Late-Breaking Original Research Session

LB1 Fertility may depend on conceptus-derived signals in lactating dairy cows. Bethany E. Liebig*[1], Milton G. Thomas[2], Kevin D. McSweeney[3], Hana Van Campen[1], Jeanette Bishop[1], and Thomas R. Hansen[1], [1]*Department of Biomedical Sciences, Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO,* [2]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [3]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO.*

Infertility and early embryonic mortality (EM) negatively affects cattle industries. Most fertility traits such as daughter pregnancy rate (DPR) and services per conception (SPC) are lowly heritable ($h^2 \leq 0.10$). Interferon tau (IFNT) is a cytokine released by the conceptus during trophoblast elongation and differentiation that decreases prostaglandin $F_{2\alpha}$ (PGF) release from the endometrium and prevents lysis of the corpus luteum (CL), thus maintaining production of progesterone. Embryo survival depends on robust IFNT release and action through inducing IFN stimulated genes (ISGs). We hypothesized that DPR and SPC could be used to select for viable embryos with greater IFNT production. Freshening dairy cows (n = 86) were sorted by genome-enhanced predicted transmitting ability (gPTA-DPR; Clarifide; Zoetis) and SPC into low fertile (LF; −2.3 gPTA-DPR; 3.7 SPC) and high fertile (HF; −1.3 gPTA-DPR; 1.4 SPC) groups. Cows were assigned to HF nonpregnant (NP), LF pregnant (LP) or HF pregnant (HP) groups (n = 7 each). Cows were estrous synchronized and time-artificially inseminated to a HF bull (+1.8 DPR). Sixteen days later, embryos were collected and typed as viable or EM based on morphology and length. Data were analyzed using protected ($P < 0.05$) t-test. There were no differences in serum progesterone concentrations (using ELISA), days in milk, number of lactations or calving date between groups. The gPTA-DPR was negatively correlated ($r = -0.52$; $P < 0.05$) with SPC. The HP cows had more viable (n = 5) embryos based on morphology than the LP cows (n = 3). Viable HP embryos were longer ($P < 0.05$) than LP embryos. Uterine flushing (UF) IFNT concentrations (using Western blot) were greater ($P < 0.05$) in HP compared with LP cows. Concentrations of IFNT in UF from viable embryos were positively correlated with gPTA-DPR ($r = 0.57$, $P < 0.05$) and explained ~46% ($R^2 = 0.46$) of the variation in gPTA-DPR. Peripheral blood mononuclear cell ISG15 mRNA concentrations (using RTqPCR) were upregulated ($P < 0.05$) in HP compared with LP cows. In conclusion, selection of dairy cows combining gPTA-DPR and SPC may improve fertility through increased production and action of IFNT. Funded by Zoetis Inc.

LB2 Low fertility in postpartum dairy cows with subclinical endometritis (SCE) is not explained by failed ovarian response to a timed AI (TAI) protocol. Roger Molina-Coto*[1], Tyler J. Stratman[1], Monica O. Caldeira[1], Scott E. Poock[2], and Matthew C. Lucy[1], [1]*Division of Animal Science, University of Missouri, Columbia, MO,* [2]*College of Veterinary Medicine, University of Missouri, Columbia, MO.*

Dairy cows with SCE have lesser fertility but the specific mechanism is unknown. We hypothesized that reduced fertility can be explained by an effect of SCE on ovarian function. The objective was to classify cows for SCE and then evaluate their ovarian response to a TAI protocol. We also examined growth of the conceptus from d 35 to 45 after TAI. Twelve weekly cohorts of Holstein cows (n = 56) were enrolled in a TAI protocol (Presynch Ovsynch) [$PGF_{2\alpha}$ (PGF1), 14 d, $PGF_{2\alpha}$ (PGF2), 14 d, GnRH, 7 d, $PGF_{2\alpha}$ (PGF3), 56 h, GnRH, 16 h, TAI] so that first TAI was 68 to 77 d postpartum. Endometrial cytology was assessed by using a cytobrush at PGF1 [30–39 d postpartum; cytobrush (CB) 1] and 1 d before PGF3 (64–73 d postpartum; CB2). The percentage of polymorphonuclear neutrophils (% PMN) was calculated as a function of total nucleated cells in the endometrial sample. Ovarian follicles and corpora lutea (CL) were counted and measured and ovulatory responses were assessed from PGF1 to 5 d after TAI. Growth of the conceptus after TAI was measured every other d from d 35 to 45 of gestation. Cows were retrospectively assigned to SCE groups based on % PMN at CB1 and CB2. Group 1 (G1; n = 29 cows) had ≤13% PMN at CB1 and ≤2%PMN at CB2; Group 2 (G2; n = 14 cows) had > 13% at CB1 and ≤2% at CB2; and Group 3 (G3; n = 13 cows) had >2% PMN at CB2. Pregnancies per AI (P/AI) were 59.3, 35.7, and 23.1% for G1, G2 and G3, respectively ($P < 0.073$). The percentage of cows with a CL at PGF3 did not differ for G1 to G3 (93, 86, and 85%, respectively; $P > 0.10$). Size of the ovulatory follicle (15.6 ± 0.6 mm), percentage of cows ovulating after TAI (88%), and diameter of the CL after ovulation (21.4 ± 0.6 mm) did not differ ($P > 0.10$) between groups. Fetal and amniotic vesicle size increased with d of pregnancy ($P < 0.001$) but did not differ for G1 to G3 ($P > 0.10$). Cows classified according to SCE tended to differ for P/AI but were similar for ovarian response to a TAI protocol. Although SCE appeared to affect the capacity to establish pregnancy, growth of the conceptus from d 35 to 45 in pregnant cows was not affected by SCE.

Key Words: endometritis, timed AI, ovary, pregnancy

LB3 Comparison of standardization methods for real-time qPCR to account for heterogeneous cell types in differing fat depots. Brandon M. Koch*, William C. Bridges, and Susan K. Duckett, *Clemson University, Clemson, SC.*

Six genes reported as adipocyte specific within the literature and a gene previously validated as a stable reference gene (REF) were analyzed using *RefFinder* to select the most stable REF to investigate the ability of cell specific genes to standardize relative gene expression across tissues varying in cell type heterogeneity. Angus × Hereford steers (n = 16) were randomly selected and samples of subcutaneous (s.c.), mesenteric (MES), and *longissimus dorsi* (LM) fat depots were collected, flash-frozen, and stored at −80°C for subsequent analysis. Lipogenic relative gene expression values were either unstandardized (RAW), or standardized with the most stable REF (Thy-1 cell surface antigen; Thy1), second most stable (glyceraldehyde 3-phosphate dehydrogenase; GAPDH), or the geometric mean of GAPDH and Thy1 (GM). Fold changes are presented in Table 1 for tissues differing in heterogeneity (high fat, MES vs. high muscle, LM). Acetyl CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl CoA desaturase (SCD) were all affected by tissue ($P < 0.05$). Standardization method and the interaction of method and tissue did not alter fold change ($P > 0.10$). This may suggest that the use of an adipocyte-specific REF does not account for variable cell type heterogeneity. More work is warranted to identify an effective method to standardize qPCR results to tissue heterogeneity, allowing for objective across tissue comparisons.

Contd.

Table 1 (Abstr. LB3). The effects of standardization and tissue on the average fold change of relative gene expression

		Gene		
Method[1]	Tissue[2]	ACC	FASN	SCD
GAPDH	MES	0.65	−3.46	−2.63
GM	MES	0.09	−3.51	−2.68
Thy1	MES	−0.01	−3.61	−2.79
RAW	MES	−0.25	−5.07	−3.88
GAPDH	LM	2.04	2.89	1.73
GM	LM	1.77	2.62	1.53
Thy1	LM	1.52	2.42	1.35
RAW	LM	1.78	3.12	1.68
	SEM[3]	1.826	7.284	6.979
P-value[4]	T	<0.01	0.02	0.04
	M	0.10	0.29	0.33
	T × M	0.11	0.35	0.42

[1]Standardized against GAPDH, Thy1, the geometric mean (GM) of GAPDH and Thy1, or raw values.

[2]High fat, MES and high muscle, LM tissue.

[3]Pooled standard error of means.

[4]Main effect of tissue (T), method (M), and the interaction of T and M.

Key Words: adipocyte, gene, standardization

LB4 Supplementation of choline increases very low density lipoprotein export from bovine primary hepatocytes. Courtney L. McCourt[1], Tawny L. Chandler*[1], Sandra J. Bertics[1], Barbara A. Barton[2], and Heather M. White[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]Balchem Corporation, New Hampton, NY.*

Supplementation of choline in dairy cattle decreases liver lipid accumulation, potentially through increased very low density lipoprotein (VLDL) export. The direct effect of choline supplementation on VLDL export has not been confirmed due to an inability to quantify bovine VLDL. In other species, VLDL is separated from low- and high-density lipoproteins based on size; however, bovine VLDL differs in density compared with nonruminant VLDL particles due to lipid profile. The objectives of this experiment were to validate a bovine-specific ELISA technique for use in quantification of bovine VLDL and determine the effect of increasing concentrations of choline chloride (CC) and dL-methionine (dLM) during fatty acid challenge on VLDL export in cell culture. Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h before treatment with CC (33, 100, 2000, 4500 μ*M*) and dLM (16, 30, 100, 300 μ*M*) within a factorial design, with 1 m*M* FA cocktail. Treatments mimicked expected physiological concentrations. After 24 h, medium was collected for VLDL quantification by ELISA. The ELISA (NeoBiolab, Cambridge, MA) is a competitive immunoassay that utilizes antibodies for apolipoprotein B100 and C, which in combination uniquely binds to VLDL particles. The assay was validated using cell culture and postpartum bovine serum samples with purified LDL and HDL spikes. Recovery of samples with spiked standards, compared with independently analyzed samples and standards, was 102 ± 1%. Cell culture data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts including fixed effect of treatment and random effect of calf. Interactions were not significant and therefore only main effects are discussed. Increasing concentrations of CC linearly increased ($P = 0.02$) VLDL export from hepatocytes. Treatment with dLM did not alter ($P = 0.7$) VLDL export. This research validates an ELISA for use in quantification of bovine VLDL. Furthermore, these data support the role of CC, but not dLM, supplementation in increasing VLDL export from hepatocytes.

Key Words: choline, methionine, VLDL

LB5 Time and dry acidulant addition substantially reduce *Salmonella* concentration in extruded dog food. Andrea Jeffrey*[1], Gregory Aldrich[1], Carl Knueven[2], Anne Huss[1], and Cassandra Jones[1], *[1]Kansas State University, Manhattan, KS, [2]Jones-Hamilton Co., Walbridge, OH.*

With the pending implementation of the Food Safety Modernization Act and increasing consumer demands, animal feed safety is under closer scrutiny. Particularly, *Salmonella* contamination has been identified as a potential biological pathogen of concern in the pet food industry, and has been the cause of several recent pet food recalls. The industry is in search of potential methods to lower the risk of *Salmonella* in animal feed. One potential method of *Salmonella* mitigation is through the use of acidifiers to reduce pH to destroy or inhibit growth of bacteria. The objective of this experiment was to determine if coating pet food with a dry acidulant powder, sodium bisulfate (SBS, Jones-Hamilton, Co., Waldridge, OH), would reduce *Salmonella* growth over time in dog foods of varying surface area, bulk density and piece density. A single formula of a dry dog food was utilized in a 4 × 3 factorial design with 4 kibble sizes (surface area of 455, 997, 1,022, or 7,337 mm^2) and 3 coating levels of SBS (0.0, 0.2, or 0.4%). The results were tested over a 14-d period with sampling day serving as a repeated measure. Kibble sizes were analyzed for surface area, bulk density, and piece density. Then, kibble was coated with varying levels of SBS and inoculated with a *Salmonella* cocktail (ATTC# 13076) on d 0 and analyzed for *Salmonella* on d 0, 1, 2, 7, and 14 by direct plating to xylose lysine deoxycholate agar. Bulk density, piece density, and surface area were not correlated with *Salmonella* quantity ($P > 0.10$), and there was no effect ($P > 0.10$) of kibble size or the interactions including kibble size on *Salmonella* concentration. However, coating kibble with SBS resulted in a 1.6 or 2.0-log reduction in *Salmonella* ($P < 0.0001$; 1.23 or 1.65 vs. 3.27 log_{10} cfu/g for 0.4 or 0.2 vs. 0.0% SBS inclusion, respectively). Time also had a substantial effect on *Salmonella* concentration, and reduced *Salmonella* by 3.41 logs by 14 d post-inoculation ($P < 0.0001$; 4.10, 3.04, 1.47, 0.97, 0.69 log_{10} cfu/g for d 0, 1, 2, 7, and 14, respectively). In conclusion, both time and the coating of kibble with a dry acidulant substantially reduce *Salmonella* concentration in the tested product. However, altering the size of kibble does not further reduce the risk of *Salmonella* in a dry extruded dog food.

LB6 A life cycle assessment framework combining nutritional and environmental health impacts of diet: A case study on milk. Katerina Stylianou[1], Martin Heller[2], Victor Fulgoni III[3], Ying Wang*[4], Gregory Keoleian[2], and Olivier Jolliet[1], *[1]Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI, [2]Center for Sustainable Systems, School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI, [3]Nutrition Impact LLC, Battle Creek, MI, [4]Innovation Center for US Dairy, Rosemont, IL.*

While there is considerable effort to understand the environmental impact of a food or diet, nutritional effects are not usually considered in food-related life cycle assessment (LCA). To address this, we developed a novel Combined Nutritional and Environmental Life Cycle Assessment (CONE-LCA) framework that evaluates and compares

in a parallel manner environmental and nutritional effects of food items or diets expressed in disability-adjusted life years (DALYs). We applied this framework in a proof-of-concept case study to investigate the environmental and nutritional human health effects associated with the addition of one serving of fluid milk to the present American adult diet. We also investigated 2 replacement scenarios where a serving of fluid milk is substituting an isocaloric portion of the average diet and sugar-sweetened beverages (SSB). Epidemiologically based nutritional impacts and benefits linked to milk intake were compared with several environmental impacts considered in LCA (global warming and particulate matter), carried to a human health endpoint. Given the evidence considered, a fluid milk consumption increase by a serving led to an overall health benefit; nutritional benefits from consumption exceed the environmental impact from production also expressed in μDALY/person/day by close to a factor 2.5. This factor was increased to nearly a factor of 5 when substitution of the average diet, and up to close to a factor 10 SSB were substituted. The initial analysis of uncertainty of the case study suggested that present findings are only suggestive; refined impact assessment factors and the inclusion of additional nutritional and environmental categories are needed to improve certainty. This case study provides the first quantitative epidemiological-based estimate of the complements and trade-offs between nutrition and environment human health burden expressed in DALYs, pioneering a new approach in LCA. We recommend further testing of this CONE-LCA approach to other food items, to characterize potential trade-offs between environmental and nutritional impacts when making recommendations about sustainable diets and food choices.

LB7 Effect of heat stress on mammary gland autophagy during the dry period. Yenny Ramirez-Lee*[1], Bahroz M. S. Ahmed[1], Sha Tao[2], Geoffrey E. Dahl[1], and Stephanie E. Wohlgemuth[1], *[1]University of Florida, Gainesville, FL, [2]University of Georgia, Tifton, GA.*

Heat stress during the dry period compromises mammary gland growth, thus negatively affecting subsequent milk yield. Cooling during the late dry period, when mammary tissue proliferates, is a common management practice. However, it neglects mammary gland involution during the early dry period, a process that is accomplished by both apoptosis and autophagy. Our objective was to evaluate the effect of heat stress on mammary gland (MG) autophagy during the early dry period. Holstein cows were dried off 45 d before expected calving and randomly assigned to 1 of 2 treatments: heat stress (HT) or cooling (CL). All cows were housed in the same barn during the dry period, but only the stall area for CL cows was equipped with soakers and fans. Rectal temperature (RT) was measured daily and respiration rate (RR) was counted thrice weekly during the dry period. MG biopsies were collected from each cow 3 d before dry-off (d −3) and on d 3, 7, 14, and 22 ± 2 after dry-off. Autophagy in MG was assessed by measuring protein expression of 2 autophagic markers Atg7 and LC3. The average THI during the dry period was 77.7, which indicated that HT and CL cows were exposed to significant heat stress. However, the cooling system effectively alleviated heat stress in CL cows by decreasing the RT (39.0 vs. 39.4°C) and RR (46.8 vs. 70.0 breaths/min) in CL relative to HT cows. Protein expression of Atg7, a marker for early autophagosome formation, did not change within or between groups. In contrast, protein expression of LC3-II, a marker of auto phagosomes, and its precursor LC3-I showed a dynamic expression pattern in MG from CL cows during the early dry period. Relative to HT cows, MG from CL cows displayed higher expression of LC3-I ($P < 0.05$) and LC3-II ($P < 0.10$) on d 7, and lower expression of LC3-II on d 14 ($P < 0.05$) and d 22 ($P < 0.10$). Our data suggest that cows undergoing chronic thermal stress throughout the entire dry period (1) lack the autophagic activity in the early dry period necessary for MG involution, and (2) lack a subsequent attenuation of autophagy, which may hinder MG proliferation in the late dry period.

LB8 Monitoring survivability and infectivity of porcine epidemic diarrhea virus within two different on-farm lagoons. Hein M. Tun*[1], John P. Carney[2], Mark Fynn[3], Lorne Grieger[4], and Ehsan Khafipour[1,5], *[1]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, [2]Manitoba Livestock Manure Management Initiative Inc., Winnipeg, MB, Canada, [3]Manitoba Pork Council, Winnipeg, MB, Canada, [4]Prairie Agricultural Machinery Institute, Portage La Prairie, MB, Canada, [5]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada.*

Porcine epidemic diarrhea virus (PEDv) has emerged as a new threat to the North American swine industry since 2013. Despite best efforts, not much is known about its survivability and infectivity within open lagoons over time. Two positive farms in Manitoba, Canada were included in the study, the first being infected 20 wk before the study and the second being identified during the initial farm study. Lagoon samples were collected over a 7-wk period during fall 2014 where active viral shedding was (lagoon 2) or was not present (lagoon 1). Lagoon sampling was conducted using a grid layout of 12 sites at 3 depths in lagoon 1, and 16 sites at 2 depths in lagoon 2. For lagoon 2, spring sampling was conducted in mid-May as described above. Survivability of PEDv was tested using duplex qRT-PCR for virulent PEDv (subgroup 2a) and the 2014 variant. 99.5% of lagoon samples collected in the fall were positive for virulent PEDv, whereas all samples tested negative for the 2014 variant. The PEDv survival in the lagoons exceeded 27 wk within lagoon 1, and viral load significantly increased over time ($P < 0.01$). The viral load was high in the initial weeks within the top and middle layers, but the reverse trend was observed in subsequent weeks with the highest levels detected within the bottom layer ($P = 0.076$). The viral load within lagoon 2 was significantly higher due to active shedding during wk 1 of sampling but decreased in subsequent weeks ($P < 0.01$). The virus infectivity was based on viral replication in Vero cells, showing 15% of samples were infective. In both lagoons, the virus was infective at the last week of fall sampling with higher infectivity in the middle layer of lagoon 1 ($P = 0.015$), and in the bottom layer of lagoon 2 ($P = 0.285$). In total, 87.5% of lagoon 2 samples were still positive for PEDv in the spring, 9 mo after the outbreak, but only 28% were infective. The data show the presence and infectivity of PED virus exceeding 9 mo in infected lagoons.

Key Words: PEDv, lagoon, survivability