

Analysis of Lysine Hydroxylation, Collagen Pyridinium Crosslinking, and Ultrastructure of Long Term *In Vitro* Fibroblast Cultures from Horses with Hereditary Equine Regional Dermal Asthenia (HERDA, aka Hyperelastosis Cutis)

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HERDA is an autosomal recessive connective tissue disorder of Quarter Horses. Affected horses have fragile, easily damaged skin and are commonly humanely destroyed early in their life because they cannot be ridden. Our investigations focus upon quantification of collagen pyridinium crosslinks, specifically deoxypyridinoline (DPD) and pyridinoline (PYD). These intermolecular bonds contribute to the tensile strength of collagen. DPD formation requires two hydroxylysine residues; PYD formation requires three. Hydroxylysine is formed during posttranslational modifications of procollagen by the enzyme lysyl hydroxylase. In normal individuals, PYD is the predominant Type I collagen crosslink. We initially determined that the DPD:PYD ratio in the urine of HERDA horses is significantly elevated, from birth, allowing diagnosis of the disease. Further analysis determined that the concentration of DPD ($p < 0.001$) and the ratio of DPD:PYD ($p < 0.0001$) were significantly higher, while the concentrations of hydroxylysine ($p < 0.0001$) and pyridinoline were significantly lower in the skin of affected horses versus unaffected controls. These findings bear similarity to those in human Ehlers-Danlos Type VI. These findings prompted an evaluation of the *in vitro* characteristics of collagen formation in long term cultures of dermal fibroblasts, which synthesize dermal collagen. Cytopreserved fibroblasts from the skin of 3 HERDA and 3 unaffected horses, propagated for six weeks *in vitro* in six well tissue culture plates (37°C, 5% CO₂) will be harvested July 5. Cells propagated on Thermanox® Coverslips will be processed using standard methods for ultrastructural analysis by TEM and SEM. Remaining wells are harvested into replicate microcentrifuge tubes. Protein content will be determined using the Wang and Smith, Lowry modification. Amino acids will be quantified on a Biochrom 20 Plus amino acid analyzer following hydrolysis in 6M HCl. Total DPD and PYD in skin are determined by reverse-phase high pressure liquid chromatography (HPLC) of the hydrolysate. Significant differences between groups will be evaluated. [Trainee stipend supported by NIH-2T35RR070710]

Changes in Thermal Gradients of the External Genitalia of Neonatal Pigs Exposed to Oral Administration of the Soy-derived Phytoestrogen Genistein and Estradiol .

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As part of a larger study investigating the role of phytoestrogen exposure of neonatal pigs on reproductive development, a subset of pigs were monitored to determine whether there were changes in external genitalia thermal signatures. Eight sows (100 d gestation) were assigned to a ration supplemented with (Trt, n=4) or without (Con, n=4) isoflavone extract (5:4:1 of genistein,

daidzein, glycitein; 1.5 mg/kg/BW/d for ~14 d) and allowed to farrow. On post natal day 7 (PND 7), male and female pigs from both sow diets were assigned to treatments: 1) control, 2) low genistein (LG; 3 mg/kg BW/d), 3) high genistein (HG; 9 mg/kg/d), and 4) estradiol (E2, positive control; 50 mg/kg/BW/d). Daily doses were administered (2x daily) by oral gavage for 7 days. Thermographic images were recorded (FLIR S60 camera) each morning on PND 7, 9, and 11 of scrotal area in males, and vulva in females and prior to euthanasia on PND 14 at which time reproductive tissue samples were recovered. Data was analyzed using ANOVA and repeated measures to determine differences in maximum, minimum, average temperatures and standard deviation of temperatures of genitalia during the course of the trial. There was no sow treatment effect on genital temperatures of males or females (testes; Con = 36.9±0.2 vs. Trt = 37.2±0.2 and vulva; Con = 37.5±0.2 vs Trt = 37.3±0.2). However, there was a day by piglet treatment effect in temperature standard deviation (i.e., measure of temperature variation) for vulva (P<0.02) and scrotal temperatures (P<0.07) associated with estradiol. Additionally, vulva width increased (P<0.0001) in estradiol-treated females over the other three treatment groups. This study demonstrates that oral administration of estradiol is bioactive in neonatal piglets, but that thermal imaging did not consistently reflect changes in tissue with tissue temperatures. [Trainee stipend supported by NIH-2T35RR070710]

Use of Bioluminescence and GFAP-luc Transgenic Mice for Evaluating the Effects of Manganese and Lipopolysaccharide on Astrocyte Activation

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Excessive exposure to manganese (Mn), an essential transition metal, is associated with specific basal ganglia neuropathology known as manganism. Manganism resembles Parkinson's Disease (PD). Besides direct effects on neuronal cells, detrimental effects of Mn on astrocytes and microglia have been implicated in its neurotoxicity. Moreover, Mn effects on the nervous system seem to be augmented in the presence of an inflammatory stimulus. To better understand the role of astrocytes in the neurotoxicity of Mn, in this study we utilized luciferase-tagged glial fibrillary acidic protein (GFAP-luc; an astrocyte-specific marker) transgenic mice that were exposed daily to Mn. After 12 days, the mice were given a single dose of LPS. Luciferase signal corresponding to the amount of astrocyte activation and eventual ensuing neuronal damage was quantified after injecting the mice with a bioluminescent substrate, luciferin. Analysis is conducted using IVIS 100 bioluminescence system that is interfaced with the Living Image software. GFAP levels were monitored at several different time points prior to LPS as well as at 4, 24, 72 hrs and one week post LPS injection. In a parallel study, we exposed male C57BL/6 mice to Mn and challenged some of them with LPS on day 12. After several behavioral analyses, mice were sacrificed at 4, 24, or 72 hrs post Mn/LPS administration. Brains, plasma, and selected organs were harvested and processed for Mn, neurochemical, and western blot analyses. When these studies are completed we expect to have determined whether (i) the GFAP-luc mice are a useful model for studying the role of astrocytes in Mn neurotoxicity and (ii) what is the nature of the interaction between Mn and the inflammogen LPS *in vivo*. [Trainee stipend supported by NIH-2T35RR070710]

PCR and Southern Blot Analysis of *Listeria monocytogenes* actA and Internalin Genes

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Listeria monocytogenes is a foodborne bacterial pathogen that employs a number of virulence-associated proteins (eg, ActA and internalins) to aid its movement in and out of the host cells. While *actA* and many internalin genes were examined previously by DNA arrays, they have not been fully characterized by PCR and Southern blot techniques. This study aimed to gain additional insight on the genetic structures in *L. monocytogenes* that underpin listerial variations of virulence among different serotypes using these molecular procedures. Oligonucleotide primers were designed from *L. monocytogenes* EGD-e *actA* and internalin genes (ie, *lmo0327*, *lmo0331*, *lmo2396*, *lmo2445* and *lmo2470*) and used in PCR for examination of 36 strains representing 12 *L. monocytogenes* serotypes and 5 non-*monocytogenes* *Listeria* species. The PCR fragments generated from the EGD-e strain were then applied as probes in Southern blot for assessment of *actA* and internalin gene structures. While *actA* and *lmo2396* gene were detected in all serotypes under investigation, *lmo0327*, *lmo0331*, *lmo2445* and *lmo2470* genes were absent in serotypes 4a, 4c and/or 7. Apart from confirming the previous DNA array results, Southern blot analysis revealed new structural details on *actA*, *lmo0327*, *lmo0331*, *lmo2396*, *lmo2445* and *lmo2470* genes. It was of interest to note that *actA* gene displayed a larger PCR band in some serotypes but a smaller one in others. *L. monocytogenes* serotypes demonstrate notable variations in their *actA* and internalin genes, with high virulence serotypes (eg, 1/2a, 1/2c and 4b) possessing more internalins than low virulence serotypes (eg, 4a and 4c), and the shared internalin genes often having distinct structures between high and low virulence serotypes. [Trainee stipend partially supported by Merck-Merial]

Identification of Mutated Genes of Attenuated *Edwardsiella ictaluri* Vaccines

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Enteric Septicemia of Catfish (ESC) caused by the bacteria *Edwardsiella ictaluri* is a devastating disease that causes high mortality rates and costs the catfish industry millions of dollars annually due to production losses and treatment costs. However, limited success has been achieved in treating this important disease. Of the available treatments and preventatives, live attenuated vaccines provide a high level of protection against ESC. We have developed several attenuated *E. ictaluri* vaccines and the focus of this study was to identify mutated genes that caused attenuation. Genomic DNA from fourteen mutants was prepared and transposon ends were amplified using single primer PCR. Sequencing of the PCR products with a nested primer revealed the locations of the transposon insertion sites in the *E. ictaluri* genome. Database and literature searches to better understand the functions of the mutated genes are underway. [Trainee stipend supported by NIH-2T35RR070710]

Identification of Herpesvirus-Host Protein Interactions using Sequential Peptide Affinity Purification and Mass Spectrometry

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Channel Catfish Herpesvirus (CCV) has a great economic impact on catfish farming, the largest aquaculture industry in the USA (\$2 billion). CCV is an obligate intracellular parasite and CCV proteins interact with both other CCV, as well as catfish, proteins during the CCV lifecycle. Protein complexes are so ubiquitous that the biological function of an unknown protein can often be inferred from the functions of interacting partners. Our goal was to introduce the sequential peptide affinity (SPA) purification system into CCV molecular biology so that it can be used as part of a strategy to identify protein-protein interactions in CCV disease. The SPA system involves fusion of the SPA tag to the target protein in such a way that protein expression is driven from its natural promoter. SPA tags allow a high degree of purification of protein complexes. We used the SPA system to purify protein complexes of CCV ORF3 (an immediate-early protein) and CCV ORF12 (a C3HC4 RING finger motif protein), which are potential CCV regulatory proteins important in regulating gene expression in disease. Also, Herpesvirus RING finger proteins are involved in reactivating quiescent genomes, stimulating lytic infection and immune evasion. We used our novel CCV bacterial artificial chromosome system to produce recombinant (r)CCVs expressing C-terminally SPA-tagged proteins (ORF3 and ORF12). We next infected cultured channel catfish ovary cells with the rCCVs. The protein complexes were isolated, digested with trypsin and analyzed by tandem mass spectrometry. To identify co-purified interacting partners of SPA-tagged CCV proteins we searched the tandem mass spectra against the CCV proteins; channel catfish proteins and translated ESTs. Successful completion of this research will result in discovery of several new specific host-pathogen interactions and determination of CCV ORF3 and ORF12 protein functions. [Trainee stipend supported by NIH-2T35RR070710]

Effect of Insecticide Exposure in Inducing Oxidative Stress in Rats

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Dieldrin, an organochlorine insecticide, and chlorpyrifos, an organophosphate insecticide, were commonly used for agricultural and domestic purposes. Some evidence suggests that exposure to these compounds results in oxidative stress by increasing the formation of free radicals, which subsequently cause an increase in lipid peroxidation. This study investigated oxidative stress as a mechanism of toxicity using two treatment protocols. Initially adult female rats were administered dieldrin at 2.5mg/kg/day for 13 days prior to breeding and chlorpyrifos at 1.0mg/kg/day during gestation (GES day 17-20). A second cohort of female rats was administered chlorpyrifos during the same gestational period to determine the effects of dieldrin pretreatment alone. The ability of the insecticides to induce oxidative stress was investigated in rat tissues (brain and liver) in pups from treated dams at postnatal days 14 and 70. Oxidative stress endpoints were measured by determining levels of lipid peroxidation (as indicated by

thiobarbituric reactive substances, TBARS) and glutathione using spectrophotometric assays and by determining the levels of the antioxidant, alpha-tocopherol (vitamin E) by UPLC. While there were no decreases in hepatic glutathione, decreases in hepatic alpha-tocopherol, and increases in lipid peroxidation suggests that dieldrin and chlorpyrifos given to pre-gestational and gestational rats causes post-natal oxidative stress in the offspring. [Partial support from NIH R03 ES014725, trainee stipend supported by NIH-2T35RR070710].

The Effect of the Lipid Peroxidation Product 4-Hydroxy-2-Nonenal on Esterase and Lipase Activities in Human THP-1 Monocytes/Macrophages

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Lipid peroxidation is a hallmark of oxidative stress. End products of lipid peroxidation include several electrophilic aldehydes; the most prominent being 4-hydroxy-2-nonenal (4-HNE). Cellular proteins can be inactivated by covalent modification with 4-HNE, which contributes to diseases such as atherosclerosis. Here we examined the effect of 4-HNE on hydrolytic activities in cultured human THP-1 monocytes/macrophages, which are catalyzed by esterases and lipases. These enzymes are important regulators of cholesterol mobilization. Dysregulated cholesterol mobilization from monocytes/macrophages contributes to phenotypic differentiation into 'foam cells' and subsequent atherosclerosis. The following results were obtained. First, when pure recombinant carboxylesterase protein was treated with increasing amounts of 4-HNE significant inhibition of hydrolytic activity was observed. Second, when THP-1 cell lysates were treated with 4-HNE, marked decreases in esterase and lipase activities were also noted. Last, intact THP-1 cells were treated with 4-HNE (25-200 μ M) up to 24-h. While cytotoxicity was noted, decreased esterase and lipase activities were observed after normalization on cell protein. Moreover, western blotting indicated induction of CE protein in cells treated with 200 μ M 4-HNE. This may indicate a compensatory response. Collectively, these results suggest that pathophysiological concentrations of 4-HNE leads to inhibition of enzymes involved in processing lipid and water-soluble esters. [Trainee stipend supported by NIH-2T35RR070710]

Detection and Quantitation of Plasma Viremia in FIV-Infected Cats during Acute Infection

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Feline immunodeficiency virus (FIV), a lentivirus, produces a syndrome in the infected cat that closely parallels human AIDS. FIV is efficiently transmitted in utero in experimentally-infected queens, frequently producing infected offspring and/or reproductive failure, similar to the increased level of spontaneous abortion which occurs in HIV-infected women. Thus, the FIV-infected queen provides an excellent model system to understand HIV pathogenesis and vertical transmission. HIV is typically detectable in the maternal circulation during pregnancy, and maternal virus load has been shown to play an important role in transplacental transmission of HIV. Likewise, we hypothesized that FIV viremia contributes to maternal-fetal transmission

the queen. Ten female cats were inoculated with FIV-B-2542 and bred during acute infection. Blood samples were collected at biweekly intervals beginning on the day of inoculation and ending on the day of cesarean delivery of fetuses at week three gestation, at which point the queen was euthanized. Blood was assayed for viremia in two ways: 1) FIV provirus in peripheral blood leukocytes from longitudinal samples was detected using standard PCR targeting a 259 bp region of the gag gene. Provirus was detected in feline blood samples by six weeks post infection (p.i.). 2) Virus load in blood plasma was quantified using real time, one step-reverse transcriptase (RT)-PCR. Total RNA was purified from plasma samples and used as template in the real time RT-PCR reactions targeting the gag gene. A standard curve was generated by in vitro transcription of a PCR-generated template. These assays are currently ongoing. To date the data show that FIV viremia appeared soon after infection and persisted throughout the duration of infection. [Trainee stipend partially supported by Merck-Merial]

Role of *Mannheimia haemolytica* Leukotoxin in Bovine Respiratory Disease

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Bovine Respiratory Disease (BRD) caused by *Mannheimia haemolytica* is a devastating disease with significant impact on both agricultural economy and animal health. The manifestation of this disease is primarily due to the effects of leukotoxin (LKT) produced by the bacteria. Molecular mechanisms of host-pathogen interactions at the onset of BRD are largely unknown. The objective of this study was to elucidate key molecular and cellular mechanisms, particularly the involvement of pro-apoptotic and anti-apoptotic genes. To accomplish our objective, we have isolated and purified LKT, exposed bovine neutrophils to this serially diluted LKT (0, 10^{-4} , 10^{-3} , 10^{-2} , and 10^{-1}) *in vitro* for various time periods (1, 2, and 3 hours), and determined the ratio of live to dead neutrophils after exposure. Next, we will evaluate the mRNA expression profiles of Bax and Bcl-2 genes, master regulators of cell apoptosis, using real-time PCR methods. The preliminary results of this on-going study suggest that a 10^{-3} dilution of isolated LKT will be of most value in studying apoptotic effects over a time course of 1 and 2 hours. The results also suggest that high concentrations of LKT (10^{-1}) cause a great extent of cell death. The significance of this study is that our results will provide a more comprehensive understanding of the mechanisms of host cell-pathogen interactions that occur with BRD. Our findings will shed light into the mystery behind BRD thus, paving the way for further studies and ultimately leading to the possibilities of developing novel treatments for BRD. [Trainee stipend partially supported by Merck-Merial]

Salmonella Sensitivity to Chlorine Dioxide

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Salmonella, a member of the family *Enterobacteriaceae*, is a gram-negative bacteria that causes enteric disease in multiple animal species. Infection occurs as a result of ingestion of the bacteria, and salmonellosis may or may not occur depending on the amount of bacteria ingested and host susceptibility. Some human infections have been associated with poultry due to the consumption of undercooked and improperly handled meat or eggs or cross contaminated food. Therefore, it is important to understand and further investigate the locations and quantities of *Salmonella* within the bird and possible interventions that could be used to reduce the risk of human infection. Ten *Salmonella* serovars identified as having been implicated in human outbreaks were used including: Typhimurium, Kentucky, Montevideo, Mbandaka, Thompson, Schwarzengrund, Alachua, Seftenberg, Braenderup, and Heidelberg. pBen276 is a plasmid that inserts bacterial luciferase genes into the *Salmonella* chromosome to allow real-time detection and quantification. Chlorine dioxide is a compound that has been used as a disinfectant/sanitizer in the processing of poultry, fruits and vegetables. A protocol was designed to test the sensitivity of *Salmonella* poultry isolates to different concentrations of chlorine dioxide. Experiments were conducted over 2 hour time periods, and bioluminescent *Salmonella* was used to determine results. [Trainee stipend supported by NIH-2T35RR070710]

***Salmonella enteritidis* Induced Expression of Gallinacin Genes in Chicken Oviduct Epithelial Cells**

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Salmonella enterica serovar Enteritidis (SE) is the most common cause of salmonellosis in humans and typically is caused by consumption of contaminated poultry meat and egg products. Current theories indicate that the bacteria are found in non-symptomatic chickens at the site of the ovary and oviduct where contamination of the egg occurs before the external shell is generated. Gallinacins are β -defensin peptides found in poultry which are expressed as part of the innate immune response to certain microorganisms. These peptides elicit signals to other immune cells for recruitment to the site of invasion. There are fourteen (14) known gallinacins in the chicken each with different expression levels based on the age of the chicken, the production status of the chicken, and the invaded tissue type; however, specific gallinacin expression is not known for all tissue types. By determining the levels of expression in a particular tissue, it can be observed which gallinacins are produced in response to a particular microorganism. In this experiment, epithelial cells of chicken oviduct isthmus, a primary site of colonization, were inoculated with SE at an MOI of 20 and two mutant strains at an MOI of 30 each in 48- well plates to determine the expression of the gallinacin genes. At one hour (T1), four hours (T4), and twenty-four hours (T24) post inoculation, RNA was extracted and reverse transcription real-time polymerase chain reaction (RT-PCR) was performed using SYBR Green as the dye marker. All gallinacin genes were consistently expressed throughout the experiment. Inoculation with the bacterial strains produced an increase in expression indicating they are all involved in response to infection in varying degrees. Data will be analyzed at a later date for relevance and frequency. [Trainee stipend supported by NIH-2T35RR070710]

Identification of Mesocortical Dopamine Neurons for Isolation with Laser Capture Microdissection

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Two functionally distinct populations of dopamine neurons are located in the ventral tegmental area, the mesocortical and mesoaccumbens dopamine neurons. Mesocortical dopamine neurons innervate the prefrontal cortex which is involved in cognitive processes such as planning, working memory, and problem solving. Mesoaccumbens dopamine neurons innervate the nucleus accumbens which is important for integrating motivation and reward. Because these two populations mediate different functions in the brain and also have distinctly different pharmacology and afferent innervations, the current hypothesis is that these neurons will have distinct gene expression profiles. In order to distinguish between these populations of neurons, we propose to use the combination of retrograde tracers, tyrosine hydroxylase immunocytochemistry and laser capture microdissection (LCM). The goal of the current project is to determine the optimal retrograde transporter to label dopamine neurons in a manner compatible with LCM. Two microliters of the retrograde tracers 5% Wheat Germ Agglutinin + AlexaFluor 594, 0.5% Cholera Toxin + Biotin, 5% Wheat Germ Agglutinin, and 0.5% Cholera Toxin + AlexaFluor 594 were dissolved in cerebral spinal fluid and injected bilaterally into the prefrontal cortex of four separate mice. After 48 h, the mice were euthanized, the brains removed, frozen on dry ice and, using a cryostat, 10 μ m sections were placed on a slide. The retrograde tracers were visualized in combination with tyrosine hydroxylase immunocytochemistry. Cholera Toxin + AlexaFluor 594 produced the best labeling of mesocortical dopamine neurons although it produced a small injection site. Properties of the other retrograde tracers are currently being analyzed. Overall, this project has demonstrated the feasibility to distinguish mesocortical dopamine neurons for isolation with LCM. [Trainee stipend partially supported by Merck-Merial]

Systematic Review of Vaccination as an Intervention for Salmonella in poultry

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Systematic reviews involve comprehensive research of a focused clinical question. These reviews require the collection of all relevant primary research using a concisely defined search strategy. Once compiled all studies are screened by multiple reviewers for relevance to the focused clinical question. Those research studies found to be relevant then undergo a structured quality assessment process, which focuses on the quality of the methods used thus placing emphasis on higher quality research. This additional analytical step unique to the systematic review process reduces bias and error that often accompanies narrative reviews. Data is then extracted from each selected article and synthesized using quantitative or qualitative means. Systematic reviews are the means by which this compilation, assessment and implementation of research can be facilitated. The following systematic review focusing on one specific area of agri-food public health research used currently available studies to determine if vaccination reduces or eliminates *Salmonella ssp.* in broilers during production and processing. Searches

were conducted using 18 databases and specific search criteria. The initial search including the terms salmonella, broilers and layers produced 2781 articles. These articles were screened for relevance then categorized by different interventions and by broiler versus layer production resulting in 1200 abstracts passed to the next level. A subset of 180 abstracts that pertained to vaccination in broilers was subjected to further screening for relevance. From this step, 53 articles were identified and are currently undergoing quality assessment. After quality assessment data will be extracted from those articles and the quantitative/qualitative analysis of the research will be conducted to determine the efficacy of vaccination as an intervention. [Trainee stipend supported by NIH-2T35RR070710]

Development of a Model for Temperament Modification in Cattle: Investigation of Fluphenazine's Effect on Animal Activity and Physiological Response

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Holstein (n=30) and Jersey (n=10) steers were allocated to fluphenazine (FLU) treatment groups based on pre-trial exit velocity (a temperament measure), body weight and hip height to examine the influence of FLU on activity patterns. Treatments (d 0) were administered intramuscularly as follows: Control (saline); Low FLU (0.026 mg FLU/kg); and High FLU (0.055 mg FLU/kg). Animals were equipped with pedometers (d -8 through d 40) to measure daily lying, standing and activity, including total steps. Ambient environmental conditions (temperature and relative humidity) were obtained throughout the trial using HOBO monitors, and respiration rate was determined each morning. Percentage of time lying, standing and being active, and number of steps, did not differ pre- or post-treatment among FLU groups, however all activity variables differed ($P < 0.0001$) over time. During an average 24 h period, steers spent more time standing (46.13%) and lying (47.92%) than active (5.90 %; 3748.26 ± 25.15 steps per day). Respiration rate changed over time (pre- vs. post-treatment), but was similar ($P < 0.10$) among treatment groups. FLU treatment did not alter animal activity, and requires further study to evaluate the efficacy of FLU as a pharmacological-based model of temperament modification in cattle. [Trainee stipend supported by NIH-2T35RR070710]

Mining Function from High-throughput Data Sets

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Sub-minimum inhibitory concentrations (sub-MICs) of antibiotics modulate bacterial metabolism and virulence. Defining the underlying molecular mechanisms of sub-MICs in bacterial virulence and adaptive mechanisms is a pre-requisite for utilizing sub-MICs in treatment strategies with multi-drug resistant strains of bacteria. Bovine respiratory disease (BRD) is a common problem of cattle and is responsible for major economic loss in the cattle industry. *Pasteurella multocida* a commensal in the bovine upper respiratory tract and is one of

the causative agents of BRD. Sub-MIC response of *P. multocida* to three different antibiotic classes, beta lactams, tetracyclines and quinolones was studied at the transcriptome level. With oligonucleotide microarrays differential expression of 415, 393 and 473 genes in response to sub-MIC amoxicillin, chlortetracycline and enrofloxacin respectively were identified. The objective of this study is to model the biological pathways and functions involved in sub-MIC responses of *P. multocida*. In order to achieve this goal we built *P. multocida* protein interaction network using gram-negative orthologs in Pathway Studio (Ariadne genomics). Differential gene expression data was overlaid onto a *P. multocida* protein interaction network. Our results indicate that all three antibiotics led to lowered expression of some antimicrobial peptide transport systems. Chlortetracycline, a protein synthesis inhibitor, resulted in lowered expression of genes involved in protein synthesis. Amoxicillin, a cell wall biosynthesis inhibitor, also exerted similar effects on the expression of genes involved in protein synthesis. Expression of genes involved in DNA repair, including *recN*, *recA*, *ruvA*, and *ruvB*, increased in response to enrofloxacin, which is likely an adaptive response to the quinolone-mediated DNA replication block by double strand DNA break repair. In summary, we identified common themes in antibiotic response as well as specific effects with each antibiotic class. [Trainee stipend supported by NIH-2T35RR070710]