Clinical and Applied Science Research Presentations
Food Animal, Group A
Phi Zeta Research Day
March 10, 2015, 1:15-3:45pm
201 Trotter Hall

1:15 – 1:30 **L.L. Schumacher** – Determining the minimum infectious dose of porcine epidemic diarrhea virus (PEDv) in the feed matrix

1:30 – 1:45 **Daniel Frese**– Effect of cattle handling technique on selected blood chemistry parameters in finishing steer

1:45 – 2:00 **Jorge C. Simroth** – Relationship Between the Prevalence and Size of Horns, and Prevalence, Anatomical Location, and Severity of Bruises on Beef Carcasses

2:00 – 2:15 **Erin Schwandt** – *"A survey of dry processed corn particle size and fecal starch in Midwestern U.S. feedlots"*

2:15 – 2:30 **Megan C. Niederwerder** – Relationship between growth performance and clinical signs of PCV2/PRRSV co-infection in nursery pigs

2:30 – 2:45 **Break**

2:45 – 3:00 **Lance W. Noll** – Culture- and PCR-based Methods for Detection and Quantification of Shiga Toxin-Producing *Escherichia coli* O157 in Cattle Feces

3:00 – 3:15 **Pius S. Ekong** – Stochastic modeling of the variability associated with concentrations of six major non-O157 Escherichia coli serogroups in cattle feces based on a multiplex real-time quantitative PCR (mqPCR) assay.

3:15 – 3:30 **Sarah Capik** – Behavioral effects of cattle handling over time

3:30 – 3:45 **Kaitlynn Abell**– Effect of vaginal temperature on behavioral patterns of *Mannheimia haemolytica* challenged beef heifer calves.

5:00 – 6:00 pm **Reception and Awards Ceremony** Frick Auditorium and Foyer, 2nd Floor, Mosier Hall

- Initiation of New Members to Phi Zeta
- Announcement & Presentation of Awards Recognizing Research & Scholarship Accomplishments
- Closing Comments

Determining the minimum infectious dose of porcine epidemic diarrhea virus (PEDv) in the feed matrix

L.L. Schumacher

J. C. Woodworth, J. Zhang*, P. C. Gauger*, Q. Chen*, M. Welch*, H. Salzebrenner*, J. Thomas*, R. G. Main*, S. S. Dritz, R. A. Cochrane, and C. K. Jones.

*Kansas State University, Manhattan and *Iowa State University, Ames.*

Our objective was to determine the minimum infectious dose of PEDv in feed using a 10-d-old pig bioassay. A PEDv isolate (USA/IN/2013/19338 P7) from a clinical case grown in cell culture was serially diluted into 9 different PEDV doses using tissue culture media. . The PEDv dilutions were mixed in swine feed to form 9 incremental dosages (5.6x10^5 to 5.6x10^-3 TCID50/g). The 4 highest dose treatments had detectable PEDV RNA (Ct of 27, 30, 33, and 37). When the PEDv was added to the feed, an increase of 9.6±0.4 SEM Ct was observed. In the bioassay, pigs inoculated with the 4 highest dose treatments had signs of PEDv replication (fecal sample Ct's ranging from 16 to 27 on d 4 and 6 after inoculation). Infection was further confirmed by histopathology and PEDv specific immunohistochemistry. No detectable PEDv RNA or lesions were noted in pigs inoculated with the other treatments. In conclusion, PEDv infectivity was correlated with PCR positive feed and the minimum infective dose of PEDv in a feed matrix was 5.6x10^1 TCID50/g (PCR Ct of 37). Interestingly, the Ct difference was approximately 10 (3 logs or 1,000-fold difference) when PEDv was blended into feed, which could be due to loss or binding of viral RNA to feed particles. This data confirms feed can be a vehicle for PEDv transfer and Ct of 37 can lead to infectivity in 10-d-old pigs. The Ct of 37 is important because it is greater than the threshold considered negative in some diagnostic laboratories. Also, this dose suggests that 1 g of acutely infected baby pig feces could contaminate as much as 450 tonnes of feed.

Effect of cattle handling technique on selected blood chemistry parameters in finishing steer

Daniel Frese

Author(s): Daniel. A. Frese, DVM, Christopher D. Reinhardt, PhD; Steven J. Bartle, PhD ; David N. Rethorst, DVM; John P. Hutcheson, PhD ; Wade T. Nichols, PhD ; Brandon E. Depenbusch, PhD; Mark D. Corrigan, PhD; Daniel U. Thomson, PhD, DVM

Angus crossbred steers (n=40; 563 ± 44 kg) were stratified by ultrasound backfat thickness and randomly assigned to the following treatment groups: 1) low stress handling (LSH) and 2) aggressive (AH). Cattle in LSH treatment were walked while AH were ran through an exercise course of 770 m two times. Cattle were restrained in a hydraulic chute, vital signs recorded, and blood samples were obtained via jugular venipuncture at baseline, 770m, 1540m and following two consecutive 1 hour rest periods following exercise. Blood was analyzed for lactate, creatinine kinase (CK), base excess, pH, cortisol, substance P, and venous P_vCO_2 and P_vO_2. Aggressively handled cattle had greater lactate, cortisol, P_vO_2, heart rate and rectal temperature than LSH cattle as well as lower blood pH, bicarbonate, base excess, and P_vCO_2 . Lactate concentrations increased 6 to 10 fold from baseline in AH cattle. Creatine kinase concentrations increased at each consecutive sample time in both LSH and AH cattle, but was not different between treatments. Four AH steers became exhausted and failed to complete the course. Significant elevation in CK, decreased P_vCO_2, and muscle tremors occurred in exhausted steers compared to non-exhausted AH cohorts. Blood-gas parameters in exhausted cattle indicate altered cardio-pulmonary physiology compared to non-exhausted cattle and warrants further investigation. Aggressive handling of cattle increases plasma lactate, CK and alters blood-gas measurements during and following exercise compared to baseline.

Relationship Between the Prevalence and Size of Horns, and Prevalence, Anatomical Location, and Severity of Bruises on Beef Carcasses.

Jorge C. Simroth

Jorge C. Simroth, Chris Reinhardt, Margaret Stephens, Steve Bartle, Dave Rethorst, and Dan Thomson.

Two major animal welfare concerns are dehorning and bruising of cattle during handling. The objective of this study was to investigate the relationship between the presence and size of horns in cattle and the prevalence, anatomical location, and severity of bruising of carcasses. Carcasses from 4,287 feedlot cattle were observed at one commercial beef packing plant in southwest Kansas. Horn measurements and bruise scoring were performed by three trained evaluators. Horn measurements taken were the length of the longest horn from base to tip and the tip to tip length. Bruises were evaluated by location and severity using the Harvest Audit Program, which divides the carcass into 9 anatomical regions. Severity was scored at three levels; minor (-): ≤ 5 cm, moderate (0): 5-15 cm, and severe (+): > 15 cm. Eighty five percent of the carcasses were beef cattle and 15% were Holsteins. Bruising and horn prevalence were 51 and 6% for beef carcasses and 70 and 11% for Holstein carcasses. Of the total number of bruises, 25.6% were severe, 35.6% were moderate, and 38.8% were minor. Majority of bruises (61.8%) occurred on the dorsal mid-line with similar bruising occurring on the left (18.6%) and right (19.5%) sides. The prevalence of bruising on the caudal third (21.8%) was a third of the prevalence of bruising that occurred on the center (60.5%) and anterior (17.6%) portions. Further research during handling including loading and unloading techniques, transportation practices, and trailer design is needed to effectively reduce the incidence of carcass bruising.

"A survey of dry processed corn particle size and fecal starch in Midwestern U.S. feedlots"

Erin Schwandt

Author(s): E.F. Schwandt, D. U. Thomson, S. J. Bartle, and C. D Reinhardt

Feedlots (n = 35) were asked to participate in a survey to evaluate dry-rolled corn (DRC) processing practices, processed corn particle size distribution, and fecal starch content in finishing cattle. The feedlot facilities were located in the central U.S. states of Kansas, Nebraska, South Dakota, Minnesota, Colorado, and Iowa. Samples of dry processed corn, the finishing diet, and fecal samples were collected from each feedlot. The average particle size of the dry processed corn across all operations was 4,223 ± 1,265 µm with a range of 1,165 to 6,823 µm. Dry rolled corn (DRC) average particle size was 4,534 ± 899 µm with a range of 2,167 to 6,823 µm. Hammermill ground corn average particle size was 1,817 ± 1,158 µm with a range of 1,165 to 3,552 µm Fecal starch content was analyzed from each operation and was 19.0 ± 6.5% with a range of 7.0 to 36.6 %. Diet composition was evaluated for by-product inclusion level [27.8 ± 13.4%]; roughage inclusion level [8.9 ± 2.0%] and NDF levels [19.3 ± 4.3%] on a dry matter basis. Data from this survey provide an indication of DRC particle size and dietary formulation practices for feedlots located in the Midwest and Northern Plains regions of the U.S. Fecal starch values indicate the amount of undigested starch in the feces, which may be influenced by corn particle size. Some feedlots may have the opportunity to increase the degree of grain processing to improve total tract starch utilization.

Relationship between growth performance and clinical signs of PCV2/PRRSV co-infection in nursery pigs

Megan C. Niederwerder

Author(s): Megan C. Niederwerder, Raymond R. R. Rowland

Average daily gain (ADG) is used in swine production as an objective measurement of overall health and performance. Both porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) infections can result in reduced ADG and increased time to market weight. Co-infections with PRRSV and PCV2 are associated with clinical signs such as pallor or jaundice, lethargy, decreased body condition, muscle wasting, pyrexia, dyspnea, open mouth breathing, rhinorrhea, diarrhea, aural cyanosis, lameness, joint effusion and coughing. To evaluate the expression of clinical disease and determine effects on growth performance, these clinical signs were documented in 95 six-week old pigs for 70 days post-infection (dpi) with PCV2 and PRRSV. Pigs with clinical disease were treated as directed by a veterinarian. Days on treatment and days with each clinical sign were plotted against 0-70 dpi ADG. As expected, pigs administered veterinary treatment for clinical disease had significantly lower ADG; ADG was 0.73±0.1 and 0.50±0.15 kg for non-treated and treated pigs, respectively ($p<0.001$). Days with dyspnea had the strongest association with reduced ADG ($r = -0.67$). Large reductions in ADG (0.213 kg) were associated with days of open mouth breathing. Days with aural cyanosis and diarrhea had the weakest association with ADG ($r = -0.14$ and $r = -0.19$, respectively). Overall, an impaired ability to breathe normally had the largest effect on growth performance. This study supports the notion that reducing causes of respiratory distress should be considered high priority for improving animal welfare and growth performance in swine production.

Culture- and PCR-based Methods for Detection and Quantification of Shiga Toxin-Producing *Escherichia coli* O157 in Cattle Feces

Lance W. Noll

Author(s): Pragathi B. Shridhar, Diana M. Dewsbury, Xiaorong Shi, Natalia Cernicchiaro, David G. Renter, and T.G. Nagaraja

Shiga toxin-producing *E. coli* O157, a major foodborne pathogen, colonizes the hindgut of cattle and is shed in the feces, which serves as a source of contamination of food and water. Cattle that shed the organism at high concentration (> 10^3 CFU/g), called 'super shedders', are responsible for increased transmission and contamination. Our objectives were to compare the detection of *E. coli* O157 in cattle feces by conventional PCR (cPCR), culture method and multiplex quantitative PCR (mqPCR), and compare quantification of *E. coli* O157 by mqPCR and spiral-plate method. Fecal samples (n=576) were suspended in *E. coli* broth and enriched for 6 hours at 40 C. Fecal suspensions before enrichment were subjected to mqPCR and spiral-plate methods to quantify *E. coli* O157. Fecal suspensions after enrichment were subjected to cPCR, mqPCR, and culture method to detect *E. coli* O157. A higher proportion of fecal samples were positive for *E. coli* O157 by mqPCR (517/576; 89.8%) than by cPCR (315/576; 54.7%) or culture-based method (247/576; 42.9%). Fecal samples that were quantifiable for O157 by mqPCR (62/576; 10.8%) were at concentrations ≥ 10^4 CFU/g of feces. Only 4.5% (26/576) of samples were positive by spiral-plate method, with the majority (17/26; 65.4%) at concentrations below 10^3 CFU/g. Our data indicate that mqPCR is superior to cPCR and culture-based methods for the detection of *E. coli* O157 in cattle feces. However, spiral plate-method may be necessary to quantify *E. coli* O157 when present at concentrations < 10^4 CFU/g in cattle feces.

Stochastic modeling of the variability associated with concentrations of six major non-O157 Escherichia coli serogroups in cattle feces based on a multiplex real-time quantitative PCR (mqPCR) assay.

Pius S. Ekong

Author(s): Pius S. Ekong, Michael W. Sanderson, Pragathi B. Shridhar, Jianfa Bai, and T.G. Nagaraja

The quantification of non-O157 STEC at various steps in the cattle production system is necessary to populate a quantitative microbial risk assessment. However, concentration data are difficult to obtain. In order to provide an estimate of variability around the concentration of non-O157 STEC detected in cattle feces, we developed a Monte Carlo simulation model in @RISK based on quantitative real-time PCR cycle threshold (C_T) value. We utilized two sets of mqPCR assays that targeted serogroup-specific genes to detect and quantify the six major non-O157 STEC in cattle feces. A linear relationship was measured between the C_T values and pre-enrichment colony counts (CFU/g) of fecal samples spiked with ten-fold serial dilutions of pooled pure cultures of O26, O103 and O111; and O45, O121 and O145 serogroups. Distributions were fitted to the intercept and slope parameters of the linear equations and the observed C_T values from the spiked fecal samples to predict the distribution of colony counts (log). The distributions of C_T values for each serogroup obtained from feces of naturally-shedding cattle were fitted into the model to predict the mean colony counts and their variability estimates. At a C_T value of 25, the models predicted a mean log count of 5.33 CFU/g for O26, O103 and O111 serogroups, with a 95% prediction interval of approximately ±0.66 log, and for O45, O121 and O145 serogroups predicted log count was 5.12 CFU/g, ±0.88 log. This result provides a method to estimate concentration of STEC in cattle feces along with their measures of uncertainty.

Behavioral effects of cattle handling over time

Sarah Capik

Author(s): Sarah F. Capik, Brad J. White, Robert L. Larson, Kaitlynn M. Abell, Ellen M. Unruh

Behavior of cattle is complex and can be altered via a variety of internal and external stimuli. The goals of this pilot study were to evaluate the potential behavioral differences in cattle that are brought through a chute and to quantify any behavioral differences, if present, in the following days. Fifty-one male crossbred beef calves were obtained from an auction market on two different days and randomly assigned to 3 pens. Calves were fitted with accelerometers and transmitters for a real time location monitoring system and allowed to acclimate to the facility for 7-10 days. Once acclimated, cattle were brought by pen to the chute where they were kept with their head tied to the side with a rope halter for approximately 60 seconds while their temperature was taken. A binomial chute score, vocalization score, and exit score was recorded for each calf. Calves were returned to their home pens and their behavior over the subsequent 3 days was compared to the baseline established prior to being handled. The project was replicated one week later. Compared to baseline, cattle traveled less distance ($p<0.05$) and spent less time lying ($p<0.01$) after going through the chute. Additionally, calves with an exit score of 1 traveled 291.6 meters (±118.3) more on average than calves with an exit score of 0. This indicates that some aspects of behavior are potentially affected for several days after cattle are brought through a chute.

Effect of vaginal temperature on behavioral patterns of *Mannheimia haemolytica* challenged beef heifer calves.

Kaitlynn Abell

Author(s): Kaitlynn M. Abell, Robert L. Larson, Miles E. Theurer, Brad J. White, Derek Mosier

The use of vaginal probes can allow for a non-invasive way to collect body temperatures in cattle, giving insight to how body temperature is associated with bovine behavior. The objective of this study was to see how temperature is associated with behavior of beef heifers challenged with *Mannheimia haemolytica*. Ten black crossbred heifer calves (mean BW 185.59 ± 11.9kg) were endoscopically challenged with *Mannheimia haemolytica* to induce a febrile response. Vaginal temperatures were recorded every hour using a remote temperature probe. Rectal temperatures from each calf were recorded at three time-points throughout the trial to determine correlation with vaginal temperature. Percent time lying, distance traveled, time spent near the hay bunk, feed bunk, water, and shed were determined for each calf via remote location monitoring. A strong positive correlation (R^2=0.71) between rectal temperature and vaginal temperature was found. Distance traveled, time spent at the shed, grain, and water were statistically significantly associated with vaginal temperature. Time spent at the hay was not statistically significantly associated with vaginal temperature. Percent time lying tended to be associated with vaginal temperature (P=0.055). As vaginal temperature increased, lying percent decreased, and time spent at the water and shed increased. Following endoscopic challenge of *Mannheimia haemolytica*, calf behavior patterns were associated with vaginal temperatures. The results from this study may improve case definition for identifying febrile calves based upon behavioral activity. Non-invasive vaginal temperature monitoring devices can aid in the understanding of bovine behavior post febrile response.