ANIMAL HEALTH RESEARCH UNIT:

Host-pathogen interactions between Ovine Progressive Pneumonia Virus (OPPV) and monocytes from sheep with varying genetic backgrounds

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

The Animal Health Research Unit at USMARC is actively involved in studying Ovine Progressive Pneumonia Virus (OPPV) and the disease it causes – one of the most economically important diseases to the sheep industry worldwide. Monocytes-macrophages and dendritic cells are the main target cells of this virus. The virus remains latent inside these cells until terminal maturation or activation at which time infectious virus is produced. Because alveolar macrophages are the first line of protection against pathogens invading the lungs, infection with OPPV can lead to secondary bacterial infections and the pneumonia from which this virus receives its name.

Our laboratory studies the innate immune response in food animals. We will be exposing monocytes obtained from sheep of differing genotypes to strains of OPPV in an effort to understand if host genotype contributes to the resistance or susceptibility to OPPV infection and disease progression. A summer student internship is available in the laboratory to assist in these pursuits. The student will have the opportunity to learn immunological techniques ranging from blood collection to immune cell isolation, tissue culture, immunological assay procedures, virus infection and titration, 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 in the Unit studying the genetic diversity of the virus and of the host.

ENVIRONMENTAL MANAGEMENT RESEARCH UNIT:

Electronic Monitoring of Swine for Improved Animal Well-Being

Scientist: T. M. Brown-Brandl, Ph.D.

Annual losses associated with heat stress in the swine industry are estimated at $299 million. Heat stress also impairs well-being. Heat stress results from a thermal imbalance caused when the animal produces more heat than can be dissipated to the environment. Heat stress causes decreases in feed intake, growth, feed efficiency, and reproduction. In extreme cases, heat stress can cause mortality. There is evidence that heat stress affects individual animals differently, but additional research is needed to better predict the variations among individual animals.

The student intern will collect physiological data and swine behavior parameters using electronic instrumentation. The instrumentation will include a video capture and animal-tracking system, a feeder monitoring system, and a thermal imaging camera system. The student will capture digital video of several groups of finishing pigs, and analyze the video using animal-tracking software. Data to be extracted with the software include the amount of time spent eating and drinking, average speed, and number of times the animal turns around. A feeder monitoring system will be used to determine feeding behavior and to validate the animal tracking software. Animals will be identified using electronic ear tags. The thermal imaging camera will be used to measure differences in the surface temperatures of individual animals. Data will be collected on individual animals in a pen of multiple animals. This data will be used to determine thermal stress tolerance for individual animals. The intern will also have the opportunity to work with other scientists in the Environmental Management Research Unit on other heat stress, air quality and manure management studies.

Air Quality in Cattle Facilities

Scientist: M. J. Spiehs, Ph.D.

Feedlot producers in the Northern Great Plains are starting to raise cattle in semi-enclosed barns instead of open lots. The barns have concrete floors covered with a deep layer of bedding material for comfort. The most commonly used bedding materials are crop residues such as corn stalks, wheat straw, and wood chips. Little is known about the potential odors and gases that can be produced from the pen surface of these barns. For the past year, Dr. Spiehs has been working with scientists and engineers from other states to determine the amount of odorous compounds (ammonia and hydrogen sulfide) and greenhouse gases (carbon dioxide, methane, and nitrous oxide) being emitted from these types of cattle facilities. Air quality instruments are used to continuously sample the air from the barns. Dr. Spiehs is also conducting lab-scale experiments to determine if the type of bedding material used in the barn will influence odor and gas emissions.

The student intern will assist Dr. Spiehs with a laboratory experiment to determine if bedding material can reduce odorous compounds and greenhouse gases in bedded manure packs. Lab-scale bedded packs will be constructed and manure, urine, and fresh bedding added at regular frequencies. Ammonia, methane, hydrogen sulfide, greenhouse gases, bedding temperature, and bedding pH will be measured once weekly. Samples of manure/bedding mixture from each lab-scale bedded pack will be collected to determine the concentration of odorous chemical compounds.

The student intern will have primary responsibility for maintaining the lab-scaled bedded packs and will work under the guidance and supervision of Dr. Spiehs. The student will assist with laboratory analysis of air and bedding samples including determining pH, dry matter, and volatile solids content of the bedding material. The student intern will also have the opportunity to travel with Dr. Spiehs to the barn sites, and will be trained on the calibration and operation of air quality collection instruments. The intern will also have the opportunity to work with other scientists in the Environmental Management Research Unit on other manure management and animal heat stress studies.

GENETICS & BREEDING RESEARCH UNIT:

Evaluating DNA variation on chromosome 5 associated with reproductive performance in cattle

Scientist: J. W. Keele, Ph.D.

The cattle genome contains genetic variation that can be utilized for improvement in reproduction. In cattle, most genetic gains result from selection of the sire as the bull can sire offspring from multiple females in one breeding season. Unfortunately, the bull cannot be directly measured for the females reproductive performance. We may, however, be able to select sires capable of producing daughters with superior reproductive performance using genetic markers. By identifying genomic regions harboring DNA sequence variation affecting performance traits, genetic markers can be developed for the cattle industry to select animals that are superior in reproductive performance. High-density genotyping arrays have been utilized to genotype multiple cattle populations across the U.S. and a population at the U.S. Meat Animal Research Center and identify genetic variation on Chromosome 5 associated with high or low reproductive performance.

The student selected for this project will evaluate regions on chromosome 5 that are associated with reproductive performance in our cattle populations. The student will accomplish this by first being shown how to use multiple genome databases to identify

candidate genes that are located within the genomic region of interest on chromosome 5. The student will then learn and use laboratory techniques including DNA extraction, polymerase chain reaction (PCR), basic sequencing protocols, and sequence analysis programs to determine if the gene sequence on chromosome 5 contains sequence variation associated with high or low reproductive performance. From this project, genetic markers will be identified for use in the cattle industry. The student selected for this project should have an interest in genetics and molecular biology, and statistical analysis. Additionally the student should have an interest in learning laboratory techniques to evaluate DNA sequence.

Evaluating DNA Variation Associated With Bovine Respiratory Disease in Cattle

Scientist: Tara McDaneld, Ph.D.

Bovine respiratory disease complex (BRDC) is the most expensive disease in U.S. cattle costing 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 SNP genotyping arrays and sample pooling) have improved capabilities for identifying host factors (bacterial and viral) associated with complex diseases including BRDC. The objective of this research proposal is to identify the bacterial and viral components that may predispose an animal to becoming susceptible to BRDC.

The student selected for this project will learn and use methods to identify bacteria and viruses in the U.S. Meat Animal Research Center disease population. The student will identify bacterial and viral species present in nasal swabs sampled from cattle to identify species potentially associated with BRDC susceptibility. The student will learn how to (1) extract DNA from nasal swabs, (2) identify bacterial and viral species present in nasal swabs through sequencing of DNA, and (3) use basic molecular biology procedures (i.e., polymerase chain reaction or PCR.

Applicants for this position should be interested in biology, and more specifically in genetics. Applicants should be willing to learn laboratory techniques and how to use DNA information to improve cattle genetics.

MEAT SAFETY & QUALITY RESEARCH UNIT:

Relationship between mitochondrial abundance and efficiency to beef top loin (longissimus) steak color stability

Scientist: D. A. King, Ph.D.

Lean color is the primary quality attribute evaluated by consumers at the retail meat case. Losses associated with discarding or discounting discolored beef products cost the industry an estimated $1 billion annually. This project will investigate relationships between beef longissimus lean color stability and mitochondrial quantity and efficiency. Beef top loin steaks will be placed in simulated retail display (appropriate lighting and temperature) with instrumental lean color measurements taken periodically. On days 0, 4, 7, and 11 of display, steaks will be removed from display and oxygen consumption and nitric-oxide metmyoglobin reducing activity will be measured. A separate sample will be used for determination of mitochondrial abundance, mitochondrial efficiency, myoglobin content, and muscle pH. Mitochondrial abundance will be measured using a quantitative real-time PCR assay. Hydrogen peroxide (H_2O_2) production during an *in vitro* assay of isolated mitochondria will be used to determine mitochondrial efficiency. Myoglobin content will be measured spectrophoto-metrically. The summer intern will assist with color measurements on steaks and all the laboratory assays. This experiment will provide insight into a potential mechanism regulating animal variation in beef lean color stability, which may lead to

strategies to manage this trait. Moreover, the results from this experiment will be used to optimize data collection protocols for future investigations of the biochemical basis for animal variation in lean color stability.

The effects of bovine immune response on *E. coli* O157:H7 colonization and fecal shedding levels

Scientist: R. Wang, Ph.D.

Shiga-toxin-producing *Escherichia coli* (STEC) O157:H7 is an important foodborne pathogen responsible for numerous disease outbreaks and clinical infections worldwide. Cattle are the principal reservoirs of STEC O157:H7 and the terminal rectum is the predominant colonization site of these bacteria in the animals. Once colonized, animals may become "normal shedders" that persistently excrete normal levels of STEC O157:H7 over prolonged periods, or become "super shedders" that periodically shed abnormally high levels of O157:H7 ($\geq 10^4$ CFU per gram of feces). The significant contribution of the super shedders to livestock contamination by STEC O157:H7 could be reduced in the 5% of the animals shedding STEC O157:H7 at abnormally high levels if colonization could be prevented.

The host immune response, especially the mucosal immune response, is the critical component of host defense system against bacterial infections. Studies have found that STEC O157:H7 colonization of the cattle terminal rectum led to mucosal damage, and triggered innate immune response as well as mucosal and serological antibody responses. The specificity and potency of the host immune response to STEC O157:H7 could play a major role in the cyclic nature of O157:H7 colonization, leading to a "decision" of a "super shedder" or a "normal shedder." Therefore, the goal of this study is to identify the specific host immune responses to STEC O157:H7 colonization in cattle, and to investigate if such responses would affect animal fecal shedding levels of STEC O157:H7. Fecal, mucosal, and serum samples will be harvested from feedlot cattle every two weeks during summer 2012. The presence of STEC O157:H7 in animal fecal samples will be determined and the animals will be sorted into STEC O157:H7 status groups of (1) super shedders, (2) normal shedders, and (3) non-shedders based on the results of O157:H7 presence in the feces. Bovine cytokines and serum/mucosal antibodies specific to O157:H7 antigens will be measured with ELISA and other immunological assays. The specific immune responses before, during, and after O157:H7 shedding will be compared between super shedders and other control animals to identify host factors that affect STEC O157:H7 colonization and fecal shedding levels. The summer intern will help collect the samples at the feedlot and conduct the laboratory assays. The information could help develop control strategies for reducing shedding and transmission of STEC O157:H7, removing super shedders from the cattle populations, and leading to a new generation of vaccine products.

NUTRITION RESEARCH UNIT:

Expression of Genes Involved in Steer Feed Efficiency

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

The U.S. Meat Animal Research Center Nutrition Research Unit is seeking a 2012 summer intern to conduct laboratory work in support of its beef 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. These tissues will be used for RNA and protein assays. 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. The successful candidate will be trained to extract RNA from several types of tissue and analyze these samples for gene expression. In addition, the intern will be responsible for creating a data set that will allow for statistical analysis. Differences in RNA transcript abundance will be used to identify metabolic processes that contribute toward variation in feed efficiency.

The Effect of Energy Source on Skeletal Muscle Growth in Swine

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

The U.S. Meat Animal Research Center Nutrition Research Unit is seeking a 2012 summer intern to conduct animal and laboratory work in support of its swine nutrition and growth program. The young pig has a high propensity for muscle growth. This is driven largely by high rates of protein synthesis, which reduces dramatically as the animal ages. Improvements in growth performance and body composition in the neonatal pig have the potential to reduce days to market and increase the carcass merit of pigs. In addition, the identification of the characteristics of the immature muscle that is responsible for the high rate of muscle protein synthesis and accretion will provide a rational underpinning for the development of new approaches for promoting the accretion of muscle mass throughout the production cycle. Previous research at USMARC has determined that a carbohydrate-based diet, compared to fat-based diet, improves the efficiency of amino acid use resulting in increased protein accretion in 10-day-old pigs. This research project is being coordinated by Dr. Oliver. The successful candidate will be trained to conduct an experiment determining the cellular mechanisms contributing to the improvement of muscle growth. In addition, the experiment will determine if the improvement in protein accretion in the young pig results in greater muscle mass at slaughter. The intern will be trained to conduct both the animal and laboratory portions of the experiment.

REPRODUCTION RESEARCH UNIT:

Influence of myostatin on ovarian follicular development in cattle

Scientist: R. A. Cushman, Ph.D.

Myostatin (e.g., growth differentiation factor-8, GDF-8) is a member of the transforming growth factor-β (TGF-β) superfamily of growth factors which regulate ovarian function in mammals. Microarray studies of cow ovaries have demonstrated that myostatin is more highly expressed in the granulosa cells of small antral follicles (1 – 5 mm) than medium (5.1 – 10 mm) or large (> 10 mm) antral follicles, suggesting a role for myostatin in early follicular development in cows. The F94L polymorphism in myostatin is associated with increased muscling; however, the functionality of the polymorphism remains questionable. Furthermore, there is evidence in the literature to suggest antagonistic relationships between carcass traits and reproductive function. Therefore, we propose to use heifers homozygous for the F94L polymorphism to investigate the role of myostatin in bovine ovarian function. Ovaries will be surgically collected from cycling heifers from each of the homozygous myostatin genotypes (n = 5/genotype) on day 4 of the estrous cycle. Ovaries will be weighed and measured, and all surface antral follicles will be counted. All small (3.1 -5 mm), medium (5.1 – 10 mm) and large (> 10 mm) antral follicles will be aspirated and pooled within heifer and follicle size category. Granulosa cells and follicular fluid will be

frozen for gene expression and steroid content analysis. The theca cells from the dominant and subordinate follicle will be frozen for gene expression analysis. The left ovary will be quartered, fixed in neutral buffered formalin, and embedded in paraffin for histological evaluation of the number of primordial, primary, and secondary preantral follicles and very small (1 – 3 mm) antral follicles. Real-time RT-PCR will be performed on the granulosa cells using primers specific for follistatin, anti-Müllerian Hormone, and Follicle Stimulating Hormone receptor. Differences in ovarian morphology and gene expression will be analyzed between myostatin genotypes using the GLM procedure of SAS. Additionally, correlation analysis will be performed to investigate the networking of these genes and their association with follicle diameter and steroid content. The summer intern would have the opportunity to be involved in performing histological evaluation and real-time RT-PCR on ovarian samples. This project will most likely also provide the summer intern with the opportunity to be involved with ovariectomies and tissue processing at the University of Nebraska-Lincoln.

Localization of estrogen receptor 1 and 2 mRNA and protein in the porcine endometrium during the estrous cycle and early pregnancy

Scientists: J. R. Miles (Project Leader) and J. L. Vallet

Between day 10 and 12 of gestation, the pig embryo undergoes a dramatic morphological transition from a spherical structure (~1 mm) to a long, thin filament (>100 mm) in a process called embryo elongation. During elongation, the embryo produces and secretes estrogen, which serves as the key molecule for maternal recognition of pregnancy and also modulates the production of proteins and growth factors within the uterus. In mammals, the physiological responses of estrogen are mediated through estrogen binding to two known estrogen receptors (ESR), ESR1 and ESR2 (formerly known as ER alpha and beta, respectively). We have previously demonstrated that *ESR1* mRNA expression in pig endometrium was increased at day 10 of the estrous cycle and pregnancy compared with day 12, 14, and 16. Furthermore, *ESR1* mRNA expression did not differ between the estrous cycle and pregnancy. In contrast, *ESR2* mRNA expression in the pig endometrium was greater at day 14 and 16 compared with day 10 and 12 of pregnancy; however, *ESR2* mRNA expression remained similar throughout the estrous cycle. This finding suggests that ESR2 may play a role in mediating the response of the endometrium to estrogen during early pregnancy. To further characterize the involvement of ESR1 and ESR2 during early pregnancy, the objective of this study is to localize ESR1 and ESR2 mRNA and protein in the endometrium during the estrous cycle and early pregnancy (day 10, 12, 14, and 16). In situ hybridization will be utilized to localize mRNA expression of *ESR1* and *ESR2* within microscopic sections of pig endometrium collected at the various time points. For localization of ESR1 and ESR2 protein, immunohistochemistry will be performed within microscopic sections of pig endometrium collected at the various time points. Students interested in this project will assist with in situ hybridization and immunohistochemistry.