

ENVIRONMENTAL MANAGEMENT RESEARCH UNIT:**Assessing Heat Stress in Feedlot Cattle: Animal Susceptibility, Environmental Conditions, and Management Effects**

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

Economic losses associated with heat stress in the United States for a single summer average \$2.4 billion over all livestock species; \$369 million of that is associated with feedlot cattle (St-Pierre et al., 2003). Heat waves are a reoccurring phenomenon over the cattle producing regions of the United States. A severe heat wave can result in the death of thousands of feedlot cattle and the loss of millions of dollars in revenue to the cattle industry, both in direct animal losses and indirect performance losses (Busby and Loy, 1996; Hahn, 1999; Hubbard et al., 1999) to a very localized area impacting a relatively small number of producers. The impact of a heat wave on a single animal is dependent on the interaction of several factors including the environment, the individual animal susceptibility, and management. These factors are interactive and each of them needs to be considered when making management decisions.

The students will be involved in collecting physiological data and behavior parameters using time-lapse cameras. Data will be collected on individual animals, as opposed to groups of animals, using these systems. This data will be used to create an understanding of an individual animal's tolerance for thermal stress. The data collected will include: 1) individual animal respiration rate; 2) continuous body temperature monitoring; and 3) collection and summarization of behavior from time-lapse cameras. Respiration rates will be collected manually, using a stopwatch and counting flank movements. Body temperature will be monitored in a select group of animals for a 3-week period using an electronic data logger. The images from the time-lapse cameras will be used to summarize time spent standing, lying, and accessing shade. The student will be working with scientists and technicians to collect and analyze the data to determine factors that impact an individual steer/heifer thermal tolerance.

Air Quality in Cattle Facilities

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

Background: Feedlot producers in the Northern Great Plains are starting to raise cattle in semi-enclosed barns instead of in open lots. The barns have concrete floors but the cattle are provided with a deep layer of bedding material for comfort. The most common bedding material used is corn stalks, although wheat straw, wood chips, and other crop residues have been used. For the past two years, Dr. Spiehs has worked with scientists and engineers from South Dakota and Iowa on a research project funded by a USDA - Agricultural Food Research Initiative grant to determine the amount of odorous compounds (ammonia and hydrogen sulfide) and greenhouse gases (carbon dioxide, methane, and nitrous oxide) being emitted from this type of cattle facility. In addition to the air quality measurements collected at the barn, Dr. Spiehs has conducted lab-scale experiments to determine if the type of bedding material used in the barn will influence odor and gas emissions. Results have shown that pine wood shavings have the lowest emissions of odorous compounds, while use of ground corn cobs and paper bedding resulted in higher concentrations of odorous compounds. However, corn stalks continue to be the most commonly used bedding due to high cost of other bedding materials.

Project Description: The Intern will be responsible for assisting Dr. Spiehs and the technician with an experiment in which various ratios of bedding material and animal waste will be mixed to determine the least amount of corn stalk bedding that can be used by the producers without negatively affecting odorous compounds and greenhouse gases in bedded packs. Lab-scale bedded packs will be constructed and manure, urine, and fresh bedding will be added at regular frequencies. Ammonia, methane, hydrogen sulfide, greenhouse gases, bedding temperature, and bedding pH will be measured once weekly. Samples of the manure/bedding mixture from each lab-scale bedded pack will be collected to determine the concentration of odorous chemical compounds. In addition, the Intern will have the opportunity to work with Dr. Spiehs and other scientists in the Environmental Management Research Unit with other on-going air quality research projects including the effect of cattle diets on air quality, and measurement of greenhouse gas concentration from the surface of feedlot pens.

Duties and Responsibilities: The Intern will have primary responsibility for maintaining the lab-scaled bedded packs and will work under the guidance and supervision of Dr. Spiehs and the technician on laboratory analysis of the air and bedding samples including determining pH, dry matter, and volatile solids content of the bedding material. The Intern will also have the opportunity to work with Dr. Spiehs on other research projects to learn about calibration and operation of air quality instruments used to measure ammonia, hydrogen sulfide, methane and other greenhouse gases.

GENETICS, BREEDING, AND ANIMAL HEALTH 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. However, currently many of our associations between SNP and reproduction are specific to breed (i.e., there is an association in one breed but not another). Additional sequencing may identify SNP with robust associations with reproduction in multiple breeds.

The student selected for this project will evaluate regions on chromosome 5 that have been identified to be associated with reproductive performance in multiple cattle populations. The student will accomplish this by first learning and using laboratory techniques including DNA extraction, polymerase chain reaction (PCR), basic sequencing protocols, and statistical and sequence analysis programs to determine if the DNA marker or gene sequence on chromosome 5 contains variation associated with high or low reproductive performance. The student will then use these techniques to generate sequence from the identified region of chromosome 5 and identify additional sequence variation (DNA markers and copy number variation) that demonstrate association with reproductive performance in multiple breeds. 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 Microbial Community Variation Associated With Bovine Respiratory Disease Complex in Cattle

Scientist: T. G. McDanel, 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 species that may predispose cattle to becoming susceptible to BRDC.

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 start collecting nasal mucosal samples from sick and control cattle and perform initial identification of bacterial species present in the nasal samples through screening the bacteria with various agar plates that select for specific strains of bacterial species. In the remaining six weeks, the student will (1) extract DNA and RNA from the nasal samples collected in 2012 from the Disease Resistant population at USMARC and (2) identify bacterial species present through initial sequencing of the DNA and evaluation of the RNA transcriptome. In cooperation with other scientists at the U.S. 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.

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.

Advancing genetic analysis software

Scientist: [R. M. Thallman, Ph.D.](#)

Genomics has the potential to greatly increase the rate of genetic improvement in livestock. The amount of data generated by new genomics technologies increases even more rapidly than computing capacity. Technologies in use at the U.S. Meat Animal Research Center for the past several years generate genetic marker data on 50,000 or 770,000 locations in the genome simultaneously. In the past year, the data generated has grown to include full genome sequence on substantial numbers of bulls, identifying genomic variation at millions of sites in the genome. GenoProb is a statistical genetics software package (written in C++) that is used by scientists from a number of institutions and countries. It was originally designed to be used on hundreds of genetic markers and with newer hardware can readily handle thousands, but not hundreds of thousands of genetic markers and not full genome sequence. GenoProb provides functionality that is not available in newer software, but new structures for data storage will be required to make GenoProb work efficiently with the new types and quantities of genomic data.

The student intern will use knowledge of programming in C++ and/or C# to expand the capabilities of GenoProb to deal with the new generation of genomics data more efficiently. In the process, the student will gain experience in the analysis of large data sets, efficient data structures, working with a large, complex program, and using source control software. The position is ideally suited to students with programming experience who are also interested in bioinformatics, genetics, livestock production, statistics, or mathematics. Interest or experience in linear algebra, and/or numerical analysis would be beneficial, but is not required. The U.S. Meat Animal Research Center is a diverse, multidisciplinary research environment. This internship will include opportunities to gain additional experience in a variety of disciplines including genetics, molecular biology, microbiology, and/or livestock production.

MEAT SAFETY & QUALITY RESEARCH UNIT:**Evaluation of immuno-magnetic particles and plating media for culture confirmation of non-O157 Shiga toxin-producing E. coli (O26, O45, O103, O111, O121 and O145)**

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

Shiga toxin-producing E. coli (STEC) of six serogroups, O26, O45, O103, O111, O121 and O145 have been declared adulterants by FSIS in certain beef products. The testing for these organisms identifies a large number of potential positive samples. In order to confirm results, culture confirmation is necessary. As an aide to isolate the suspect STEC from samples, immuno-magnetic particles have been commercially released by different companies for use in immuno-magnetic separation (IMS). There are three sources of these IMS beads, SDIx, BioControl and Dynal. A fourth company, BioMeriux, may have available an alternative purification system that uses serogroup specific bacteriophage fibers instead of antibodies. Further, there are multiple media, such Biolog Rainbow agar, ChromAgar STEC, STEC Differentiation Agar (USMARC) and washed sheeps blood agar that presumably allow selective growth of the STEC strains, or yield a specific phenotype of colony that correlates with STEC strains. Questions have been raised as to the performance of the IMS products and if there are benefits of one product over another, as well as the most appropriate media to use in order to culturally confirm the presence of STEC. This proposal plans a side-by-side evaluation of the available STEC IMS beads and plating medias.

Currently, studies are underway to examine the enrichment and detection of different STEC serotypes in beef samples that compare enrichment broth types, ratios, and levels of interfering background bacteria. During these studies, freezer aliquots will be saved for the STEC IMS bead comparison to follow. In total, there will be 180 enrichment broths available for use in this comparison. Three distinct isolates of each STEC serotype will be used. The STEC will have been inoculated at a low level and the screening results already known for each broth. Frozen broths will be thawed, properly diluted and then divided for IMS using each commercial reagent. The purified IMS beads will be serially diluted and spiral plated on to the various selective agars for the isolation of STEC serotypes. Recovery of STEC and the number of non-suspect STEC will be counted to determine the levels of STEC isolated and evaluate any nonspecific cross reactions with other bacteria present, as well as evaluate the selectivity and specificity of each medium. The goal will be to identify the best performing method for culture confirmation of each STEC serotype from samples considered potentially positive.

The planned work fits well into a 9 to 12 week period, and will generate immediately useful data that can stand alone or be incorporated into another publication. The laboratory experience offered by this work is mostly cultural microbiology, but the intern will be involved with all facets of the work ranging from the media and reagent preparation, to a limited amount of molecular testing using PCR on final isolates. Some of the skills that will be learned include proper biological safety cabinet use, reagent preparation, media sterilization/preparation, immuno-magnetic separation systems, simple PCR and gel electrophoresis, and statistical data analysis/presentation.

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 (H₂O₂) production during an in vitro assay of isolated mitochondria will be used to determine mitochondrial efficiency. Myoglobin content will be measured spectrophotometrically. 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.

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 2013 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 some of the 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 tissues samples. 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 between efficient and inefficient steers will be used to identify metabolic processes that contribute toward variation in feed efficiency.

Feed Additives in Feedlot Cattle

Scientist: K. E. Hales, Ph.D.

The U.S. Meat Animal Research Center Nutrition Research Unit is seeking a 2013 summer intern to conduct work in support of research on the use of β -agonists in feedlot cattle. The first objective of this research is to determine if feeding β -agonists affects heat stress in feedlot cattle. Synthetic β -adrenergic agonists are chemically similar to dopamine, norepinephrine, and epinephrine and have been studied and applied in livestock because of their marked effects on growth rate. Most notably, β -agonists increase the rate of skeletal muscle accretion. The β -agonist zilpaterol hydrochloride increases daily gain, feed efficiency, and dressed yield in feedlot cattle. Zilpaterol hydrochloride has only been recently approved for use in the United States, but no research has been published on possible effects on heat stress and the mechanism in which the rate of skeletal muscle accretion increases. Our hypothesis is that zilpaterol hydrochloride decreases heat stress because it is a bronchodilator, and the rate of muscle accretion is increased because of a decrease in protein degradation, and thus, a decrease in the animal's maintenance requirements. Symptoms of heat stress will be measured including respiration rate, panting score, and temperature in finishing cattle. The second objective of this research is to determine if feeding β -agonists reduce the animal's maintenance requirement through decreasing protein degradation. Nutrient balance will be determined after 5 days of total urine and fecal collections to determine if nitrogen balance and utilization are affected. Additionally, at the end of the nutrient balance collections, fasting heat production will be

measured on cattle after a 48-hour fast on control cattle and cattle that have been orally drenched with a β -agonist. The successful candidate will be trained in a variety of metabolism, energetic, and nutrition procedures. The intern will be responsible for data collection, laboratory analysis, and statistical analysis.

REPRODUCTION RESEARCH UNIT:**Effect of glucosamine supplementation during pregnancy on placental histoarchitecture and fetal plasma fructose and glucosamine concentrations.**

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

The pig has a diffuse epitheliochorial placenta, which distinguishes it from cotyledonary placentas present in sheep and cattle. Over most of its surface, the placenta of the pig consists very simply of a folded epithelial bilayer, made up of endometrial luminal epithelium on the maternal side, and trophoblast epithelium on the fetal side. Maternal and fetal capillaries are adjacent to the epithelial cells on the maternal and fetal sides of the bilayer, respectively. The bilayer is surrounded and embedded in stromal tissue on the fetal side, essentially providing support and structure for the fetal side of the placenta. Previous research indicates that glycosaminoglycans like hyaluronan and heparan sulfate are primary components of fetal stromal tissue. These in turn are polymers of different sugars, approximately half of which is glucosamine. Our previous research has shown that the depth of folding of the epithelial bilayer may contribute to improved function of the pig placenta, and that stromal development in placenta of small fetuses may be inadequate to support the development of deeper folds. In this experiment, we hypothesize that providing supplemental glucosamine will support stromal development, leading to deeper folds in placenta of small fetuses. In order to ensure the presence of small fetuses, we have unilaterally hysterectomized-ovariectomized (UHO) pigs, which results in moderate intrauterine crowding. The UHO pigs will be fed supplemental glucosamine from day 85 to 105 of pregnancy, and then fetal blood samples and placental tissues will be collected at slaughter on day 105. We anticipate that most of the tissues will be collected before students begin work for the summer, but it is possible that the last few animals will be collected in late May or early June. The student will primarily help with preparing placental tissues for histology, and then measuring histological sections for various structural features, which is done using computer assisted morphometry. In addition, the student will help with fructose and glucosamine assays of fetal plasma. Fructose is a substrate for glucosamine synthesis, so our hope is that supplemental glucosamine might affect fructose concentrations. Finally, the student will also help with assay of hyaluronan in placental tissues using a commercially available kit. The results will demonstrate whether glucosamine supplementation during late pregnancy can improve placental development, particularly in placenta of small fetuses. Aside from improvements in litter size, if this treatment is successful it could contribute to increased birth weight of piglets. Low birth weight is well known to be associated with poor preweaning survival.