

Physiology and Endocrinology: Nutrition and Immunology

370 Assessment of oxidative stress biomarkers in exhaled breath condensate and blood of dairy heifer calves from birth to weaning. R. Ranade¹, S. Talukder¹, G. Muscatello¹, and P. Celi*^{1,2}, ¹Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia, ²Melbourne School of Land and Environment, The University of Melbourne, Parkville, VIC, Australia.

This study reports preliminary data on systemic and respiratory biomarkers of oxidative stress (OS) in healthy dairy calves from birth to weaning. Blood and exhaled breath condensate (EBC) were sampled from 19 female calves within 24 h from birth, and then at 6, 12 and 18 weeks of age. Values of reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and advanced oxidation protein products (AOPPs) were determined in blood, while EBC samples were assayed for hydrogen peroxide (H₂O₂) levels. The degree of OS was estimated by the ratio of ROMs/BAP (U.Carr./μmol/L) multiplied by 100 to give an OS index (OSI). Changes in OS biomarkers were analyzed by a linear mixed model using Genstat version 14. The elevated BAPs observed in healthy calves may be significant in facilitating rapid metabolism associated with growth and development in the young calf. The observed decrease of AOPP is of interest as it is a marker of protein oxidation and is also considered to mediate pro-inflammatory responses, therefore suggesting that the calves recruited in this study did not experience any subclinical inflammatory conditions. This study successfully measured OS biomarkers in blood and EBC in the dairy calves. Our findings suggest that the calves enrolled in this study did not experience OS, and therefore the redox homeostatic control mechanism was adequately developed from birth to weaning. In conclusion, our study reports the physiological concentrations of some novel OS biomarkers in calves which may be considered as a reference standard when comparing further studies.

Table 1. Oxidative stress biomarkers in calves

	Calf age (wk)				SE	P-value
	1	6	12	18		
ROMs (U.Carr.)	179	161	154	160	8.5	0.218
BAP (μmol/L)	3814 ^a	3313 ^b	3250 ^b	3832 ^a	115	<0.001
OSI (arbitrary units)	4.7	4.9	5.1	4.3	0.3	0.326
H ₂ O ₂ in EBC (μmol/L)	23.8 ^{ab}	23.1 ^{ab}	16.2 ^c	45.0 ^d	1.5	<0.001
AOPP (μmol/L)	28.9 ^a	20.1 ^b	15.7 ^b	14.6 ^b	1.4	<0.001

^{a-c}Within rows, means followed by different letters are significantly different at *P* = 0.05.

Key Words: oxidative stress, calves, EBC

371 Prenatal transportation alters the metabolic response of Brahman bull calves exposed to a lipopolysaccharide challenge. J. A. Carroll*¹, N. C. Burdick Sanchez¹, M. C. Roberts^{2,4}, D. M. Price^{2,4}, B. P. Littlejohn^{2,4}, R. C. Vann³, T. H. Welsh Jr.⁴, H. D. Hughes⁵, J. T. Richeson⁵, and R. D. Randel², ¹Livestock Issues Research Unit, USDA-ARS, Lubbock, TX, ²Texas A&M AgriLife Research, Texas A&M University System, Overton, ³MAFES-Brown Loam, Mississippi State University, Raymond, ⁴Texas A&M AgriLife Research, Texas A&M University System, College Station, ⁵Department of Agricultural Sciences, West Texas A&M University, Canyon

This study was designed to determine if prenatal transportation influences the metabolic response to a postnatal LPS challenge. Pregnant Brahman cows (n = 96) matched by age and parity were separated into transported (TRANS; n = 48; transported for 2 h on gestational d 60, 80, 100, 120 and 140) and non-transported control groups (CONT; n = 48). From these treatments, bull calves (n = 16 per trt) were identified at weaning (176 ± 2 d of age) to subsequently receive a LPS challenge. We previously reported an effect of TRANS on temperament (TEMP); therefore bulls were also grouped based on TEMP score [Calm (C); Intermediate (I), or Temperamental (T)]. On d -2 bulls were transported by trailer from Overton to Lubbock, TX. On d -1, bulls were fitted with indwelling jugular cannulas and placed in individual stalls. On d 0 blood samples were collected at 0.5-h intervals from -2 to 8 h, and again at 24 h relative to LPS challenge (0.5 μg/kg BW) at Time 0. Serum was analyzed for glucose, insulin, nonesterified fatty acids (NEFA), and blood urea nitrogen (BUN) concentration. All variables increased post-LPS (*P* < 0.01). Glucose was 8 and 13% greater in TRANS than CONT pre- and post-LPS (*P* < 0.01), and was 11 and 8% greater in T than C and I bulls pre-LPS (*P* < 0.01). Post-LPS glucose was affected by TEMP, with this response influenced by prenatal transportation (*P* < 0.01). Insulin tended (*P* = 0.09) to be greater in TRANS than CONT bulls pre- and post-LPS. Post-LPS insulin was greatest in the C TRANS and I CONT bulls (*P* < 0.01). Pre-LPS NEFA was 18% greater in CONT than TRANS bulls (*P* = 0.02), and was greatest in I bulls (*P* < 0.01). Post-LPS, NEFA was also greater in I bulls (*P* < 0.01). The BUN was greater both pre- and post-LPS in the C TRANS bulls (*P* < 0.01). In summary, both prenatal transportation and TEMP had significant effects on the metabolic response before and after LPS, with prenatal transportation altering the observed metabolic responses within temperament groups. The altered metabolic response in the TRANS bulls may help explain differences observed in markers of the acute phase response in these bull calves following the LPS challenge.

Key Words: cattle, immune, transportation

372 Effects of an intramammary lipopolysaccharide challenge on metabolism and mammary immune response in hyperketotic dairy cows. M. Zarrin*^{1,2}, H.A. van Dorland¹, O. Wellnitz¹, and R.M. Bruckmaier¹, ¹Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland, ²Department of Animal Science, Yasouj University, Yasouj, Iran, ³Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Bern, Switzerland.

Energy requirement and inadequate feed intake induces negative energy balance (NEB) in early lactating dairy cows. Elevation of ketone bodies is a frequent metabolic adaptation during NEB. Previous studies showed positive correlation between high plasma BHBA concentration and risk and severity of mastitis. The objective of this experiment was to study the effects of an intramammary lipopolysaccharide (LPS) challenge accompanied by hyperketonemia on metabolism and mammary immune responses in mid-lactation dairy cows. Thirteen dairy cows were randomly assigned to 2 treatment groups including an intravenous Na-DL-β-OH-butyrate infusion (HyperB, n = 5) to achieve elevated plasma BHBA concentrations (1.7 mmol/L), and a 0.9% saline solution infusion (Control, n = 8) for 56 h. After 48 h of infusion, 2 udder quarters were stimulated with 200 μg of *Escherichia coli* LPS. Blood samples were taken hourly during the infusion. Udder biopsies were taken at 48 h after the start of infusions immediately before the LPS challenge, and at the end of infusions. Blood samples were analyzed for blood metabolites,

cortisol, glucagon, and insulin concentrations. The mRNA abundance of candidate genes was determined by RT-qPCR in udder tissues. Difference between before and after LPS challenge in the measured parameters and area under the curve were evaluated by ANOVA with treatment as fixed effect in the model. Intramammary LPS challenge increased somatic cell count (SCC), rectal temperature, plasma glucose, cortisol, glucagon, and insulin concentration in both groups, but SCC, plasma glucose, and glucagon concentration remained lower in HyperB than the control ($P < 0.05$). IL-8, IL-10, citrate synthase, mRNA abundance increased during the LPS challenge in LPS quarters in HyperB ($P < 0.05$). RANTES mRNA abundance decreased ($P < 0.01$), and toll-like receptor 4 mRNA abundance tended to decrease in control group ($P = 0.08$). The results demonstrate that BHBA infusion affects metabolism and immune response of mammary gland to LPS challenge which may reflect the increased susceptibility for mastitis during spontaneous hyperketonemia.

Key Words: cow, ketone body, LPS

373 Effects of supplemental amino acids and chromium propionate on metabolism and neutrophil function in peak lactation dairy cows. K. Yuan*, C. F. Vargas, L. K. Mamedova, E. C. Titgemeyer, and B. J. Bradford, *Kansas State University, Manhattan.*

The study objective was to evaluate effects of chromium propionate (CrPr, KemTRACE Chromium Propionate 0.04%, Kemin Industries Inc.), rumen-protected Lys (LysiPEARL, Kemin Industries Inc.) and Met (MetiPEARL, Kemin Industries Inc.), or both on metabolism and neutrophil function in dairy cows near peak lactation (38 ± 15 DIM, 43.0 ± 7.0 kg/d milk). Forty-eight individually fed Holstein cows (21 primiparous, 27 multiparous) were stratified by calving date in 12 blocks and randomly assigned to 1 of 4 treatments within block. Treatments were control, CrPr (8 mg/d Cr), RPLM (10 g/d Lys and 5 g/d Met, intestinally available), or CrPr plus RPLM. Treatments were premixed with ground corn and top-dressed at 200 g/d for 35 d. On d 21 and 35 of treatment, blood samples were collected for analysis of plasma metabolites and hormones, and blood neutrophils were isolated from cows in 6 blocks for analysis of transcript abundance (TA) of pro-inflammatory cytokines. Tumor necrosis factor α (TNF α) and interleukin 1 β (IL-1 β) TA in neutrophils in the basal state or after 12 h of lipopolysaccharide (LPS) activation was quantified by qPCR. Data were analyzed as a complete block design with a factorial arrangement of treatments and sample day as a repeated measure using ProcMIXED in SAS. Plasma glucose, NEFA, and glucagon were unaffected ($P > 0.10$) by treatments. Plasma insulin was increased ($P = 0.01$, by 13%) by RPLM. Basal TNF α TA in neutrophils was decreased by CrPr ($P = 0.03$, by 80%) on d 21 but not on d 35. After LPS activation, CrPr tended ($P = 0.09$) to increase neutrophil TNF α TA. These results suggest that CrPr supplementation may decrease basal inflammatory status, but improve innate immune response upon activation. Basal IL-1 β TA in neutrophils was decreased ($P = 0.03$) by RPLM, and the decrease in response to RPLM was greater when CrPr was provided (RPLM \times CrPr, $P = 0.04$, by 78%). No treatment effect ($P > 0.10$) was observed for IL-1 β TA after LPS activation. Collectively, results indicate that supplemental CrPr and RPLM provided for 5 wk had minimal effects on metabolism but may modulate neutrophil function in lactating cows.

Key Words: lysine, methionine, chromium

374 Polymorphonuclear leukocyte transcriptomics in transition Holstein cows fed two levels of dietary energy prepartum. M. J. Khan*, D. E. Graugnard, S. L. Rodriguez-Zas, and J. J. Loor, *University of Illinois, Urbana.*

Efficient activation of polymorphonuclear neutrophils (PMN) is central for an effective immune response. The periparturition period is characterized by marked changes in inflammatory status that are functionally related with impaired immune responses in the cow. Whole-transcriptome adaptations of PMN to preparturition dietary energy level and change of physiological state remain unknown. We used a bovine microarray (Agilent) for transcript profiling of PMN RNA isolated on -14 and 7 d around parturition from cows (8/diet) fed a control (high-straw, CON; NEL = 1.34 Mcal/kg) or moderate-energy (OVE; NEL = 1.62 Mcal/kg) diet during the entire dry period. The ANOVA model included diet, day, and diet \times day as fixed effects, and cow within diet as random effect. The raw P -values from ANOVA were adjusted using a False discovery rate (FDR) to minimize false-positives. At an FDR $P < 0.01$ there were 318 (downregulated = 151, upregulated = 167) and 1,015 (downregulated = 354, upregulated = 661) differentially expressed genes (DEG) at d -14 and d 7. The KEGG pathways analysis using the Dynamic Impact Approach (DIA) uncovered major changes in metabolism at d 7 due to the effect of OVE vs CON. Amino acid metabolism and Metabolism of Terpenoids and Polyketides were the most impacted and downregulated pathways among metabolism categories. Of the top-5 most-impacted pathways in OVE compared with CON on d -14 and 7, we observed inhibition of "phenylalanine, tyrosine and tryptophan biosynthesis," "Terpenoid backbone biosynthesis," "Fat digestion and absorption," and "Phenylalanine metabolism"; whereas, "Glycosaminoglycan biosynthesis" was activated. "Cell growth and Death" was the most impacted activated pathway in cellular process KEGG category. Results suggest that overfeeding energy prepartum had profound effects on PMN transcriptomics, which may be functionally related to their function.

Key Words: PMN, microarray, bioinformatics

375 Efficacy of ampicillin trihydrate for therapy of metritis in lactating dairy cows. F. S. Lima*, A. Vieira-Neto, G. S. F. M. Vasconcelos, R. S. Bisinotto, N. Martinez, L. F. Greco, L. D. P. Sinedino, R. D. Mingoti, K. N. Galvão, C. A. Risco, W. W. Thatcher, and J. E. P. Santos, *University of Florida, Gainesville.*

Objective was to compare ampicillin trihydrate (AMP) with ceftiofur hydrochloride (CEFT) as treatment for metritis in dairy cows. Holstein cows had rectal temperature (RT) measured daily for the first 12 DIM, and fever was characterized by RT $> 39.4^{\circ}\text{C}$. Vaginal discharge (VD) was scored at 4, 6 and 8 DIM, and on any day a cow had fever. Cows with VD score 5 (reddish/brownish foul smell) were diagnosed with metritis (MET). Within cows with MET ($n = 528$), they were segregated as those with ($n = 216$) or without ($n = 312$) concurrent fever. Cows with metritis were blocked by parity and type of metritis (with or without fever), and assigned randomly to receive 11 mg/kg of AMP ($n = 259$) or 2.2 mg/kg of CEFT ($n = 269$) daily for 5 d. A cohort of healthy cows (NOMET, $n = 268$) was selected randomly as controls. Cows with metritis had RT measured on d 1 to 7, and d 12 after initiation of treatments, and VD scored on d 5, 7, and 12. Cure was characterized by VDDIM, VD was scored for diagnosis of clinical endometritis (CE, VD > 2 , mucopurulent discharge). Data were analyzed using PROC GLIMMIX of SAS. Efficacy of treatments is depicted in the Table. Cure on d 5 and 7 increased for AMP compared with CEFT. Cure of metritis was less when cows had concurrent fever (Table). Cows receiving AMP had decreased ($P = 0.03$) prevalence of CE than those treated with CEFT (58.5 vs. 67.8%). Prevalence of CE was greater ($P < 0.01$) for cows previously diagnosed with MET than NOMET (63.2 vs. 27.2%). Treatment did not affect the proportion of cows that left the herd by 60 DIM (AMP = 3.8 vs. CEFT = 7.2%). Ampicillin is an efficacious alternative for therapy of metritis in dairy cows.

Table 1.

Parameter	AMP		CEFT		P-value		
	No Fever	Fever	No Fever	Fever	TRT	Fever	TRT × Fever
Cows, no.	152	107	160	109	—	—	—
RT, ¹ °C	39.04	39.31	39.02	39.22	0.09	0.01	0.20
Fever, ² %	12.5	33.2	12.9	28.2	0.47	0.01	0.34
Cure, %							
d 5	48.4	25.6	28.4	18.5	0.01	0.01	0.33
d 7	69.0	49.7	57.3	34.6	0.01	0.01	0.69
d 12	88.0	79.2	90.6	81.1	0.50	0.01	0.82

¹RT = mean RT from d2 to 12 after enrollment.

²Fever = incidence of fever.

Key Words: dairy cow, ampicillin, metritis

376 Use of digital infrared thermography (IRT) and oxidative stress (OS) biomarkers as a diagnostic tool to diagnose interdigital dermatitis in sheep. S. Talukder*¹ and P. Celi^{1,2}, ¹Faculty of Veterinary Science, The University of Sydney, Narellan, NSW, Australia, ²Melbourne School of Land and Environment, The University of Melbourne, Parkville, VIC, Australia.

This study reports preliminary data on the use of digital infrared thermography (IRT) and biomarkers of oxidative stress (OS) to diagnose the degree of interdigital dermatitis (ID) lesions in sheep. Interdigital space skin temperatures were obtained from healthy (n = 6) and ID affected (n = 11) crossbred rams with a FLIR T620 series infrared camera and images were analyzed using ThermoCAM Researcher Professional 2.9 software. Interdigital space lesions were scored using a 5 point scoring system (0–4). Blood were sampled from 17 rams and plasma was analyzed for reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and advanced oxidation protein products (AOPP). The degree of OS was estimated by the ratio of ROMs/BAP (U.Carr./μmol/L) multiplied by 100 to give an OS index (OSI). Footrot scores were used to stratify the rams in 3 groups: Group A (0 to 0.5), B (0.6 to 1.5) and C (≥1.5). Changes in OS biomarkers and skin temperature were analyzed by a linear mixed model using Genstat version 14. We observed that skin temperature was significantly higher in rams with ID lesions ($P < 0.05$; Table 1). It was noted that ROMs and OSI biomarkers did not differ significantly between feet with ID lesions; a trend for higher BAP and AOPP concentrations in rams with footrot score > 1.5 was noted. In conclusion, IRT was reliable in detecting elevated temperature associated with ID in sheep.

Table 1. Oxidative stress biomarkers and interdigital space skin temperature in sheep

	Groups			SE	P-value
	A	B	C		
ROMs (U.Carr.)	119.7	148.1	118.9	25.5	0.60
BAP (μmol/L)	4012.0	4523.0	3688.0	315.0	0.09
OSI (arbitrary units)	3.0	3.3	3.5	0.1	0.59
AOPP (μmol/L)	17.3	11.9	19.2	2.6	0.08
Max Temp (°C)	35.7 ^b	36.6 ^a	37.0 ^a	0.7	0.04
Min Temp (°C)	30.2 ^b	31.5 ^a	31.2 ^a	0.5	0.02
Average Temp (°C)	33.7 ^b	34.9 ^a	35.1 ^a	0.7	0.05
Footrot score	0.06 ^c	1.3 ^b	2.2 ^a	0.3	0.01

^{a-c}Within rows, means followed by different letters are different at $P = 0.05$.

Key Words: infrared thermography, oxidative stress, footrot

377 Effect of cattle temperament as determined by exit velocity on lung respiratory lesions and liver disease. T. B. Schmidt*¹, J. W. Dailey², J. W. Waggoner³, A. H. Voyles⁴, C. D. Alexander⁴, J. O. Buntyn¹, K. I. Domenech¹, M. Schneider⁴, and J. A. Carroll², ¹University of Nebraska-Lincoln, Lincoln, ²USDA-ARS, Lubbock, TX, ³Kansas State University, Garden City, ⁴Garden City Community College, Garden City, KS.

The objective of this trial was to use exit velocity as a means of determining temperament of cattle to evaluate the effect of temperament on animal health. At the time of processing, exit velocity and BW were recorded on 20 pens of cattle (2,877 hd) at a commercial feedlot. Infrared sensors affixed to the alleyway at a distance of 2.75 m were used to remotely trigger the start and stop of a timing system. Exit velocity (m/sec) was recorded and cattle were placed into pens for the duration of the feeding period. Cattle were classified as temperamental (TEMP) and non-temperamental (NTEMP) based upon exit velocity. Twenty percent of each pen was classified as TEMP based the fastest exit velocity; the remaining 80% was classified as NTEMP. At the conclusion of the feeding period, cattle were transported to a commercial abattoir and harvested. At the time of harvest, the livers and lungs of each animal were evaluated. Livers were assessed based upon the Elanco Liver System and lung lesions were assessed based upon a hedonic scale of 1–4; 1 = no lung lesions, 2 = plauritis lesions, 3 = portions/lobes of lung missing, and 4 = collapsed/consolidated lesions. No difference ($P = 0.18$) in liver abscesses was observed; 4.9 and 3.8% of NTEMP and TEMP cattle were positive for liver abscesses, respectively. Overall, 39 and 31% of N-TEMP and TEMP cattle exhibited lungs lesions, respectively. Assessment of lesions revealed that 64, 14, and 22% of the lesions observed in NTEMP cattle were scored as 2, 3, and 4, respectively. For TEMP cattle, 68, 12, and 20% of lesions observed were scored as 2, 3, and 4, respectively. Based upon the scale for lung lesions, lesions were greater ($P = 0.006$) for NTEMP cattle compared with TEMP cattle (1.61 ± 0.02 vs. 1.46 ± 0.05). Results of this trial indicate that based upon temperament, there is no difference in occurrence of liver disease; however, cattle classified as NTEMP had decreased hedonic lung lesion scores, indicating that N-TEMP cattle may be more susceptible to respiratory challenge. These data could be utilized to develop alternative health management strategies for temperamental cattle in feedlots.

Key Words: temperament, lung, lesion

378 Relationship between cattle temperament as determined by exit velocity and carcass merit in beef cattle. T. B. Schmidt*¹, J. W. Dailey², J. W. Waggoner³, A. H. Voyles⁴, C. D. Alexander⁴, J. O. Buntyn¹, K. I. Domenech¹, M. Schneider⁴, and J. A. Carroll², ¹University of Nebraska-Lincoln, Lincoln, ²USDA-ARS, Lubbock, TX, ³Kansas State University, Garden City, ⁴Garden City Community College, Garden City, KS.

The objective of this trial was to use cattle temperament, as determined by exit velocity only, as a means to evaluate the effect of temperament on carcass merit and the possible utilization of exit velocity alone as a sorting tool within the feedlot. At the time of processing, exit velocity and BW were recorded on 20 pens of cattle (2,877 hd) at a commercial feedlot. Infrared sensors affixed to the alleyway at a distance of 2.75 m were used to remotely trigger the start and stop of a timing system. Exit velocity (m/sec) was recorded and cattle were placed into pens for the duration of the feeding period. Cattle were classified as temperamental (TEMP) and non-temperamental (NTEMP) based upon exit velocity.

Twenty percent of each pen was classified as TEMP based the fastest exit velocity; the remaining 80% was classified as NTEMP. At the conclusion of the feeding period, cattle were transported to a commercial abattoir and harvested. Data collected included: HCW, REA, back fat, KPH, and marbling scores. At the time of processing, BW was greater ($P < 0.001$) for NTEMP cattle compared with TEMP (326 ± 1.43 kg vs. 319 ± 2.88 kg). At harvest, NTEMP cattle had greater HCW ($P < 0.001$; 370 ± 1.70 kg vs. 365 ± 3.43 kg), back fat ($P < 0.001$; 1.30 ± 0.004 cm vs. 1.22 ± 0.008 cm) and yield grade ($P = < 0.001$; 2.35 ± 0.02 vs. 2.15 ± 0.04) when compared with TEMP cattle. Temperamental cattle had larger ($P = 0.03$) REA compared with NTEMP (101 ± 0.04 cm² vs. 99 ± 0.08 cm²). Overall, 81.5% of TEMP cattle had a yield grade ≤ 2 compared with

77% for NTEMP cattle. Marbling scores were greater ($P = 0.003$) for NTEMP cattle compared with TEMP (417 ± 1.65 vs. 406 ± 3.34). Percent of carcasses grading USDA Standard were 3.79% for TEMP compared with 1.52% for NTEMP cattle and percentage of N-TEMP carcasses receiving a quality grade of Choice was 54.34% compared with 49.1% for TEMP cattle. The results of this trial indicate that temperamental cattle produce lighter weight carcasses with decreased USDA YG and decreased marbling. Based upon these results, the use of exit velocity as an indicator of temperament may be a feasible tool for sorting cattle upon arrival at the feedlot.

Key Words: temperament, carcass, merit