalin and embedded in paraffin for evaluation of changes in rate of primordial follicle activation. Primordial, primary, and secondary follicles will be counted and measured in a minimum of 5 sections per animal per dose of SLIT2. Histological data will be analyzed using the MIXED Procedure of SAS with day of culture (0 or 2) and treatment (0, 5, 50, or 500 ng/mL of SLIT2) and the interaction as fixed effects, and animal as a random effect.

Duties and Responsibilities: The incumbent will be responsible for performing sectioning, staining, histological evaluation of bovine ovarian tissue from cortical cultures, counting follicle populations, and analyzing data.

Expression of genes involved in swine feed efficiency

Scientist: W. T. Oliver, Ph.D.

Background: The U.S. Meat Animal Research Center Nutrition and Environmental Management Research Unit is seeking a 2016 summer intern to conduct laboratory work in support of its feed efficiency program. The efficient conversion of nutrients to edible product is paramount for the profitability of the swine industry. Feed efficiency has been improved by, among other strategies, superior genetics, superior nutrition, and the use of feed additives. However, the incomplete and inefficient utilization of nutrients continues to have an adverse effect on efficiency of production. Research is underway to determine the genetic factors that lead to variation in efficiency in swine 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 swine. Tissue

samples from several organs expected to have involvement with feed efficiency have been collected from pigs 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 swine tissue samples. This research project is being coordinated by Dr. Oliver.

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 pigs will be used to identify metabolic processes that contribute toward variation in feed efficiency.

GENETICS, BREEDING, AND ANIMAL HEALTH RESEARCH UNIT

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

Scientist: T. McDaneld, Ph.D.

Background: 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 species that may predispose cattle to becoming susceptible to BRDC.

Project Description: The student selected for this project will identify bacterial 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, 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 2013 Disease Resistance population last summer. In the remaining six weeks, the student will (1) extract DNA from the nasal samples collected in 2015 from the Disease Resistance population at USMARC (2) identify bacterial species present in the nasal samples through initial 16S sequencing of the DNA and (3) 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.

Investigation of the diversity of the gamma delta T lymphocyte pathogen-binding receptor family among sheep breeds

Scientist: T. Smith, Ph.D.

Background: Cells of the immune system recognize disease-causing pathogens and respond in a manner to stop the infection. While we know how conventional cells of the immune system do this, for some non-conventional cells such as gamma delta ($\gamma\delta$) T cells this process is less clear. It has shown that bovine $\gamma\delta$ T cells bear lineage-specific transmembrane glycoproteins coded by a multigenic family and that these molecules function both as pattern recognition receptors (PRR) and signaling co-receptors for cellular activation. For example, while a subpopulation known as WC1.1^{+} $\gamma\delta$ T cells respond early

and participate in a recall response to the spirochetes *Leptospira interrogans* and *Leptospira borgpetersenii,* other subpopulations that express a different set of the WC1 genes do not respond. Other experiments showed that WC1 molecules on γδ T cells that respond to *Leptospira* bind the bacteria. Thus, we hypothesize that WC1 is essential for the recognition of bacterial pathogens by γδ T cells and that the WC1 family members expressed by a particular cell will determine its ability to respond to an infection. WC1 genes are also found on gamma delta T cells of sheep and goats as well as pigs. We are now evaluating the diversity of WC1 genes in sheep.

Project Description: A new sequence and build of the sheep genome is underway at MARC that has many advantages over other attempts at this. Since the organization of these genes is known for cattle, we will use the new genome build to analyze their organization in the sheep genome using a reference animal. Using RT-PCR we will also evaluate expression of the annotated genes among reference animals. However, diversity of these important pathogen-detecting receptors may occur among sheep breeds and thus using PCR we will be able to use DNA archived from over 80 sheep breeds found in the US to determine diversity of the most distal and variable domain of the WC1 molecules.

Duties and Responsibilities: The incumbent will be responsible for performing RT-PCR assays and sequencing of products using samples from various sheep breeds, developing evolutionary trees and analyzing the data as well as annotating the new genome sequence of sheep generated at MARC for sheep WC1 genes.

MEAT SAFETY & QUALITY RESEARCH UNIT

Characterization of geospatially related Salmonella strains, isolated from beef cattle and their corresponding pre-harvest feedlot environments

Scientist: D. M. Harhay, Ph.D.

Background: Bovine peripheral lymph nodes (PLNs) have been identified as a potential source of human exposure to *Salmonella enterica*, when adipose trim containing contaminated PLNs is incorporated into ground beef. Results of recent studies suggest that *Salmonella* on cattle hides may gain entry to bovine PLNs via transdermal abrasions such as insect bites and wounds. Once within the lymphatic system, *Salmonella* have the ability to persist or even thrive, without obvious negative impact on cattle health and performance. Furthermore, at harvest *Salmonella* located within bovine tissues are protected from current carcass antimicrobial interventions. As a result, the beef industry perceives the need to investigate ways to mitigate the risk of *Salmonella* contained within the lymph nodes of cattle in the production environment. An important knowledge gap to understanding how bovine PLN become infected with *Salmonella*, is to determine if differences exist between the *Salmonella* isolated from feedlot environments (pen surface material, tank water and feed) and those isolated specifically from cattle (hides, feces and PLN) residing in those environments. To address this knowledge gap, samples were collected from the hides, feces and PLN of cattle (n=200) and their corresponding environments. All samples were analyzed for the presence of *Salmonella* resulting in the collection of over 500 isolates.

Project Description: The student selected for this project will determine the serotypes of a subset of *Salmonella* isolated in this project, which will be further characterized using methods including pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance phenotyping. Genotypic variation among the strains will also be accessed via whole genome sequencing using the illumina platform. This project builds upon ongoing *Salmonella* characterization studies in our laboratory, using established tools and methods. Accordingly, the scope of the project is in keeping with what can be completed in an eight-week timeframe.

Duties and Responsibilities: Applicants for this position should enjoy laboratory work and have a strong attention to detail. They should also have taken coursework in Microbiology, Molecular Biology and Bacterial Genetics and have an understanding of basic microbiological laboratory techniques, including aseptic technique and safe handling of bacterial pathogens. Potential candidates should also have a desire to learn new laboratory techniques and computational methods for analyzing genome sequence data.

Developing a high-throughput, accurate measure of degradation of proteins important to improved tenderness during aging of 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 7, 14, 21, 28, 35, or 42 days postmortem. Raw samples will be obtained and then 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, along with the raw samples, will be 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 quadrupole LC-MS analysis of myofibrillar proteins associated with tenderness variation such as desmin, troponin-T, titin, nebulin, and vinculin. This approach may provide a faster, more repeatable measure that will provide a more accurate indication of the role of postmortem protein degradation to meat tenderness at different postmortem aging times than has been possible with Western blotting.

Duties and Responsibilities: The intern will be responsible for cooking steaks, conducting slice shear force, preparing the raw and cooked muscle samples, running the Western blots and LC-MS analysis on muscle extracts, and comparing the results relative to measures of meat tenderness.

REPRODUCTION RESEARCH UNIT

Evaluating expression of candidate genes affecting age of puberty in the pig

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

Background: Reproductive efficiency has a great impact on the economic success of pork production. Gilts comprise a significant portion of breeding females and gilts that reach puberty earlier tend to stay in the herd longer and be more productive. About 10 to 30% of gilts never farrow a litter and one of the most common reasons for removal is failure of gilts to reach puberty, defined as the female's first estrus in the presence of a mature boar. Failure to show estrus is a significant part of the problem because when the ovaries of nonpubertal gilts are examined, many have had one or more ovarian cycles. We have conducted genome wide association studies (GWAS) and identified several genomic regions that are associated with age at puberty and delayed expression of estrus or ovulation. These regions contain candidate genes that likely have an important role in the expression of estrus behavior and attainment of puberty. The goal of this project is to understand if genetic differences associated with variation in expression or function of these candidate genes are related to phenotypic differences in pubertal traits.

Description: The candidate genes identified from GWAS have been evaluated for variation discovered in genomic sequence from founder animals. Variants have been identified that result in protein coding changes (nonsynonymous SNPs or deletions), and that may affect exon splice sites, miRNA 3'UTR binding sites and potential promoter transcription factor binding sites. These changes within regulatory sites are predicted to affect expression levels of genes or function of the gene products. The objective for the 8-week project is to quantify expression levels of several candidate genes using quantitative real-time polymerase chain reaction (QPCR) assays.

Duties and responsibilities: The intern will assist in collecting tissues from animals at slaughter and will be responsible for extracting RNA to quantify gene expression. The intern will learn how to perform QPCR, create datasets for statistical analysis, and compare expression levels to identify how variation in gene expression leads to differences in pubertal phenotypes. The intern will also have the opportunity to assist with DNA extraction for genotyping assays to discover further genetic variation within candidate genes.

Effect of glucosamine supplementation on litter size in a commercial setting

Scientist: J. Vallet, Ph.D.

Background: Development of the microscopic architecture of the pig placenta likely contributes to the efficiency of nutrient transport by the placenta of the pig. In a previous study in gilts, we demonstrated that supplementation of gilt diets with glucosamine during late gestation promotes beneficial changes in the microscopic architecture of the pig placenta and also appeared to improve uterine capacity. Last summer, with funding from the Nebraska Pork Producers and in collaboration with a commercial farm in Diller NE, we supplemented sows (parity 2 through 8) with glucosamine during late gestation to determine the effect of supplementation on litter size, piglet birth weights and weaning weights. Results indicated an increase in total piglets born of about .4 piglets, which was not statistically significant, but results also indicated some detrimental effects of the supplementation on stillbirth rates in very late parity (7 and 8) sows. In both experiments, the dose of glucosamine used was 10 g per day, but the sows used in the commercial trial were substantially heavier than the gilts used in our first experiment. To determine whether the dose of glucosamine used in the sow experiment may have been insufficient to result in beneficial increases in the larger parity 2 through 6 sows, we wish to repeat this experiment with supplementation of sows with 20 g of glucosamine per day, and measure litter size, birth and weaning

weights in the same commercial herd as the first experiment. Management at the commercial herd is willing to host and help undertake this second experiment to determine if a beneficial dose of glucosamine is possible.

Project description: Approximately 200 parity 1 through 5 sows will be mated using artificial insemination using the normal management used at the Diller farm over a two week period (100 sows per week). Beginning on day 85 of gestation, 100 sows will receive 20 g per day glucosamine supplementation and 100 sows will receive 20 g per day glucose supplementation in their daily feed as a top dress. The commercial farm uses farrowing induction to control the day of farrowing, such that farrowings will occur on two consecutive Thursdays and Fridays in early June. Resulting piglets will be counted, ear tagged and weighed to record birth weights. Four weeks later, tagged piglets from each farrowing week will be weighed again at weaning (all piglets weighed on a single day). Thus, litter size and weight data collection will be complete by late July, allowing time for statistical analysis of the data.

Duties/responsibilities: Diller farm staff will mate animals and will take care of top dressing feed on a daily basis. Student will be responsible for helping collect birth and weaning weight data at the Diller farm, which will require one or more overnight stays in Beatrice during birth and weaning weight collections. Diller farm staff will provide assistance with these activities as well, and supervisor will also participate in these activities. Student will be responsible for entering data into the computer and for statistical analysis of the data, with guidance from supervisor. In addition to these experiment specific activities, student will help with other ongoing projects at USMARC as time permits. At the end of the experiment, student will present the results from the experiment.