environment and handling that the experimental group experienced. It was concluded that social facilitation increased the number of visits to the feeders, but had no effect on the stress and weight gain of recently weaned lambs.

Key Words: Welfare, Weaning, Lambs

M10 Bone quality, behavioural repertoire, and physical condition of laying hens housed in conventional, modified and furnished colony battery cages. M. J. Jendral*[1], D. R. Korver[1], J. S. Church[2], and J. R. Feddes[1], [1]*University of Alberta*, *Edmonton, Canada*, [2]*Alberta Agriculture, Food and Rural Development*, *Edmonton, Canada*.

The welfare of White Leghorn hens housed in conventional (CON) (30cm x 45cm) (n=84), modified (MOD) (n=84) (60cm x 45cm) and furnished colony (120cm x 110cm) (n=24) cages was investigated by evaluating bone quality, behavior and physical condition. All cages provided 450cm^2 floor space/hen. CON and MOD each housed 3 hens, and MOD contained a perch (30cm x 5cm) and nestbox (NB) (24cm x 45cm), providing an additional 360cm^2 of nest area/bird. Colony units, which housed 26 hens, contained a perch (120cm x 5cm), NB (60cm x 55cm) and were furnished with (CWDB) or without (CWODB) a dustbath (DB) (60cm x 20cm), providing each hen with 126cm^2 NB space, and in CWDB, 46cm^2 DB area. Video recordings at 35 and 60 wks were examined for locomotory behavior, and hen physical condition was scored at 31 and 65 wks. At 65 wks, hens were euthanized and right femur, tibia and humerus were excised for analysis. Data were analyzed using GLM for mixed effects, and scored condition values were compared by chi-square analysis. Effects were significant at $P<0.05$. CON hens exhibited lower femoral and tibial total mineral density and mass, cortical area and mass, and breaking strength than CWDB, CWODB or MOD hens, but higher density and cross sectional area of bone in the trabecular space. Total and cortical humeral density, mass and breaking strength were higher in CWDB and CWODB than in CON and MOD. Birds in CON cages exhibited more pacing, standing and escape behaviors, but fewer bouts of walking, wing flapping, stretching and ruffling than hens in furnished cages. CWDB and CWODB hens perched, jumped and walked more than hens in MOD. Average and total feather condition scores were higher for MOD and CON hens, as were the proportion of hens with higher scores for individual body regions, and head and vent wounds. Foot and claw condition were improved in furnished cages, and keel bone scores were lowest for MOD hens. These finding suggest that while group size impacts hen welfare, MOD and colony cages provide amenities that encourage movement, performance of natural behaviours and improved bone condition.

Key Words: Layer, Welfare, Behaviour and Condition

Animal Health - Livestock and Poultry: Bovine I

M11 Osteopontin expression during the periparturient period in dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* infection. E. L. Karcher*[1], D. C. Beitz[1], and J. R. Stabel[2], [1]*Iowa State University*, *Ames*, [2]*USDA-ARS-National Animal Disease Center*, *Ames, IA*.

Investigation of the role of osteopontin (Opn) in Johne's disease is of interest based upon its ability to influence cytokine expression and to improve host defense against mycobacterial infections. The objective of this study was to characterize Opn expression and secretion by peripheral blood mononuclear cells (PBMCs) isolated from periparturient dairy cows naturally infected with Mycobacterium avium subsp. paratuberculosis (MAP). Twenty-five multiparous Holstein cows were assigned to 3 groups based upon their infection status: 1) noninfected healthy cows (n = 8), subclinically infected cows (n = 10), and clinically infected cows (n = 7). Blood was collected from the jugular vein from 3 wks pre- through 5 wks post-calving. PBMCs were isolated from the buffy coat fractions of whole blood. PBMC were cultured for 24 h with and without MAP whole cell sonicate (MPS). RNA was extracted from cells, and converted to first-strand cDNA. Real-time PCR was performed on each sample to evaluate the expression of Opn. RT-PCR data was evaluated using $2^{-(\Delta\Delta Ct)}$ with samples calibrated within treatment to mean ΔΔCt value at one day after calving. Immunoblot analysis was performed for detection of Opn protein from cultured PBMCs. PBMCs isolated from subclinical cows expressed greater amounts of Opn mRNA compared with control ($P<0.06$) and clinical ($P<0.05$) cows. Expression was higher prepartum, followed by a decline at calving that was consistent until 21 days postpartum. MPS-stimulated PBMCs from subclinical cows expressed less Opn mRNA than control and clinical cows ($P<0.05$). There was no effect of parturition on expression from stimulated cells, regardless of treatment group. Immunoblot analysis of Opn detected a predominant band at 50 kDa and three minor bands at 62, 37, and 24 kDa. The data indicate an ability of MAP infection and parturition to modulate Opn expression.

Key Words: Periparturient, Osteopontin, *Mycobacterium avium* subsp. *paratuberculosis*

M12 Development of a novel enzyme-linked immunosorbent assay for the diagnosis of Johne's disease. S. Eda*[1], A. J. Branscum[2], Y. Kaneko[1], M. C. Scott[1], and C. A. Speer[1], [1]*University of Tennessee*, *Knoxville*, [2]*University of Kentucky*, *Lexington*.

Johne's disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), has a significant economic impact on the US dairy cattle industry. Use of enzyme-linked immunosorbent assays (ELISA) to identify cattle for further fecal culture testing or for culling is listed as a recommended method for JD control in dairy and beef herds. However, several recent reports estimated diagnostic sensitivities of currently available ELISAs to be only 13.5 to 27.8%. For example, by using a Bayesian non-gold standard analysis, the diagnostic sensitivities of two current ELISAs were estimated to be 26-27%. Recently, it was predicted that if the diagnostic sensitivity of currently available ELISAs could be improved to 80%, then their use could result in an effective reduction of JD prevalence, higher level of milk production, and higher annual net revenue per cow. We developed a novel ELISA, called EVELISA, for the detection of MAP infections in cattle and is highly sensitive identifying 97.4% of fecal-culture positive cattle compared to a currently marketed ELISA that identified 50%. However, when 37 serum samples from a herd with a high rate of false-positives were tested by the EVELISA as well as a currently available ELISA, both ELISAs found more than 70% of the samples to be positive for JD. The false-positive rate of the

EVELISA was reduced significantly to 26.1% when the serum samples were pre-absorbed with *M. phlei*. By using the fecal culture method as the gold standard, empirical diagnostic sensitivity of the EVELISA using *M. phlei* absorption (absorbed EVELISA) was 97.1%, whereas that of a current ELISA was 48.5%. Moreover, a Bayesian non-gold standard analysis revealed that the absorbed EVELISA had a significantly higher level of diagnostic sensitivity (82%) than that of a current ELISA (22%). These data indicate that this novel ELISA is a rapid, inexpensive, specific, and highly sensitive test for JD which may improve the effectiveness of JD control measures.

Key Words: *Mycobacterium avium* subsp. *paratuberculosis*, Johne's disease, Enzyme-linked Immunosorbent Assay

M13 Effect of pasteurization on bacterial count and immunoglobulin G levels of bovine colostrum. J. A. Elizondo Salazar*, S. C. Donaldson, B. M. Jayarao, and A. J. Heinrichs, *The Pennsylvania State University*, *University Park*.

On-farm pasteurization of colostrum has received considerable attention in recent years, primarily to reduce bacterial pathogens present in colostrum. Application of this practice has been reported to result in significant health benefits for calves and economic returns for producers, however little information is available on the effect of pasteurization on immunoglobulin G (IgG) concentration. A study was conducted to determine the effect of low-temperature long-time pasteurization on the bacteriology and IgG levels in colostrum. First milking colostrum was collected from 28 primiparous and multiparous Holstein cows. Each sample was thoroughly mixed and two 10mL aliquots were analyzed. The first aliquot served as the control while the second aliquot was heated for 30 min at 62.8°C. The treated and untreated colostrum samples were examined for standard plate count (SPC), preliminary incubation count (PIC), coagulase-negative staphylococci (CNS) count, environmental streptococci (ES) count, coliform (CC) count, gram-negative noncoliform (NC) count, Streptococcus agalactiae (SAG) count, and Staphylococcus aureus (SA) count. IgG1 and IgG2 levels were measured using radial immunodiffusion. The results of the study showed that pasteurization resulted in a significant reduction of SPC, CC, NC, ES, CNS, SA, and PIC. Pasteurization also resulted in denaturation of 24% of colostral total IgG. The preliminary findings of the study suggest that gram-negative bacterial numbers (CC, NC) and gram-positive mastitis pathogens such as SA, CNS, and ES are considerably reduced.

Table 1. Bacterial count and immunoglobulin G levels of bovine colostrum before and after pasteurization.

	Unpasteurized	Pasteurized	SEM	*P*
Bacterial Count, cfu/ml				
SPC	16,161.4	21.4	4084.0	0.001
PIC	11,317.9	12.9	2985.9	0.001
CC	10,293.6	3.6	3749.6	0.001
NC	2,162.1	0.0	629.9	0.001
ES	3,784.3	0.0	2520.9	0.001
CNS	43,113.6	2.9	22724.4	0.001
SA	3,627.1	0.0	2,524.7	0.022
Immunoglobulin, mg/ml				
IgG_1	84.8	73.8	2.7	0.009
IgG_2	4.3	2.9	0.4	0.014
Total IgG	89.1	76.7	2.8	0.005

Key Words: Colostrum, Pasteurization, Immunoglobulin G

M14 Measuring bovine colostrum specific gravity using two hydrometers at various temperatures. A. J. Heinrichs*, S. A. Belegundu, C. M. Jones, and J. A. Elizondo Salazar, *The Pennsylvania State University*, *University Park*.

An indirect measure of colostrum IgG quantity has been to measure specific gravity of colostrum and apply the data to a predetermined equation. This method has been used to some degree on farms for the past 25 years, utilizing a glass hydrometer with a specific calibration chart for colostrum at 22°C. While it is known that the relationship between specific gravity and IgG is variable, this method remains one of the few rapid, on-farm means to evaluate colostrum quality. Two different hydrometers were studied in this experiment, one a standard glass hydrometer fitted with the colostrum IgG calibration chart, the other a plastic hydrometer with a similar specific gravity range. Holstein colostrum samples (n = 146) were analyzed at 15.6, 21.1 and 26.7°C to determine correlations between the 2 hydrometers over a temperature range that is frequently observed when samples are tested on-farm. Specific gravity measurements for the glass hydrometer were calculated from IgG values recorded from the instrument's scale. The correlation for specific gravity between glass and plastic hydrometers was 0.96 over all temperatures and was 0.94, 0.97 and 0.98 at 15.6, 21.1 and 26.7°C, respectively. Specific gravity was different between 15.6 and 26.7°C for both glass and plastic hydrometers. This study shows that a plastic hydrometer can be equally effective in estimating colostrum specific gravity as a glass instrument, making it a viable option for on-farm use. The potential for improved durability of this tool may encourage expanded use of the hydrometer to estimate colostrum IgG content and thus improve passive transfer in calves.

Key Words: Colostrum, Specific Gravity, Immunoglobulin G

M15 Changes in protein expression in *Escherichia coli* as a consequence of growth in milk whey. J. D. Lippolis* and T. A. Reinhards, *National Animal Disease Center / ARS/ USDA*, *Ames, IA*.

Understanding changes in protein expression by bacteria as they adapt to their environment and the pressures exerted by the host immune system to eliminate the bacteria will become a foundation to research into better therapeutics for treatment of bacterial infections. Shotgun Proteomics, using amine-reactive isobaric tags (iTRAQ) was used to quantify protein changes in Escherichia coli (mastitis isolate) grown in either Luria-Bertoni broth or milk whey. Changes in expression for over 264 proteins were obtained, 74 proteins that were down-regulated when the bacteria were grown in whey, 66 that were up-regulated and the rest were unchanged. Several proteins of immediate interest were those involve in iron transport. Iron(III) dicitrate transport protein (FECA) and Iron(III) dicitrate-binding periplasmic protein (FECB) were both up-regulated in E. coli when grown in whey by approximately 3.0 fold. An innate mechanism to limit bacterial growth is the sequestration of free iron by proteins such a lactoferrin. Therefore, necessary for successful growth in milk, bacteria must increase expression of proteins that bind and internalize iron. Our proteomic profiling suggests E. coli responded to the milk environment by increasing its own iron-binding proteins. Two proteins associated with osmotic regulation were also up-regulated when the E. coli was grown in whey. Osmotically-induced protein Y (OSMY) and Osmotically-inducible lipoprotein E (OSME) were up-regulated 4 and 5 fold respectively. There is evidence that osmotic regulation plays an important role in bacterial virulence by either affecting expression

of virulence genes or affecting bacterial growth in vivo. These data demonstrate that quantitative shotgun proteomics has great potential to provide new insights into how bacteria thrive in milk and may provide new insights into antibiotic therapies.

Key Words: Mastitis, Proteomics, Infection

M16 Results of milk samples submitted for *Mycoplasma* spp examination from California dairies between 1999 and 2005. D. F. Resende*, R. G. S. Bruno, P. V. Rossito, K. Glenn, and J. S. Cullor, *University of California*, *Davis*.

The objective of this study was to study the prevalence of *Mycoplasma* spp in California, utilizing data from milk samples submitted to the Milk Quality laboratory located at the Veterinary Medicine Teaching and Research Center (VMTRC - UC Davis) in Tulare - CA with the majority of milk samples representing San Joaquin Valley. A total of 350,412 records of microbiological testing of individual animal milk samples (n=267,357) and bulk tank milk samples (n=83,055) submitted for screening of *Mycoplasma* spp between 1999 and 2005, including milk samples obtained from mastitis cases, routine herd checks and routine creameries samples, were reviewed. Bulk milk samples represent approximately 1100 dairies, and individual cow samples represent approximately 120 dairies. Samples were cultured on Mycoplasma agar plates (UC Davis media lab) and results were recorded as no growth, contaminated and positive. In a subset of samples Fluorescent Antibody Staining was performed for *Mycoplasma*spp identification. All data was analyzed using Minitab software (Minitab 14). 9,167 bulk tank milk samples, 6,405 individual cow samples and about 40% of the dairies tested positive for *Mycoplasma* spp. *Mycoplasma* spp prevalence in bulk milk tank samples was higher in 1999 (P<0.005) when compared to the other years (2000-2005), and prevalence in individual cow samples was higher in 2001(P<0.01). There was no difference among the other years (P<0.005). Incidence of *Mycoplasma* spp was not influenced by season (P>0.20). *Mycoplasma bovis* was the most common isolated species when compared to other *Mycoplasma* species (581 vs. 126 cases /year; P<0.005).The vast majority of both bulk milk tank samples and individual cow samples were characterized as no growth. In summary, among years, 1999 had the highest incidence of *Mycoplasma* spp and season did not affect this incidence. *Mycoplasma bovis* was the most abundant isolated species of *Mycoplasma* in bulk tank milk and individual cow samples.

Key Words: Mycoplasma, Milk Quality, Milk Samples

M17 Evaluation of Direct Fecal PCR and Serum ELISA for the Detection of *Mycobacterium avium* subsp. *paratuberculosis*. D. L. Clark*, J. J. Koziczkowski, R. P. Radcliff, R. A. Carlson, and J. L. E. Ellingson, *Marshfield Clinic*, *Marshfield, WI*.

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease in cattle. The disease causes diarrhea, reduced milk production, poor reproductivity, emaciation, and eventually death. Culture on Herrold's egg yolk agar (HEYA) is considered the gold standard for diagnosis of Johne's in cattle. Although sensitive and specific, the method can take up to 16 weeks due to slow growth of MAP. Currently serum ELISA is used to screen herds for Johne's disease but positive tests must be confirmed with culture or PCR. The current research sought to evaluate an in-house direct fecal PCR procedure and directly compare it to ELISA using culture as the gold standard. Serum and fecal samples were collected from cows (n=250) with unknown Johne's status. Fecal samples were processed for culture on HEYA and direct PCR. Serum samples were tested using the Parachek™ serum ELISA. Overall 67/250 (26.8%, 95% CI 21.4-32.8) animals were culturally confirmed as being infected. PCR and ELISA detected 74/250 (29.6% 95% CI 24-35.7) and 25/250 (10% 95% CI 6.6-14.4) respectively. Culture and PCR were able to detect more positive animals than ELISA ($P < 0.0001$). Overall, direct fecal PCR was 70.2% sensitive and 85.3% specific when using culture as the gold standard. The ELISA method was 31.3% sensitive and 97.8% specific. When culture reported < 10 CFU, the sensitivity and specificity of PCR and ELISA were 57.1% and 85.3%, and 4.8% and 97.8% respectively. When culture reported 11-39 CFU, the sensitivity of PCR and ELISA were 75% and 50% respectively. When culture reported > 40 CFU, the sensitivity of PCR and ELISA were 100% and 88.2% respectively. Specificity could not be calculated at these levels because there were no negative samples. Direct PCR out performed ELISA in diagnosing infected animals and was not significantly different when compared to culture. The direct fecal PCR method described here is economical, easy to use, and more accurate than the ELISA method. These data support the use of PCR as an alternative to culture for screening herds for prevalence and diagnosis of Johne's disease.

Key Words: Johne's, PCR, Paratuberculosis

M18 Effect of vitamin E and selenium administration on concentration of malondialdehyde in udder milk. P. Wicheanson[1], V. Harnpanichpun[2], V. Chupia[3], P. Vinitchaikul*[3], and W. Suriyasathaporn[3], [1]*Sixth year student, Faculty of Veterinary Medicine, Chiang Mai University*, *Muang, Chiang Mai, Thailand*, [2]*Dairy Product Research and Development Unit, Chiang Mai*, *Muang, Chiang Mai, Thailand*, [3]*Faculty of Veterinary Medicine, Chiang Mai University*, *Muang, Chiang Mai, Thailand*.

In response to mastitis, phagocytes generate superoxide to kill invading microorganisms, causing an increase of oxidative reaction in the udder. Although essential for survival, the undesirable repercussion of inappropriate or excessive oxidative reaction, the so-called oxidative stress, can cause tissue degeneration and consequently decrease in milk production. To reduce oxidative stress, it is possible that external administration of antioxidants might decrease oxidative stress in udder. The objective of this research was to determine the effect of vitamin E and selenium administration (Vit E-Se), an antioxidant, on concentrations of malondialdehyde (MDA), a oxidative stress marker, in udder milk. Fifty-eight dairy cows from six small holder farms in Chiang Mai were used. Milk samples were collected at morning milking. Twenty ml of commercial Vit E-Se with concentrations of 17 mg/ml of vitamin E and 1.67 mg/ml of selenium were administered after morning milking. Milk samples were collected at 6, 24, 72 hour after Vit E-Se administration. The samples was measured MDA with modified Smith's method. Due to data collected from the same cow, repeated measure analysis was used (Proc Mixed, SAS 8.0). MDA after Vit E-Se were compared with MDA before administration. Cow factor was defined as a repeated factor. Result shown that mean and SEM of MDA at before and after administration at 6th, 24th and 72nd hours were 1,370 +45, 1,133+28, 1,232+18 and 1,252+23 ppb, respectively. Concentration of MDA at before was higher than those after administration (p<0.05) There was no significant difference

of MDA between 24 and 72 hour after Vit E-Se. In conclusion, vitamin E and selenium administration result in decrease oxidative stress in udder.

Key Words: Vitamin E and Selenium, Dairy Milk, Malondialdehyde (MDA)

M19 Effect of feeding an immunostimulatory feed supplement (OmniGen-AF) during the dry period on somatic cell scores (SCS) in early lactation Holstein cows. H. T. Ballantine*[1], J. D. Chapman[2], Y.-Q Wang[4], and N. E. Forsberg[3,4], [1]*Ballantine Consulting*, *Hiram, GA*, [2]*Prince Agri Products*, *Quincy, IL*, [3]*Oregon State University*, *Corvallis*, [4]*OmniGen Research*, *Corvallis, OR*.

In previous studies, the feeding of a proprietary ingredient blend, OmniGen-AF (OG), (OmniGen-AF; Prince Agri Products) has been observed to increase the expression of markers of innate and acquired immunity. The goal of this study was to assess the hypothesis that OG, when fed to dry cows, had potential to reduce somatic cell scores (SCS) of cows in the subsequent lactation. The study was conducted on a commercial dairy in the southeast and included 114 Holstein cows. Cows were randomly assigned to one of two dry cow feeding regimens which consisted of a control ration program (0 g/h/d; n=46 cows) or a treatment ration program which consisted of feeding OG at 28 g/h/d to far-off dry cows and 56 g/h/d to close-up cows (n=68 cows). Animals were assigned to treatments as they entered the dry period over a period of 8 months. At freshening, all animals were managed similarly (i.e., no OmniGen-AF was fed during lactation). Milk production and SCS values were evaluated from monthly DHIA records and differences between treatment groups were assessed using ANOVA including treatment and parity as main effects. Addition of OG to the rations of dry cows had no effect on summit milk ($P>0.05$) which averaged 34.8 kg/day. SCS at the first DHIA test were not different ($P>0.05$) between the control (SCS=4.75) and OG-fed cows (SCS=4.45). SCS differences were detected between treatments at the second ($P=0.008$) and third ($P=0.022$) DHIA tests between control (SCS=5.31 and 4.78) and OG-fed cows (SCS=4.16 and 3.88). No differences in SCS were noted ($P=0.13$) between control and OG-fed dry cows animals at the fourth DHIA test. These results suggest that feeding OG during the dry period may be of benefit in reducing SCS in fresh cows in the subsequent lactation.

Key Words: OmniGen-AF, Somatic cells, Immunity

M20 Effect of intramammary treatment with Pirlimycin hydrochloride on antibiotic sensitivity of Gram-positive subclinical mastitis pathogens. M. D. Apparao, L. Oliveira, C. Hulland, and P. L. Ruegg*, *University of Wisconsin*, *Madison*.

The objective of this study was to evaluate the effect of intramammary treatment with pirlimycin hydrochloride on antibiotic sensitivity of subclinical mastitis pathogens. Cows (n = 254) from six WI dairy herds were screened for subclinical mastitis using the California Mastitis Test (CMT). Cows with at least one CMT positive quarter (n = 211) were randomly assigned to receive 50 mg intramammary pirlimycin in each positive quarter once daily for two consecutive days (labeled treatment) or no treatment. Duplicate aseptic milk samples were collected from all CMT positive quarters before treatment (pre-treatment) and 21 days after treatment (post-treatment). Target pathogens (Staphylococci spp and Streptococci spp.) isolated from milk samples were identified to the species level using laboratory procedures as defined by the NMC (1999). Treatment failures were defined as recovery of the same pathogen from pre and post-treatment milk samples. Cure was defined as absence of the causative pathogens or recovery of a different pathogen from the post treatment sample. Minimum Inhibitory Concentrations (MIC) were determined for Gram-positive pathogens using broth microdilution. Statistical analysis was done using PROC Univariate of SAS 9.1. Resistance was observed in 31% and 24% of pre and post treatment isolates, respectively. There was a significant difference between the pre- and post treatment MIC values for control quarters but no significant difference was observed for treated quarters. Microbiological cures were 37% and 52% for control and treated quarters, respectively. There was no association between results of antibiotic sensitivity testing and microbiological cures. Labeled usage of intramammary Pirlimycin had little short-term effect on the antibiotic sensitivity of Gram-positive subclinical mastitis pathogens but the outcomes of the sensitivity tests were poor predictors of microbiological cure of Gram-positive mastitis pathogens.

Key Words: Mastitis, Sensitivity Test, Antibiotics

M21 The effect of uterine infusion of ceftiofur in the immediate postpartum on lactation and reproduction in dairy cows. R. G. Bruno*, M. F. Sa Filho, F. S. Lima, V. J. A. Magalhaes, and J. E. P. Santos, *Veterinary Medicine Teaching and Research Center, University of California Davis*, *Tulare*.

Objectives were to evaluate the effect of intrauterine antibiotic treatment on lactation and reproduction in dairy cows. Holstein cows, 379, were randomly assigned to no treatment (CON, n=188), or a single uterine infusion (INF, n=191) of 500 mg of ceftiofur hydrochloride (Spectramast DC, Pfizer Animal Health) at 2 d in milk (DIM). Cows were presynchronized with two PGF2a injections at 37 and 51 DIM. Cows observed in estrus after the second PGF2a were inseminated, and those not in estrus received timed AI at 75 DIM. Ovaries were examined by ultrasonography at 35 and 42 DIM to determine cyclicity by the presence of a CL in at least one of the two examinations. Pregnancy was diagnosed at 38 and 66 d after the first AI. Yields of milk and milk components were measured once a month for the first 200 DIM. Survival and reproductive performance were evaluated for the first 240 DIM. Treatment did not affect ($P>0.10$) yields of milk and milk components and they were 39.5 and 39.0 Kg/d for milk, 1.39 and 1.35 kg/d for fat, and 1.18 and 1.17 Kg/d for true protein for CON and INF, respectively. Cows at high risk for uterine disease (retained placenta, milk fever, assisted calving, or twin calving) produced less ($P<0.01$) milk, fat and true protein than those at low risk. Infusion did not influence ($P=0.14$) prevalence of cyclic cows (50.9 vs 58.6%). Pregnancy rates at first AI were 30.4 and 29.7% for CON and INF ($P=0.88$). The median days open were similar ($P=0.18$) for CON and INF (170 vs 183 d). Similarly, survival time did not differ ($P=0.12$) between treatments and averaged 209 and 208 for CON and INF, respectively. Cows with subclinical endometritis had marked reduction ($P=0.02$) in first AI pregnancy rate (22.5 vs 34.3%) and extended ($P=0.01$) interval to pregnancy (189 vs 164 d). In summary, a single intrauterine infusion with 500 mg of ceftiofur did not improve lactation performance, resumption of ovulation, pregnancy rate at first AI and time to pregnancy, but cows with subclinical uterine inflammation had smaller pregnancy rate and extended interval to pregnancy.

Key Words: Ceftiofur, Dairy Cow, Reproduction

M22 Association of milk antimicrobial proteins with mastitis in dairy cattle. M. D. Person*, C. N. Person, T. D. Lester, and R. W. Rorie, *University of Arkansas*, *Fayetteville*.

Dairy cattle vary considerably in their susceptibility to mastitis, perhaps due to innate levels of milk antimicrobial proteins. This study evaluated the relationship between level of antimicrobial proteins in milk and incidence of mastitis. Milk samples were collected from 81 Holstein cows with at least three consecutive months of DHI records for somatic cell count (SCC). Composite milk samples were analyzed for the antimicrobial proteins, lysozyme, glucosaminidase, lactoferrin and lactoperoxidase. Based on SCC history and criteria established by DHIA, mastitis status of each cow was categorized as either new (SCC > 200,000 for the first time during current test date), chronic (SCC > 200,000 on two or more consecutive test dates), previous (classified as chronic previously, but current test SCC < 200,000), or no infection (SCC < 200,000). Levels of each of the milk antimicrobial proteins were then compared for cows in the four mastitis categories. Cows with new or chronic mastitis infections had higher (P = 0.0002) glucosaminidase levels (18.82 and 16.25 U, respectively) than cows with previous or no infection (9.31 and 9.26 U, respectively). Likewise, lactoferrin levels were higher (P = 0.008) for cows with new or chronic mastitis than for those with previous or no mastitis (141.78 and 99.92 versus 74.94 and 79.55 mg/L, respectively). No correlation was found between either lactoperoxidase (range of 970.77 to 1124.17 U/L) or lysozyme (range of 86.48 to 94.17 U/L) milk levels, and the mastitis categories (P = 0.6248 and 0.6830, respectively). Results suggest that glucosaminidase and lactoferrin increase in response to mastitis infection, rather than innate high levels of these proteins prevent mastitis since cows without mastitis had lower levels of these proteins than cows with infection. It is possible that a cow's ability to produce these proteins quickly in response to mastitis might reduce the severity and duration of infection.

Key Words: Mastitis, Cattle, Antimicrobial proteins

M23 Reduction of mortality and morbidity and increase in milk production in dairy livestock by plasmid-mediated growth hormone releasing hormone treatment during a period of high temperatures and humidity. P. A. Brown*, A. S. Khan, and R. Draghia-Akli, *ADViSYS Inc*, *Woodlands, TX*.

South Texas dairy farmers usually use a seasonal calving program to avoid the heat stress during the summer months. Pregnant Holstein heifers were relocated from South Dakota to Texas and calved June through September. The average temperatures and relative humidity (RH%) in South Texas resulting in high heat indexes (Table 1). In South Dakota, the climatic conditions would have been more favorable for calving (Table 1), We evaluated the potential of a plasmid-mediated growth hormone releasing hormone (GHRH) treatment to mitigate heat stress and its effects on the morbidity, mortality and milk production of treated animals and their offspring. In the third trimester of their pregnancy, thirty–two of the 52 heifers received 2.5 mg of a myogenic plasmid expressing a GHRH analog with increased half-life (pSP-HV-GHRH), while 20 heifers were used as controls. Body condition scores of treated animals vs. controls were statistically significantly better (3.55 vs. 3.35 at 60-80 days in milk (DIM), P < 0.0001), correlating with maintenance of IGF-I levels (P < 0.04) and body weights (r = 0.63). Hoof pathology was reduced with treatment. Involuntary culling of the pSP-HV-GHRH-treated animals was reduced by 40%. Mortality of heifers was significantly decreased (3% vs. 20% in controls, P < 0.003), while calves from treated heifers were 25% less likely to die at birth (18.8% vs. 25%), and from birth-to-260 days (0% vs. 20%). Overall calf mortality was reduced by 47%, P < 0.02. Calves from treated heifers were heavier at 260 days post-natal (P < 0.05). Milk production was increased by 10 to 22% (27.45 ± 0.89 kg/d vs. 23.2 ± 1 kg/d) in GHRH-treated animals compared to non-treated controls up to 300 DIM. This study demonstrates that treatment with a GHRH-expressing plasmid during the third trimester of pregnancy greatly improved the production, viability and general welfare of dairy cattle exposed to a four month period of high temperatures and humidity during late gestation and calving.

Climatic conditions in south Texas and South Dakota

	TEMP (°F)	Mean RH%	HEAT INDEX (°F)
Climatic conditions in Texas			
JUNE	90	70	106
JULY	89	65	99
AUG	91	64	105
SEPT	87	68	97
Climatic conditions in South Dakota			
JUNE	84	71	91
JULY	89	71	104
AUG	81	74	85
SEPT	76	73	76

Key Words: Dairy, Production, Heat Stress

M24 Factors affecting death rate of lactating cows in Dairy Herd Improvement herds. R. H. Miller, H. D. Norman*, M. T. Kuhn, and J. R. Wright, *Agricultural Research Service, USDA*, *Beltsville, MD*.

Frequencies of deaths of lactating cows of all breeds from 2001 through 2005 were estimated from an approximate 10% sample of US Dairy Herd Improvement herds (based on units position of herd code). Herds with <400 lactations across years were excluded. Because the trait is binomially distributed (0 = live; 1 = dead), PROC GENMOD of SAS was used. To prevent failure to converge, herds with a death rate of <0.3% were excluded. Data from 998,599 lactations (793 herds) were analyzed. The model included herd, year that lactation ended, month that lactation ended, and parity (1, 2, …, 7, ≥8). Actual death rate was 3.9% per lactation. All effects were significant ($P < 0.0001$). Chi-square values for herd, year, month, and parity were 14,826, 1,447, 209, and 6,990, respectively. Parameter estimates from GENMOD were expressed as least squares means on the binomial scale. Differences among months were small, but frequencies were slightly higher for lactations that ended in February and March. Death rate steadily increased with parity; estimates of parity differences relative to first parity were 0.8, 2.1, 3.1, 3.8, 4.3, 4.5, and 5.8% for parities 2, 3, 4, 5, 6, 7, and ≥8, respectively. Cows were 4 times more likely to die during eighth lactation and later than during first lactation. Death rates increased from 2001 to 2005; estimates of year differences relative to 2001 were 0.1, 0.5, 1.9, and 2.1% for 2002, 2003, 2004, and 2005, respectively. The sharp increase in frequency from 2003 to 2004 may have resulted from changes in regulations for disposal of downer cows.

Key Words: Death Rate, Longevity, Herdlife

M25 Identification of Monascus purpurea (red yeast) contamination of silages in the mid-West. G. Seiler[1], Y. Wang[2], and N. E. Forsberg*[2,3], [1]*Heartland Veterinary Services*, *Goddard, KS*, [2]*OmniGen Research*, *Corvallis, OR*, [3]*Oregon State University*, *Corvallis*.

The goal of this study was to identify a red mold common in silages on dairies in the mid-West and to determine whether it had potential to account for health challenges on dairy farms. This type of red mold is widely reported by dairy practitioners and can achieve sizes exceeding one foot in diameter. The red aspect of the mold is found in the interior of the ball and may not be visible from the exterior. The rationale for completing this study was that appearance of the mold coincided with appearance of several clinical signs which might be attributed to the presence of this mold. Clinical signs included multiple births, birth deformities, early embryonic mortality and toxic metritis. No changes in milk production were associated with the mold. A sample of molded silage was recovered and applied to Sabouraud culture plates containing antibiotics. Red-colored colonies were recovered and DNA was isolated using a Qiagen kit procedure. DNA from the isolated mold was amplified using pan-fungal primer sequences which specified a 550 bp fragment extending from the 16S ribosomal DNA to the 28S ribosomal subunit. The product spanned both the ITS-1 and -2 domains. The PCR product was sequenced and identified via a BLAST search. Identity of the DNA was Monascus purpurea (100% match to database), also known as "red yeast". Implications to the feeding of M. purpurea-infected silage are not certain but a review of the literature indicates that this yeast secretes citrinin (a nephrotoxin and mild hepatotoxin). Red yeast has been used as a nutritional supplement for humans to reduce cholesterol. Several cholesterol-lowering compounds are present in the yeast (monocolins) some of which are patented for cholesterol-lowering properties. These have been reported to effectively reduce liver HMG-CoA reductase activity. Others have also reported a similar mold in silages known as Monascus ruber. M. ruber lies within the same clade as M. purpurea and, like M. purpurea, produces citrinin and monocolins. Implications of Monascus spp. to livestock health warrant additional study.

Key Words: Silage, Monascus, Mold

M26 *Neotyphodium coenophialum* exposure reduces carcass mass and ribeye area, but not meat quality of growing steers grazing high versus low endophyte infected forages. K. R. Brown*[1], R. B. Cox[1], G. A. Anderson[1], G. K. Rentfrow[1], L. P. Bush[1], J. R. Strickland[2], J. A. Boling[1], and J. C. Matthews[1], [1]*University of Kentucky*, *Lexington*, [2]*Forage-Animal Production Research Unit, USDA-ARS*, *Lexington, KY*.

Steers that graze toxic endophyte-infected tall fescue before undergoing a finishing feed regimen generally have compromised growth during finishing and carcass characteristics at slaughter. The potential effects of consumption of toxic endophyte-infected tall fescue on growth, carcass quality, and postmortem parameters of growing Angus steers slaughtered off of pasture were examined. Steers were randomly allotted by weight to either a low-endophyte (LE; 6.8% infection) mixed grass fescue pasture (n = 9; BW = 266 ± 10.9 kg; 5.7 ha) or a high endophyte (HE; 62.8% infection) fescue pasture (n = 10; BW = 267 ± 14.5 kg; 5.7 ha) for at least 85 d. Shrunk BW was measured at d 0, 36, 57 and 85, and carcass parameters taken at slaughter (d 89, 91, 98, 103, or 105). ADG was greater ($P < 0.01$) for LE than for HE (0.40 vs -0.05 kg, respectively) from d 0 to 36, but no treatment difference was observed for ADG or BW for the overall 85 d period. However, BW at slaughter ($P < 0.05$; 338 vs 313 kg), hot carcass BW ($P < 0.01$; 172 vs 148 kg), and dressing percentage ($P < 0.01$) of LE steers were greater than for HE. Although 12th rib backfat thickness did not differ between LE and HE, the REA of LE steers was greater ($P < 0.01$; 60.3 vs 51.7 cm^2). No differences in pH, a^* , b^* , or shear force of REA steaks were observed between LE and HE on d 7, 14, or 21 postmortem. Although no treatment effects were observed for hue angle and chroma values, REA steak L^* values of LE steers were 1.3 to 2.0 units higher ($P < 0.04$) than for HE at d 7, 14, and 21. These results indicate that steers grazing fescue pastures with a high percentage of endophyte infection have reduced carcass mass and REA, but not indices of meat quality after 85–105 d of exposure.

Key Words: Endophyte, Fescue, Growing Steers

M27 Plasma metabolite and mineral levels of dry cows out–wintered on brassica forages. P. Gazzola*[1,2], L. Boyle[1], P. French[1], A. Hanlon[2], and F. Mulligan[2], [1]*Teagasc*, *Fermoy, County Cork, Ireland*, [2]*University College Dublin*, *Belfield, Dublin, Ireland*.

Maintaining cows on plots of brassica forages during the winter (out–wintering) is an attractive low–cost management system. The aim of this study was to evaluate plasma metabolite and mineral levels of dry cows out-wintered on kale (Maris Kestrel) and swedes (Marian) to cows housed indoors fed grass silage. Cows (n=22 per treatment) were blocked according to parity, liveweight, body condition score (BCS) and calving date, and randomly assigned to treatment. Cows were scored for BCS fortnightly. Blood samples were collected from cows within 1 wk of their expected calving date, and then again 1 wk post–calving. Non–esterified fatty acids (NEFA), beta–hydroxy butyrate (BHB) and glucose were evaluated from blood plasma to measure metabolic status. Minerals measured include calcium (Ca), phosphorous (P), magnesium (Mg), copper (Cu) and glutathione–peroxidase (GSH–Px). Data was tested for normality using SAS (9.1) and analyzed using a mixed model which included the number of days prior to/post bleeding as a factor, block as a random variable and the pre–calving values as a co–variate for post–calving values. Indoor cows had a higher BCS (3.67) prior to calving compared to cows on swedes (3.32) but not to kale (3.40; SE 0.125; $P<0.05$). No difference in NEFA, BHB or glucose was detected at either the pre or post calving stage. Out-wintered cows on kale had lower plasma Cu levels (15.0 µmol/L) compared to swedes (19.1) and indoor cows (20.2; SE 0.93; $P<0.001$). Alternatively, cows on kale had a higher ($P<0.05$) GSH–Px levels (96.4 units / g HB) compared to swedes (80.7) but not indoor cows (86.4; SE 5.4). No difference was detected for Ca, P or Mg at the pre or post stage. Metabolite levels did not indicate any negative effects of out-wintering cows on kale or swedes. Cows out-wintered on kale are at risk of copper deficiency copper.

Key Words: Brassicas, Dry Dairy Cow, Metabolic and Mineral Status

M28 Grazing high versus low endophyte-infected tall fescue reduces contractility of bovine lateral saphenous veins. J. L. Klotz*[1], K. R. Brown[2], L. P. Bush[2], J. C. Matthews[2], J. A. Boling[2], and J. R. Strickland[1], [1]*USDA-ARS, FAPRU*, *Lexington, KY*, [2]*University of Kentucky*, *Lexington*.

Cattle that graze toxic endophyte-infected tall fescue are continuously exposed to a myriad of toxins that are known to negatively affect cardiovascular tissues. As part of a larger study documenting the physiologic impact of grazing endophyte-infected tall fescue in growing cattle, the objective was to examine the vasoconstrictive activities of 5-hydroxytryptamine (5HT), α-methylserotonin (ME5HT; a $5HT_2$ receptor agonist), D-lysergic acid (LSA), and ergovaline (ERV) as affected by consumption of 2 levels of toxic endophyte infected tall fescue. Segments (2-3 cm) of the cranial branch of lateral saphenous vein were collected at time of slaughter from steers following an 89-105 d grazing period of either a low endophyte-infected (LE) mixed grass pasture (6.8% infection; n=8; BW=336±9 kg) or a high endophyte-infected (HE) tall fescue pasture (62.8% infection; n=8; BW=317±9 kg). Veins were sliced into 2-3 mm sections and suspended in a myograph chamber containing 5 mL of oxygenated Krebs-Henseleit buffer (95% O_2/5%CO_2; pH=7.4; 37°C) and allowed to equilibrate at 1 g of tension for 90 min. Increasing concentrations ($1x10^{-11}$ to $1x10^{-4}$ *M*) of 5HT, ME5HT, LSA, and ERV were administered every 15 min. Data were normalized (%) to contractile response induced by a reference dose of norepinephrine ($1x10^{-4}$ *M*) and data for each treatment were analyzed for effects of concentration and endophyte level. Maximal contractile intensities ($1x10^{-4}$ *M*) were greater ($P<0.05$) for steers grazing LE pastures than HE pastures for 5HT (73.3 vs 48.9±2.1%), ME5HT (52.7 vs 24.9±1.5%), and ERV (65.7 vs 49.1±2.6%). Onset of contractile response did not differ for 5HT and ERV, but onset of ME5HT contraction was delayed ($P<0.05$) in HE steers to 10^{-4} compared to 10^{-5} *M* in LE grazing steers. Grazing of HE pastures for 89-105 d appears to induce functional alterations in blood vessels, as evidenced by reduced contractile capacity and altered serotonergic receptor activity.

Key Words: Bovine, Fescue, Vasoconstriction

M29 Ergocryptine and ergonovine induced contractile responses in fescue naïve bovine lateral saphenous veins. J. L. Klotz*[1], B. H. Kirch[1], G. E. Aiken[1], L. P. Bush[2], B. C. Arrington[2], and J. R. Strickland[1], [1]*USDA-ARS, FAPRU*, *Lexington, KY*, [2]*University of Kentucky*, *Lexington*.

α-Ergocryptine (ERP; ergopeptine alkaloid) and ergonovine (ERN; ergoline alkaloid) are two alkaloids found in endophyte-infected tall fescue. Various alkaloids found in endophyte-infected tall fescue have been shown to elicit different effects in the grazing animal. As part of an ongoing characterization of vascular response generated by different alkaloids, the objective this study was to examine the vasoconstrictive potentials of ERP and ERN using bovine lateral saphenous veins (cranial branch) biopsied from fescue naïve cattle. Segments (2-3 cm) of vein were surgically biopsied from healthy cross-bred yearling cattle (n=6; 280 ±26 kg). Veins were trimmed of excess fat and connective tissue, sliced into 2-3 mm sections and suspended in a myograph chamber containing 5 mL of oxygenated Krebs-Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 37°C). Tissue was allowed to equilibrate at 1 g of tension for 90 min prior to initiation of treatment additions. Increasing doses of ERP or ERN ($1x10^{-11}$ to $1x10^{-4}$ *M*) were administered every 15 min following buffer replacement. Data were normalized as a percent of contractile response induced by a reference dose of norepinephrine ($1x10^{-4}$ *M*). Exposure of vein segments to increasing concentrations of ERP and ERN did not result in appreciable contractile response until $1x10^{-7}$ *M*. The two alkaloids did not differ in potency, but did in contractile intensity, with the $1x10^{-4}$ *M* response to ERN and ERP reaching maximums of 68.5 ±7.7% and 42.9 ±7.9%, respectively. The contractile response to increasing concentrations of ERN and ERP were opposite from previous evaluations of ergoline (e.g. lysergic acid) and ergopeptine (e.g. ergovaline) alkaloids using this bioassay, where the ergopeptine generated the greatest contractile intensity. This experiment demonstrates two additional causative agents that may be involved in the loss of vascular plasticity concomitant with consumption of toxic endophyte-infected tall fescue.

Key Words: Alkaloid, Bovine, Vasoconstriction

M30 Defining cutoff points for subclinical endometritis at different stages of lactation. K. N. Galvão*, S. B. Brittin, M. Frajblat, and R. O. Gilbert, *Cornell University*, *Ithca, NY*.

The objective was to define cutoff points for subclinical endometritis at different stages of lactation based on uterine cytology. Holstein cows (555), from 7 different herds, had uterine cytology performed at 21, 35, and 49±7 d in milk (DIM) and the proportion of each leukocyte type out of 200 cells counted, including epithelial and excluding erythrocytes, was recorded. Receiver operating characteristic (ROC) curves were performed at each time point to select the best cutoff point, for each leukocyte type, to predict pregnancy by 150 DIM. After selection of a cutoff point for significant predictors, time to conception was evaluated using Kaplan-Meier survival analysis. A Cox model including the effects of parity, BCS, PGF2a treatment before first AI, cyclicity by 49 DIM, plus each significant leukocyte predictor separately, was performed for 382 cows with complete information. Open cows were censored at 300 DIM. At 21 DIM, none of the leukocytes were significant predictors of pregnancy. The proportion of neutrophils (PMN) at 35 and 49 DIM tended to be a significant ($P=0.08$) predictor of pregnancy and the cutoff point was 3.0% PMN in both time points. This cutoff point resulted in significant ($P<0.01$) differences in time to conception at 35 DIM and in a tendency ($P=0.10$) at 49 DIM. Median d to conception was longer for cows with more than 3% PMN at 35 DIM (151 vs 121 d) and at 49 DIM (159 vs 131 d). For the Cox model that included PMN cutoff points, cyclicity by 49 DIM was the only significant variable and cyclic cows had increased ($P=0.01$) hazard of conceiving. The mere presence of one macrophage (LMN) was a significant ($P=0.04$) predictor at 49 DIM. The median d to conception was longer ($P<0.01$) for cows having LMN (169 vs 130 d) and resulted in lower ($P<0.01$) hazard of conceiving using the Cox model. Lymphocytes (SMN) were not significant ($P>0.15$) predictors at any stage. We infer from these data that leukocytes at 21 DIM, LMN at 35 DIM, and SMN at any stage are not diagnostic for subclinical endometritis and that the cutoff point for diagnosing subclinical endometritis should be 3% PMN at 35 and 49 DIM or the presence of LMN at 49 DIM.

Key Words: Endometritis, Dairy cow