However, in the greenhouse barn the actual number of cows within 10 m of each end was not different from expected ($p \geq .05$). These results indicate that at 30°C and at 8°C there was no difference in cow comfort based on animal distribution between the two types of barns. However, at -3°C the cows in the greenhouse barn utilized more of the floor space thereby reducing crowding and increasing cow comfort. This could be due in part to the radiant heating that occurred within the greenhouse barn but not the steel-framed barn.

Key Words: Dairy Cattle Behavior, Greenhouse Barn, Temperature

108 Factors affecting cow preference for stalls with different freestall bases in pens with different stocking rates. A.M. Wagner-Storch and R.W. Palmer*, *University of Wisconsin-Madison*.

Stall use was monitored using a closed circuit television system in a 4-row, 104-stall freestall barn. Stall status was recorded four times each day, 1400, 2000, 0400, and 0900 h, for a 9-mo period, 5/9/01 to 2/9/02. Two measures of cow preference, stall with cow lying or stall occupied (cow lying or standing in stall). The objective was to compare percentages of cow preference measures for each factor affecting use of stalls with different freestall bases. Six factors were analyzed: freestall base, distance to closest water, stall located at the end of a stall type section (END vs. NotEND), row of stalls (INTERIOR vs. EXTERIOR (wall side)), inside barn temperature (TEMP), and length of time animals exposed to freestall bases (XPOSR). One pen had a low stocking rate (LowSR, 66%), five different freestall bases [mattresses-MATR1 and MATR2, waterbeds (WATR), soft rubber mats (SRMAT), and concrete (CONC)], and cows were milked with a robotic milker. The other pen had a 100% stocking rate (100%SR), the same five freestall bases, plus a sand (SAND) freestall base, and cows were milked 2X in a herringbone parlor. Each pen was analyzed separately because of different stocking rates and cow movement control. Freestall bases were grouped with 3 to 7 stalls/section and randomly placed in each row. Results show both sides of the barn having significant differences ($P < .05$) between all freestall bases for lying and occupied. Stall usage for the 100%SR side for lying was SAND (69%), MATR1 (65%), MATR2 (57%), WATR (45%), SRMAT (33%), and CONC (23%), whereas, occupied was MATR1 (88%), MATR2 (84%), SAND (79%), SRMAT (65%), WATR (62%), and CONC (39%). Stall usage for the LowSR side for lying was MATR1 (45%), MATR2 (39%), WATR (26%), SRMAT (12%), and CONC (10%), whereas, occupied was MATR1 (60%), MATR2 (55%), WATR (34%), SRMAT (19%), and CONC (16%). Lying and occupied was highest ($P < .05$) for stalls 1) the farthest distance from water, 2) NotEND, and 3) on EXTERIOR for both sides. Lying and occupied varied for XPOSR and TEMP for each freestall base. Cows spent more time on mattress bases, but highest percentage lying was in sand bases. Cows may prefer to stand on mattress bases to concrete alleys. Location appears to impact lying in or occupying stalls.

Key Words: Freestall Base, Cow Preference, Stocking Rate

109 Rubber flooring affects behaviour of dairy cows, especially animals with hoof injuries. J Fregonesi, F Flower, T Vittie, C Tucker, and DM Weary*, *Animal Welfare Program, Faculty of Agricultural Sciences, University of British Columbia*.

Flooring surfaces can affect the comfort of animals housed indoors, such as dairy cows in free-stall barns. The impact of flooring features may be particularly important for animals with hoof injuries. In the current experiment, four groups (each of 12 cows) were alternately housed in sections of a free- stall barn with either rubber flooring or grooved concrete covering the area in front of the feed alley. Each group was followed for a 3-wk period on each surface, and individual behavioral responses were scored using 24 h time-lapse video recordings. Cows on the concrete surface spent 12.9 % of the available time standing inactive. This value increased to 15.5 % when cows were on the rubber flooring (s.e. = 0.4 %, $P<0.001$). The increased standing time on the rubber was due in part to reduced time lying in the free stall (52.1 vs. 53.7 % lying, s.e. = 0.4, $P< 0.05$). Cows spent 20% of their time feeding, regardless of the flooring. The presence and severity of hoof lesions was assessed after hoof trimming. Cows with more lesions spent less time lying in the stall ($r = -0.42$, $P<0.01$), and more time standing with their front two feet in the free stall ($r = 0.64$, $P<0.001$), particularly when on concrete flooring (floor * lesion interactions, $P< 0.05$). Cows with lesions also spent less time feeding ($r = - 0.39$, $P<0.01$), but there was no interaction between this effect and the flooring. In conclusion, flooring for dairy cattle can have important effects on their behavior, especially for those cows with injured feet.

Key Words: Flooring surface, Behavior, Welfare

110 Effects of stall surface on occupancy and postural changes in dairy cows. D. C. Lay Jr.*[1], L. L. Timms[2], and D. R. Thoreson[2], [1]*ARS-USDA-Livestock Behavior Research Unit, West Lafayette, IN,* [2]*Iowa State University, Ames, IA*.

A great deal of concern is allotted toward dairy cow comfort in order to optimize both cow welfare and milk production. Toward this end, producers are utilizing various stall surfaces in order to optimize cow comfort, while at the same time decreasing health concerns. Experiment 1 was designed to determine which surface the cow preferred. We compared 6 different free-stall surfaces: A. 2" rubber mat-Dynamatrix®; B. Sand; C. Mattress - AgroMatic®; D. Mattress - Pasture Mat #1®; E. Sand with Sand Saver; F. Mattress - Pasture Mat #2®. Our goal was to allow the cows to choose the surface on which they preferred to lie. Therefore, a free stall barn was built to include 60 free-stalls that were randomly assigned to receive one of the six stall surface types. The barn was stocked at 95% capacity. The study was conducted between July and December, during which 7 d of observations were collected during each of three separate study periods. Data collected included whether the stall was occupied and the cows body position in the stall. Experiment 2 was conducted using tie stalls which were either bedded with sand or used a mattress (Pasture Mat®) for flooring ($n = 8$/trt). Data were collected for 17 d (Rep 1), when the flooring was new, and then again, two years later, for a 22 d period (Rep 2) to record cow position. During Rep 2, data were collected for 8 days, cows were moved to the alternate flooring, and data were again collected 6 d later for an 8 d duration. Data for Exp. 1 shows a distinct seasonal affect, with cows occupying stalls in treatment B>E>C,D>F>A ($P < 0.0001$) during July, but this pattern changed to D,E,F>A,B,C ($P < 0.0001$) by late September. A very similar pattern of usage was seen for late November. Data from Experiment 2, Rep 1, found that cows on mats were more likely to be found lying ($P < 0.001$), compared to cows on sand. However, by Rep 2, we found no differences in resting behavior between treatments ($P > 0.10$). Collectively, these data indicate that cows do have a preference for the type of surface on which they lie, and that these preferences can change during the season. However, behavior of cows in tie stalls may not be fully indicative of these preferences.

Key Words: dairy, stall, comfort

Animal Health
Immunology and Management

111 Immunological and growth performance responses of finishing steers supplemented with menhaden fish oil. T. J. Wistuba*, E. B. Kegley, and M. E. Davis, *University of Arkansas, Fayetteville AR / USA*.

Inclusion of fish oil in ruminant diets may fortify the fatty acid composition of meat and modulate the immune system. Therefore, an experiment was conducted to determine the effects of supplemental menhaden fish oil on growth performance and immune function of beef calves. The 72-d study used 20 crossbred steers (438 ± 28 kg initial BW; 2 calves/pen; 5 pens/dietary treatment). Dietary treatments consisted of either a control (75% corn, 11% soybean meal, and 10% cottonseed hull) diet or the control diet with 2% fish oil. Steers were weighed on d 0, 1, 21, 42, 63, 72, and 73. On d 0, 21, 42, and 63 all calves were bled by jugular venipuncture, and *in vitro* blastogenic response of peripheral lymphocytes to phytohemagglutinin (PHA), concanavalin A (CONA) and pokeweed mitogen (PWM) was measured. Fish oil supplementation decreased ADFI (14.52 vs. 13.28 kg, $P < 0.05$, as-fed); conversely, it had no effect on ADG or gain/feed (2.08 vs. 1.89 and 0.14 vs. 0.14;

P > 0.10). There was no effect of fish oil on mitogen stimulation of isolated lymphocyte proliferation on d 0, 21, or 63. However there was a treatment x time interaction (P < 0.01) because lymphocytes isolated on d 42 from calves fed the control diet with 2% fish oil had a smaller proliferation response to stimulation with CONA (P < 0.01) and PWM (P < 0.01) and tended to have a smaller response to stimulation with PHA (P < 0.08) than lymphocytes from calves fed the control diet. Since CONA predominantly stimulates T cells, PWM predominately stimulates B cells, and PHA stimulates both T and B cells, this change indicated that fish oil supplementation on d 42 limited the proliferation of both sets of lymphocytes. Skin-fold response to intradermal injection of PHA on d 71 did not differ among treatments. Fish oil supplementation in this trial had no negative effects on growth performance or feed efficiency. Results indicated that fish oil supplementation did modulate the immune system on d 42. More research may be required to document or elicit the exact immunological changes that occur at the cellular level.

Key Words: Menhaden fish oil, Finishing cattle, Immune response

112 *In vitro* cytotoxicity of aflatoxins B1, M1, ochratoxin A and protective effects of antioxidants. A. Baldi*, E. Fusi, R. Rebucci, L. Pinotti, F. Cheli, and V. Dell'Orto, *Department VSA, University of Milan, Italy.*

The aim of this work was to investigate the capability of different antioxidants to reduce the *in vitro* cytotoxicity of aflatoxin B1 (AFB1), M1 (AFM1) and ochratoxin A (OTA). Five cell lines were used: MDCK (Madin Darby Canine Kidney), LLC-PK1 (Pig Kidney), AML-12 (Mouse Liver Hepatocytes), SKNMC (Human Neuroblastoma) and BME-UV1 (Bovine Mammary Epithelial). Since mycotoxin biotransformation comprises cytochrome P-450 mediated reactions, the cells were preliminarily tested for their cytochrome P-450 activity evaluated by ethoxyresorufin O-deathylation (EROD) method. The effects of mycotoxins on cell viability were evaluated by the cellular methylthiazoltetrazolium (MTT)-cleavage activity. AFB1, AFM1 and OTA, were added to the culture medium at different concentrations (0.07, 0.15, 0.30, 0.60, 1.25, 2.5, 5, 10, 20, 40 μg/ml) and biological effects were evaluated at different incubation times (24, 48 and 72 h). The concentration of the toxin giving 50% cytotoxicity (LC_{50}) was determined. AFB1 LC_{50} after 24 hours was: BME-UV1 =32.3μg/ml, AML-12=89.8μg/ml, SKNMC=24.5μg/ml. LC_{50} of AFM1 on BME-UV1 was 6μg/ml. OTA cytotoxicity was: BME-UV1 =.8μg/ml, AML-12 =26.5μg/ml, SKNMC=26.8μg/ml, LLC-PK1 =26.7μg/ml and MDCK =14.5μg/ml. AFB1 treatment caused a time-dependent effect with a significant decrease in LC_{50} values within 72h. No significant time dependent effect was observed in AFM1 and OTA cytotoxicity. In order to evaluate inhibitory effects of antioxidants on cytotoxicity, BME-UV1 cells, the most sensitive, were cultured in combination with LC_{50} doses of mycotoxin in presence or absence of Retinol, α-Tocopherol and β-Carotene (0.01 mM and 0.001 μM). α-Tocopherol treatment induced a significant decrease in cytotoxicity at LC_{50} dose (51.5±5.57 vs 39.2±4.21; P<0.05), no significant effects of Retinol and β-Carotene on *in vitro* cytotoxicity was found. To conclude α-Tocopherol was able to inhibit by 12% OTA,but not AFB1 and M1 cytotoxicity. Supported by MURST 2000 COFIN Baldi.

Key Words: Cytotoxicity, Mycotoxins, Antioxidants

113 *In vitro* evaluation of the oxidative damage induced by mycotoxins. E. Pavoni[1], B. Bertasi*[1], M.N. Losio[1], and A. Baldi[2], [1]*IZS, Brescia - Italy,* [2]*Department VSA, University of Milan - Italy.*

The aim of this work was to evaluate the involvement of reactive oxigene species (ROS) in the Ochratoxin A (OTA) and Aflatoxin B1 (AFB1) cytotoxicity. Madin, Darby canine kidney (MDCK), pig kidney (LLC-PK1), mouse liver hepatocytes (AML-12) and human neuroblastoma (SKNMC) cell lines were used for OTA. SKNMC, AML 12 and bovine mammary epithelial (BME-UV1) cell lines were tested for AFB1. Cells were incubated with different concentrations of OTA (0, 5, 10 and 20 μg/ml) and AFB1 (0, 2.5, 5, 10, 20 and 40 μg/ml). ROS production was measured at different times (24, 48 and 72 h) by dichlorofluorescein (DCF) method. OTA-induced ROS production in MDCK and LLC-PK1 cells was significantly higher (P<.05) than the one induced in the nervous SKNMC cell line. ROS production by OTA was increased in a dose dependent manner in MDCK and SKNMC cells. A time dependent effect was observed for OTA-treated cells, with a significant (P<.05) increase in ROS production within 48 h. No significative (P>.05) variation of ROS production was observed in OTA-AML 12 treated cells. Quantitative analysis of ROS production in OTA-treated (20 μg/ml) cells is summarized in table. No significant (P>.05) variation of ROS production was observed in AFB1-treated cell lines. To conclude, the results show that OTA cytotoxicity is strongly related to oxidative damage, mainly detected in renal cell lines, derived from the *in vivo* target tissue. Conversely, results suggest that AFB1-cytotoxicity does not appear to be direcly associated to oxidative mechanism detected by ROS test. Supported by MURST Cofin 2000 - Baldi grant.

	ROS, fluorescence units±SE		
	24h	48h	72h
MDCK	6369±6.1^a	9658±6.4^b	9976±7.2
LLC-PK1	5187±17.4^a	8662±13.4^b	8995± 17.6
SKNMC	4021±6.7^a	8124±4^b	9021± 9.3
AML 12	1113±36	—	—

$^{a\,b}$ Means on the same row with different superscripy are different (P<.01).

Key Words: mycotoxins, ROS, cytotoxicity

114 Effect of intravenous infusion of increasing amounts of lipopolysaccharide on plasma macro-mineral, vitamin D, and protein concentrations in lactating dairy cows. M. R. Waldron*[1], B. J. Nonnecke[2], T. Nishida[1], R. L. Horst[2], M. R. Foote[3], and T. R. Overton[1], [1]*Cornell University, Ithaca, NY,* [2]*National Animal Disease Center (NADC), USDA ARS, Ames, IA,* [3]*Iowa State University, Ames, IA.*

Four multiparous lactating cows (175-220 DIM) were used in a 4x4 Latin square design to assess the effects of increasing doses (0.0, 0.5, 1.0, 1.5 μg/kg BW) of lipopolysaccharide (LPS; *E. coli* 0111:B4) on plasma concentrations of macro-minerals, vitamin D, and protein. Treatments were dissolved in 100 ml of sterile saline and infused intravenously over a period of 100 min. Blood was sampled immediately before infusion (0 h), at 60-min intervals for 8 h, and at 24 and 48 h postinfusion. Vitamin D metabolites were analyzed in 0, 2, 6, 24, and 48 h samples only. Parallel response trends were observed for all doses of LPS administered; therefore, LPS response data were combined and analyzed as either 0 μg/kg BW LPS (CTL) or all doses of LPS combined (TRT). Plasma calcium (9.52 vs. 8.57 mg/dl, SE=0.36) and phosphorus (5.81 vs. 4.26 mg/dl, SE=0.34) concentrations decreased after LPS infusion ($P < 0.05$ and $P < 0.005$, respectively), but differences in plasma magnesium concentrations were not significant (2.16 vs. 2.21 mg/dl, SE=0.04; $P > 0.20$). Plasma 25-OH vitamin D_3 (80.4 vs. 75.9 ng/ml, SE=3.5) was not different ($P > 0.20$), whereas 1,25-$(OH)_2$ vitamin D_3 (50.9 vs. 42.1 pg/ml, SE=3.8) tended to decrease ($P = 0.08$) after LPS infusion. Differences in plasma protein concentration (7.72 vs. 7.55 g/dl, SE=0.10) after LPS administration approached a trend ($P = 0.17$). These data suggest that the inflammatory response elicited by LPS alters plasma macro-mineral and vitamin D concentrations that are important for calcium homeostasis and metabolic health of lactating dairy cows.

Key Words: Lipopolysaccharide, Minerals, Vitamin D

115 Endotoxin (LPS) administration uncouples growth hormone (GH) regulation of insulin-like growth factor-1 (IGF-1): decreased signal transduction STAT-5b phosphorylation. T. H. Elsasser* and S. Kahl, *USDA-ARS, Growth Biology Laboratory, Beltsville, MD.*

We employed immunohistochemical assessment of phosphorylation of STAT-5b and the induction of an endogenous, proinflammatory cytokine-driven inhibitor of JAK-2 kinase, CIS-SOCS-3, to determine whether signal transduction processes are affected by LPS challenge (to mimic an immune stress) when in the host response to stress GH regulation of IGF-1 is lost. Liver tissue was obtained by transcutaneous biopsy 6 h after the administration of LPS (*E. coli* 055:B5, 3 μg/kg BW, i.v. bolus) or saline to heifers (mean BW 334 ± 12 kg, synchronized to diestrus). The 6-h point was chosen based on previous data showing that this was the earliest time after LPS that plasma IGF-1 was consistently and significantly decreased. STAT-5b phosphorylation capacity was tested by administering recombinant bovine GH (Monsanto,

Co., Inc., 50 mg/heifer, i.v. bolus, n = 8) 5 to 8 min prior to obtaining the biopsy cores. Fixed and deparaffinized biopsy tissues were incubated with primary antibody for phosphorylated STAT-5b (pSTAT-5, Tyr-PO_4-694, B-D Transduction Labs) and CIS-SOCS-3 (IBL Co., Ltd). Antigen visualization on slides was accomplished by avidin-biotin complex enhanced horseradish peroxidase deposition of diaminobenzidine. Plasma IGF-1 was measured by RIA. Plasma IGF-1 concentrations in LPS heifers were depressed 32% at 6 h ($P < 0.05$) compared to controls. The percentage of hepatocytes staining for pSTAT-5 increased 5-fold ($P < 0.05$) after GH challenge in heifers not receiving LPS. In contrast, pSTAT-5 was not detected in tissue from LPS-challenged heifers. CIS-SOCS-3, which inhibits the JAK-2 kinase phosphorylation of STAT-5b, was present in control heifers only in Kupffer cells and unaffected by GH challenge. LPS challenge resulted in intense increased staining in the Kupffer cells but also in hepatocytes. The data suggest that perturbed phosphorylations in the GH signal transduction pathway contribute to the uncoupling of GH control of IGF-I.

Key Words: Growth hormone, Signal transduction, Endotoxin

116 Growth, feed intake, and acute phase protein response of two genotypes and genders to an acute challenge with lipopolysaccharide (LPS). J. W. Frank*[1], R. W. Ratliff[1], G. L. Allee[1], R. D. Boyd[2], and M. A. Mellencamp[2], [1]*University of Missouri - Columbia,* [2]*PIC USA, Inc.*

This study was conducted to evaluate the response of two genetic lines of pigs to increased levels of endotoxin challenge. In addition, C reactive protein was measured to determine its potential use as a biomarker of immune response. Thirty-six pigs (BW = 21.3 kg) were allotted by genotype (Line 1 and Line 2) and sex (B and G) to one of three LPS treatments. Lines 1 and 2 were generated by mating two dam lines to one sire line. Treatments were an i.m. injection of 0 (LPS-0), 25 (LPS-25), or 50 ug LPS/kg BW (LPS-50). Pigs were penned individually and weighed on d 0 and 7. Feeders were weighed daily to establish baseline feed intake (ADFI -48 to 0 h relative to injection). Acute feed intake response (AFIR) is ADFI 0 to 48 h after injection divided by baseline intake. Serum was harvested at time 0 and 48 h after injection to measure C reactive protein. LPS-0 pigs grew faster and consumed more feed than LPS-25 or LPS-50 pigs (0.79 vs. 0.51 and 1.15 vs. 0.93 kg/d, respectively; $P<0.001$). There was also a sex x genotype x LPS interaction for ADG ($P<0.04$). Line 1 B and Line 2 G had similar decreases in ADG at LPS-25 and LPS-50 compared to LPS-0, whereas Line 1 G and Line 2 B had decreased ADG with increased LPS dose. A sex x genotype x LPS interaction was also observed for AFIR ($P<0.05$). Line 1 B and Line 2 G responded similarly to both LPS doses, while Line 1 G and Line 2 B had decreased AFIR with increased LPS dose. Three of twelve B and no G died following the LPS challenge ($P<0.10$). Line 1 had higher baseline C reactive protein concentrations than Line 2 (171 vs. 83 ug/ml; $P<.05$). No differences were observed for C reactive protein 48 h after challenge. A net change in C reactive protein of #71 and 19 ug/ml resulted for Line 1 and 2, respectively ($P<0.11$). Based on these data, C reactive protein may not be a good indicator of immune stimulation as a result of an LPS challenge. Additionally, barrows and gilts within genetic lines may respond differently to an endotoxin challenge.

Key Words: Pigs, LPS, Genotype

117 Effect of dexamethasone (DEX) and insulin-like growth factor-1 (IGF-1) on pokeweed mitogen (PWM)-induced lymphoproliferation and immunoglobulin production. A.L. Delgado*[1], T.H. Welsh, Jr.[2], and J.C. Laurenz[1], [1]*Texas A&M University-Kingsville,* [2]*Texas A&M University-College Station.*

This study investigated the effect of IGF-1 and the synthetic glucocorticoid, DEX, on PWM-induced lymphoproliferation and immunoglobin M (IgM) production in vitro. Blood was obtained from male, crossbred pigs (n=3 pigs/experiment; 35-45 days of age) and lymphocytes isolated by density gradient centrifugation. Lymphocytes were plated in 96-well plates at 1 X 10^5 cells/well in DME/F12 containing 10% fetal bovine serum, 2 mM L-glutamine, 10 uM 2-mercaptoethanol, PWM (0 to 1000 ng/mL), DEX (0 to 10^{-6} M) and/or IGF-1 (0 to 200 ng/mL). Cultures were incubated for 96 h and proliferation determined using the CellTiter proliferation assay (Promega, Madison, WI). In replicate cultures, supernatants were removed and IgM production determined using an ELISA. As expected, PWM induced dose-dependent increases ($P<.01$) in proliferation and IgM production with maximal effects occurring at 12.5 and 160 ng/ml, respectively. DEX dose-dependently inhibited ($P<.01$) PWM-induced (3.1 and 12.5 ng/mL) proliferation with 74 and 50% inhibition occurring at 1 x 10^{-7} M. Similarly, DEX dose-dependently inhibited ($P<0.01$) PWM-induced (20 ng/mL) IgM production with maximal 50% reductions in IgM production occurring at 10^{-6} M. In contrast, in cultures treated with higher concentrations of PWM (>80 ng/mL) DEX (10^{-7} M) augmented IgM production. IGF-1 did not affect ($P=.21$) PWM-induced lymphoproliferation. Similarly, IGF-1 did not affect ($P>.05$) IgM production at higher levels of PWM (160 and 320 ng/mL), but did dose-dependently increase ($P<.05$) IgM production at lower concentrations of PWM (80 ng/mL) with maximal effects at 25 ng/mL IGF-1 (2837 ± 740 vs 4725 ± 907 ng/mL IgM, respectively). In addition, IGF-1 provided modest protection ($P<.05$) against DEX-mediated suppression of PWM-induced lymphocyte proliferation and IgM production and enhanced ($P<.05$) the DEX-mediated augmentation of IgM production at higher concentration of PWM (>80 ng/mL). Collectively, these results demonstrate that IGF-1 can enhance lymphocyte function and reduce the suppressive effects of glucocorticoids suggesting that IGF-1 may be useful to modulate immune function in young pigs.

118 Toxicity of ergovaline on Caco2 cells as assessed by MTT, alamarBlue, and DNA analysis. N.W. Shappell*, *ARS-USDA.*

The exact mechanism of fescue toxicity has yet to be established, but it has been associated with an inability to thrive. Ergovaline has been identified as the major ergopeptine alkaloid associated with fungal infections of tall fescue. The gastrointestinal (G.I.) toxicity of ergovaline was evaluated in a Caco2 cell system which mimics the G.I. epithelium. Cells were plated at 1 X 10e3 cells per well (96 well plates) in DMEM, with 9.1% fetal bovine serum. Ergovaline in methanol was tested at 1 X 10 e-4 to -11M beginning on da 1, 8, and 18 in culture. Acute and chronic toxicity was assessed after 24 and 72h of exposure to ergovaline. Treatment periods were chosen to study undifferentiated, semi-differentiated, and completely differentiated cells. Cell toxicity was assessed by MTT (thiazolyl blue) reduction (mitochondrial succinate dehyrdogenase activity), alamarBlue assay (cytochrome oxidase activity), and total DNA. Undifferentiated cells were sensitive to 0.1 mM ergovaline after acute exposure(74%, 56%, and 53% of control values for MTT, alamarBlue, and DNA respectively, $P \leq 0.0001$) or chronic exposure (6%, 13%, and 0.3% as indicated above). By da 11 in culture, cell toxicity to ergovaline had decreased, and after 24h of exposure a ∼ 12% increase in MTT was seen (1 nM, 10 nM, and 0.1 mM - $P \leq 0.02$, 0.01, and 0.0002, respectively). After 72h of exposure to 0.1 mM ergovaline, all three parameters were reduced ∼20 to 30% (MTT 26%, $P \leq 0.0001$, alamarBue 31%, $P \leq 0.0001$, and DNA 16 %, $P \leq 0.006$). Fully differentiated cells exhibited increased MTT activity (∼20%) again, after 24h exposure at all concentrations except 0.1 nM, while alamarBlue activity was decreased at all concentrations (∼15%). A ∼15% elevation in MTT was found after 72h exposure from 1 nM to 10 μ M ergovaline, while both MTT and alamarBlue activity decreased ∼13% with 0.1 mM ergovaline. No change in DNA was found until 72h of exposure, when DNA was reduced ∼12% over most concentrations. These findings indicate variable sensitivity of G.I. cells to ergovaline, dependent on the state of differentiation. Ergovaline (0.1 mM) is toxic to undifferentiated cells, while differentiated cells are much more resistant to its toxic effects.

Key Words: ergovaline, toxicity, DNA

119 Effects of segregated early weaning on systemic and enteric T lymphocyte subpopulations in pigs. D. C. Brown*, C. V. Maxwell, M. E. Davis, G. F. Erf, and S. Singh, *University of Arkansas, Fayetteville.*

Systemic and gut T lymphocyte subpopulations were compared between pigs reared in on-site and off-site facilities. Crossbred pigs were weaned at 19 ± 2 days of age and allotted to one of two facilities based on initial BW (5.94 ± 0.07 kg on-site; 5.87 ± 0.07 kg off-site). Pigs in each group were divided into four weight groups, allotted into equal subgroups (2 or 3 pigs/pen) and stratified based on sex and litter. All pigs received common diets and were managed similarly. On d 1, 3, 11, and 24 post-weaning, one pig from each weight group was randomly sacrificed (n=4 per facility) and blood mononuclear cells and intraepithelial lymphocytes (IEL) were isolated for single and double stain analysis using flow cytometry techniques. Compared to other days sampled the percentage

of blood T lymphocytes were greater ($P \leq 0.01$)on d 24 post-weaning, the percentage of CD4+ cells was higher ($P \leq 0.05$) on d 1 post-weaning, and double positive cells (CD4+CD8+) were lower ($P \leq 0.10$) on d 3 post-weaning in pigs from both facilities. On-site pigs had a higher ($P \leq 0.10$) percentage of CD4+ IEL at d 1 post-weaning compared to off-site pigs on d 1, 3, 11 and 24 post-weaning. After d 1post-weaning, the percentage of CD4+ IEL decreased ($P \leq 0.05$) in on-site pigs and the percentage of CD8+ IEL in the off-site pigs increased ($P \leq 0.05$). The percentage of CD8+ IEL then decreased ($P \leq 0.05$) on d 24 postweaning for off-site pigs. The percentage of CD8+ IEL was higher ($P \leq 0.05$) for the off-site pigs on d 3 and 11 postweaning than on-site pigs on d 3 post-weaning. Off-site pigs had a higher ($P \leq 0.05$) percentage of IEL positive for the gamma/delta T cell receptor on d 1post-weaning compared to on-site pigs on d 1, 11 and 24 post-weaning and off-site pigs on d 3 and 11 post-weaning. Double positive and CD8+CD4- IEL were lower ($P \leq 0.05$) on d 1 and 3 post-weaning than d 11 and 24 post-weaning. These data suggest that the differing nursery environment alters systemic and enteric T cell subpopulations.

Key Words: T cell subpopulations, Intraepithelial lymphocytes, Nursery pigs

120 Bacterial colonization of the neonatal pig gut is altered by enteral versus parenteral feeding. R. B. Harvey*[1], K. Andrews[1], R. E. Droleskey[1], K. V. Kansagra[2], B. Stoll[2], D. G. Burrin[2], K. J. Genovese[1], T. S. Edrington[1], R. C. Anderson[1], and D. J. Nisbet[1], [1]*Food and Feed Safety Research Unit, USDA-ARS, College Station, TX USA,* [2]*USDA-ARS-Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX USA.*

Sepsis in preterm human infants has been associated with total parenteral nutrition (TPN), and it has been suggested that sepsis may occur due to translocation of gut luminal bacteria. In this study, we compared the effects of TPN versus enteral (ENT) feeding on intestinal bacteria translocation. Newborn, colostrum-deprived pigs (<24 h old) were fitted with intravenous catheters and divided into two groups. One group (n = 13) received TPN through intravenous feeding and the second group (n = 14) was orally fed a commercial pig milk-replacer (ENT); the nutrient intake did not differ between groups. After 7 d of treatment, pigs were euthanized and jejunum (J), ileum (I), cecum (C), liver, spleen, mesenteric lymph node, and blood were evaluated using restrictive media and serial dilutions to determine the presence and concentrations of enteric bacteria. ENT pigs had increased bacterial concentration and diversity of intestinal tract enterics compared to TPN pigs. The majority of positive samples in the TPN group were cultured from the C, with infrequent isolation from the J or I. Bacterial genera commonly isolated from the intestinal tract included *Enterococci*, *Pediococci*, *Enterobacter*, *Staphylococci*, *Klebsiella*, *Citrobacter*, and *Clostridia*. The ENT group had 1/14 positive for *Clostrium difficile* toxin A compared to 5/13 for the TPN group. Translocation of bacteria from the intestinal tract to tissues or blood was similar (8/14 ENT, 6/13 TPN) between groups. We conclude that ENT pigs have increased concentrations and diversity of intestinal bacteria compared to TPN pigs. TPN pigs may be at higher risk for colonization by C. difficile.

Key Words: Enteric bacteria, Colonization, Parenteral feeding

121 Development of a novel paradigm for the real-time monitoring of bacterial pathogenicity in swine. S. Willard*[1], P. Ryan[1], R. Bailey[2], M. Lawrence[2], C. Estill[2], S. Gandy[1], and D. Lay[3], [1]*Dept. of Animal and Dairy Science, Mississippi State University, Mississippi State, MS,* [2]*College of Veterinary Medicine, Mississippi State University, Mississippi State, MS,* [3]*USDA-ARS, West Lafayette, IN.*

The objective of this study was to evaluate whether photonic reporters (e.g., luciferase) incorporated into relevant Salmonella strains could be used as indicators of bacterial infection (both in incidence and severity) within the living pig. To develop this paradigm, neonatal pigs (n = 12) were removed from the sow between 1 and 7 days of age, and placed on an antibiotic-free milk replacer for the duration of the trial. Pigs were anaesthetized for whole body imaging using a Telazol-xylazine-ketamine cocktail, placed in dorsal recumbency and the ventral surface of non-infected pigs imaged (10 min) using a photon counting camera (background image). Following this, one of two experiments were conducted. In Experiment 1, a dose response study was conducted in which pigs were challenged via esophageal intubation with increasing doses of a Salmonella anatum bacterial isolate engineered to express the luciferase protein (Salmonella-lux; 1.5, 4.5 and 7.5 billion CFU). This was done to determine the level of photonic activity (relative units; RU) detectable through the stomach and ventral surfaces of the living pig. In Experiment 2, pigs were imaged (10 min accumulation of photons) pre- and post-challenge (Time 0: Salmonella-lux at 2 billion CFU), the pigs were then recovered and re-imaged at 24, 48 and 72 h post-Salmonella-lux challenge. A 17.7-fold increase ($P < .05$) in photonic emissions from the addition of 1.5 billion CFU (14,882.7 ± 3,965.5 RU) to 7.5 billion CFU (263,596.8 ± 95,905.6 RU) of Salmonella-lux was noted in the dose response study. Detectable photonic emissions in the stomach were highest ($P < .05$) immediately post-infusion (2.5-fold above pre-challenge), while photonic emission from the lower gastrointestinal tract were highest ($P < .05$) at 24 h (43.7-fold above pre-challenge). By 72 h post-challenge, no difference ($P > .10$) between the 72 h post-challenge and pre-challenge (background) photonic emissions were noted from infected pigs. In summary, photon-emitting bacteria can be detected through the ventral surfaces of the neonatal pig, providing a unique model from which to assess bacterial pathogenesis in the living pig.

Key Words: Salmonella, Swine, Biophotonics

122 Effect of ketoprofen, local anesthesia, and caudal epidural anesthesia during castration of beef cattle. S. T. L. Ting*[1,2], B. Earley[1], J. M. L. Hughes[2], and M. A. Crowe[2], [1]*Teagasc, Grange Research Centre, Dunsany, Co. Meath,* [2]*Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4, Ireland.*

The effect of burdizzo castration alone or in combination with ketoprofen (**K**), local anesthesia (**LA**), or caudal epidural anesthesia (**EPI**) on cortisol, acute phase proteins, immune function, and performance of beef bulls was determined. Fifty Holstein x Friesian bulls (13 mo of age; BW = 307 5.3 kg) were assigned to one of five treatments: 1) untreated control (**C**); 2) burdizzo castration (**BUR**); 3) BUR following K (3 mg/kg BW i.v.; **BUR + K**); 4) BUR following LA (8 mL into each testis followed with 3 mL s.c. along the incision line of 2% lidocaine HCl; **BUR + LA**); and 5) BUR following EPI (0.05 mg/kg xylazine HCl and 0.4 mg/kg lidocaine HCl as caudal epidural; **BUR + EPI**). The area under the plasma cortisol against time curve was lower ($P<.05$) in BUR + K than in BUR, BUR + LA and BUR + EPI animals. On d 1 post treatment, plasma haptoglobin (**HAP**) levels were higher ($P<.05$) in the castration groups except in BUR + K. On d 3 and 7, HAP levels were higher ($P<.05$) in BUR + LA and BUR + EPI than in C. Plasma fibrinogen (**FIB**) levels were higher ($P<.05$) on d 3 in all castration groups than in C. On d 7, FIB levels remained elevated ($P<.05$) in BUR + LA compared with C and other castrated groups. On d 1 and 3, Con A-induced interferon-γ production was lower ($P<.05$) in BUR + LA than in C, but was not different ($P>.05$) to BUR and BUR + EPI. ADG from d -1 to 35 was lower ($P<.05$) in BUR, BUR + LA and BUR + EPI, but not in BUR + K compared with C. In conclusion, burdizzo castration increased plasma cortisol and acute phase proteins, suppressed immune function and reduced growth rates. LA prolonged the increase in acute phase proteins and suppression of cell-mediated immunity. K was more effective in reducing cortisol than LA or EPI. Systemic analgesia with a non-steroidal anti-inflammatory drug was more effective in minimising inflammatory responses associated with castration than local or epidural anesthesia.

Key Words: Cattle, Castration, Epidural Anesthesia

123 Effect of forage condensed tannins on gastrointestinal parasite infection in grazing wether goats. B.R. Min*[1], W. Pomroy[2], S.P. Hart[1], and T. Sahlu, [1]*E (Kika) dela Garza Institute for Goat Research, Langston University, OK 73050, USA,* [2]*Veterinary and Biomedical Science, Massey University, Palm/N, NZ.*

The objective of this study was to evaluate effects of dietary condensed tannins (CT) in Serica lespedeza (SL; *Lespedeza cuneata*; 4.6% extractable CT/kg DM) on total fecal egg output (TFEO; eggs/d) and stage of larvae development compared with non-CT-containing forage (rye/crabgrass (RC); 0.6 g extractable CT/kg DM) in grazing wether goats. A grazing trial (cross over) involving 11 naturally parasite-infected (>1,200 eggs/g) goats (47±3.3 BW) were randomly selected 1 mo after Ivermectin treatment (0.2 mg/kg BW) failure. Larval culture of pre-treatment feces showed that 86-97% of larvae were *Haemoncus*, with the remainder being *Trichostrognylus* and *Ostertagia*. Periods lasted

15 d, with fecal samples taken on d 0, 5, and 15. The number of eggs/g feces were determined by a modification of the McMaster technique. Larvae were cultured for 10 d at 27°C by placing 20 g of fresh feces inside a small glass container within a larger container holding free water (20 ml) to maximize humidity. Larvae were collected using a modified Baermann′s procedure and counted. Mean fecal egg counts (2,722 *vs* 1,162 eggs/g) and TFEO (173 *vs* 45 x 104 eggs/d) were lower ($P<0.01$) for RC *vs* SL. Larvae development from eggs to infective stage of larvae (L3) by 15 d was 88% (3,432 *vs* 421 larvael/20 g feces; $P<0.001$) lower for RC *vs* SL. In conclusions that CT in forages such as SL may reduce pasture contamination with infective larvae and be a valuable tool for parasite control.

Key Words: condensed tannins, gastrointestinal parasite

124 Minimally invasive diagnostic procedures and measures of test performance in BVD infected cattle. N Cook*[1], A Schaefer[1], S Tessaro[2], D Deregt[2], G Desroche[3], and P Dubeski[4], [1]*Agriculture and AgriFood Canada,* [2]*Animal Disease Research Institute,* [3]*Public Works, Edmonton,* [4]*Alberta Agriculture Food and Rural Development.*

The objective was to compare minimally invasive measures for diagnosis of bovine viral diarrhea (BVD). Heifers (n = 10) were infected with 2 * 10^7 TCID type 2 strain 24515A BVD virus, with 5 seronegative animals as controls. Infrared thermographic (IRT) images of the eye were recorded each day for 21 days. Saliva samples were collected every third day. Clinical assessment was conducted every day and utilized a 4-symptom classification system, each on a scale of 0 # 4. Scores for clinical assessment identified infected animals from Day 9 post-infection. Infrared images of the maximum eye temperature were statistically higher for infected animals by Day 7 post#infection ($p<0.001$) and remained statistically different from control levels up to Day 11 ($p<0.05$). Salivary cortisol levels were elevated in infected animals by Day 7 post-infection ($p<0.004$) and remained so until Day 14 ($p<0.02$). Comparisons between tests were made using measures of test performance. Clinical assessment score = 2 had a maximum test efficiency of 50%. Maximum test efficiency for IRT eye temperature was 60.3% at 32.4C and for salivary cortisol was 83% at 8 nmol/L. Diagnostic tests were compared at referent levels giving maximum test efficiency. IRT eye temperature provided a test sensitivity of 98.5%, compared to 87.8% for salivary cortisol and 19.7% for clinical assessment. However, IRT test specificity was 10% compared to 100% for clinical assessment and 47.2% for salivary cortisol. Risk factor (RF) was represented as the ratio of false negative to true negative test results. Clinical assessment RF = 1.05 was higher than for salivary cortisol (RF = 0.35) and IRT eye temperature (RF = 0.2). Minimally invasive diagnostic tests are capable of earlier diagnosis of BVD infection than clinical assessment. Additionally, measures of test performance vs. test referents can be used to objectively manage risk associated with diagnostic testing.

Key Words: Bovine Viral Diarrhea, Infrared Thermography, Salivary Cortisol

Breeding and Genetics
Genetic Prediction and Selection in Cattle

125 Detection and adjustment of abnormal test day yields. G. R. Wiggans*, P. M. VanRaden, and J. C. Philpot, *Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

Milk, fat, and protein yields on test-day (**TD**) were investigated to develop a method to detect abnormal yields for exclusion from calculation of lactation yields as an alternative to using producer-reported sick code. A TD yield was considered to be abnormal if it was < 60 or > 150% of predicted TD yield. These limits were selected to identify the low and high 1% of the distribution. The new cow specific upper limit replaced the previous limit of 123 kg milk. Predicted TD yield was calculated from previous TD yield adjusted by a daily change (slope) based on days in milk (**DIM**), DIM^2, previous TD yield, and interaction between DIM and previous TD yield. To accommodate changes in slope at peak yield, separate coefficients were estimated for ≤50 and >50 DIM. Because yield from more than one TD may be abnormal, the last previous normal TD yield was retained for assessing subsequent TD yield. To be identified as abnormal, TD yields that were abnormal based on the previous TD yield also were required to be abnormal when checked against a prediction based on last previous normal TD yield and subsequent TD yield. Herd mean was used when fewer than three TD were recorded and to determine an acceptable range for component percentages. The procedure was applied in reverse for the first test with the predicted yield calculated from the second TD. When the outlier detection method was applied to >93 million TD records of cows that calved in 1997 or later, 1.8% of milk, 3.4% of fat, and 1.9% of protein TD yields were identified as abnormal. The higher percentage of outliers for fat reflects its greater variability. Lactation yields were calculated after replacing abnormal TD yields with a floor or ceiling of 60 or 150% , respectively, of yield predicted from the last previous normal TD. For cows that had lactation records with one abnormal TD yield or more and a subsequent lactation record, the correlation between consecutive lactations increased from 0.692 to 0.693 for milk (561,063 lactations), from 0.653 to 0.660 for fat (951,387 lactations), and from 0.686 to 0.694 for protein (488,653 lactations). The outlier detection method identifies both high and low abnormal TD yield and improves the correlation between consecutive lactation yields.

Key Words: Test-day yield, Abnormal yield, Outlier detection

126 Examination of methods to correct for preferential treatment among AI bull dams. N.R. Zwald* and K.A. Weigel, *University of Wisconsin - Madison, Madison, WI.*

The objective of this study was to quantify the magnitude of preferential treatment among dams of AI sires, and to identify methods that can be used to reduce selection errors. Accurate selection of the most elite animals from the population is essential in maximizing genetic progress. Preferential treatment in some dairy herds has made this selection increasingly complex. To examine this problem, test-day data from dams of 1,972 AI bulls born between 9/1/94 and 9/1/97 and their herd-mates were obtained. These data included 641 herds, 188,031 cows, and 1,634,441 test-day records from cows calving between 1/1/90 and 12/31/97. Data were analyzed using a standard sire model, sire-maternal grandsire model, animal models with various assignment of management group classes, and test-day models. Breeding values of bull dams for milk, fat, and protein were regressed on daughter yield deviations of their sons to determine the magnitude of preferential treatment, and to determine which model best corrects for preferential treatment in bull dam herds. Correlations between initial sire PTA and pedigree values were calculated for AI sires receiving their first evaluation during 1998. Correlation between initial PTA was calculated for the following pedigree information at bull birth date: parent average (0.37), sire PTA milk (0.42), dam PTA milk (0.29), maternal grandsire PTA milk (0.36), sire-MGS PTA milk (0.44). This research shows that considerable preferential treatment occurs in many herds, which can lead to incorrect selection of bull dams and inflated parent averages on many young sires.

Key Words: Preferential treatment, Test-day model

127 Data sub-setting and assessment of bias in estimation of genetic correlations among countries. H. Jorjani*, *Interbull Centre, Dept. of Anim. Breed. and Genet., Swedish University of Agricultural Sciences.*

The objective of this study was to assess the effects of different data sub-settings for simultaneous estimation of genetic correlations among a large number of countries. For this purpose dairy cattle populations in 6 countries, with heritability values of 0.15-0.40 and genetic correlations between 0.50 and 0.90, were simulated. Exchange of selected young bulls among countries started at generation 2 and continued until the end of experiment at generation 10. Two different population structures, small and mixed, with 20 bulls and 2000 cows, and 20-640 bulls and 2000-64000 cows per generation, respectively, were simulated. For estimation