472 Effects of rumen-protected methionine fed to lactating Holstein cows during a heat stress challenge on mammary explant response to lipopolysaccharide. D. N. Coleman*1, M. Vallati-Riboni1, R. T. Pate1, D. Luchini2, F. C. Cardoso1, and J. J. Loor1, 1University of Illinois, Urbana, IL, 2Adisseo, Alpharetta, GA.

The objective was to investigate the effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on the response of mammary gland explants to lipopolysaccharide (LPS). Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM of TMR as top dress) or TMR without RPM] in a crossover design. The study was divided into 2 periods with 2 phases per period. During phase 1 (9d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets. Group 2 (n = 16) remained in TN (THI = 61 ± 4) but was pair-fed to HS counterparts. After a 14d washout and 7d adaptation period, the study was repeated (period 2). Environmental treatments were inverted relative to phase 2 in period 1 (sequence), while the dietary treatments remained the same. Mammary tissue was harvested at the end of phase 2. Twenty-five mg of tissue per cow was incubated with 0 or 3 μg/mL of LPS for 2h. Statistical analysis was performed using the MIXED procedure of SAS (Cary, NC). Regardless of Met supplementation, incubation with LPS increased mRNA abundance of interleukin-8, 6, 1α, C-X-C motif chemokine ligand 2 (CXCL2), tumor necrosis factor α, nuclear factor kappa B subunit 1 (NFκB1) and toll-like receptor 2 (Tlr2) (P < 0.001). An environment × LPS interaction was observed for NFκB1 (P = 0.03); expression was greater in LPS-treated explants from non-HS compared with HS cows, mRNA abundance of CXCL2, NFκB1, inducible nitric oxide, nitric oxide synthase 2, and superoxide dismutase 2 (P < 0.05) decreased with HS. While LPS did not alter abundance of genes in NFE2L2 signaling (P > 0.10), explants from HS cows had lower abundance of NFE2L2 (P < 0.001) and cullin 3 (P = 0.04) (an inhibitor of NFE2L2). Overall, preliminary evaluation indicates that HS reduced immune and antioxidant responses while RPM did not attenuate the inflammatory response induced by LPS in vitro.

Key Words: amino acid, immune response, mammary gland

473 Dairy cows that are less resilient to metabolic stress have increased markers of subacute inflammation, oxidative stress and endotoxemia when calving during climatic heat stress. N. Nemes-Navon1,2, G. Kra1, N. Ben-Aharon1, S. Yakoby1, and M. Zachut*1, 1Department of Ruminant Science, Institute of Animal Science, Volcani Center, Rishon LeZion, Israel, 2Bar Ilan University, Ramat Gan, Israel.

Cows that are less resilient to metabolic stress exhibit increased adipose tissue (AT) lipolysis and oxidative stress postpartum (PP). We hypothesized that increased AT lipolysis will be related to elevated subacute inflammation and higher release of endotoxins from AT to blood PP, and that seasonal heat stress may exacerbate these effects. The study included 24 multiparous dairy cows calving during winter (W, n = 12) or summer heat stress (S, n = 12) at the Volcani research farm (Israel). Cows were categorized retrospectively to those with low (LWL) or high weight loss (HWL) during the first month PP, indicating on metabolic resilience. Blood samples were obtained twice a week during the transition period for tumor necrotizing factor α (TNF-α), oxidative stress marker (malondialdehyde, MDA) and LPS-binding protein (LBP). Subcutaneous AT biopsies were collected at 7 d PP during S for immunoblots of LBP and TNF-α. Data were analyzed by PROC MIXED (SAS). Blood TNF-α was 6.7-fold higher in S vs. W (P < 0.0009), and was 1.8-fold higher in HWL than in LWL cows during S (P < 0.05), but not between HWL and LWL cows at W. Blood MDA was 5-fold higher in S than in W (P < 0.0001), and was 2-fold higher in HWL than in LWL (P < 0.05) during S, and tended to be higher in HWL vs. LWL at W (P < 0.1). Across seasons, blood LBP was 1.7-fold higher in HWL than in LWL at 7 d PP (P < 0.05). In AT of S cows, the abundances of LBP (P < 0.05) and TNF-α (P < 0.001) were higher in HWL than in LWL. Together, cows that were less resilient to metabolic stress had a higher inflammatory response and increased signs of endotoxemia in blood and AT specifically during heat stress. Seasonal heat stress has a dramatic effect on the degree of oxidative stress, subacute inflammation and immune function in transition cows.

Key Words: metabolic stress, heat stress, inflammation

474 Effects of intralipid infusion on metabolism and inflammation in immune-challenged lactating cows. E. A. Horst*1, L. M. van den Brink, E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, and L. H. Baumgard, Department of Animal Science, Iowa State University, Ames, IA.

Objectives were to evaluate the effects of Intralipid (IL; 20% i.v. fat emulsion; Fresenius Kabi, Uppsala, Sweden) infusion on metabolism, inflammation, and liver lipid content following an i.v. lipopolysaccharide (LPS) challenge in lactating cows. Cows (765 ± 32 kg BW; 273 ± 35 DIM) were enrolled in 2 experimental periods (P); during P1 (5d) baseline data were obtained. At the start of P2 (2d), cows were assigned to 1 of 2 treatments: 1) control + IL (CONIL; 3 mL of sterile saline; n = 5) or 2) LPS + IL (LPSIL; 0.375 μg/kg of BW LPS; n = 5). Directly following i.v. bolus (saline or LPS) administration, IL was i.v. infused continuously (200 mL/h) for 16h. Cows were fasted for 16h during P2. Liver biopsies were obtained on d1 of P1 and at 16 and 48h postbolus. Effects of treatment, time, and treatment × time interactions were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Mild pyrexia (0.8°C) was observed for 5.5h postbolus in 5.5h bolus in LPSIL relative to CONIL cows (P < 0.01). LPS increased circulating insulin (4-fold) during the IL infusion period relative to CONIL cows (P < 0.01). Circulating glucagon increased 3-fold in LPSIL cows from 8 to 12h post-LPS relative to CONIL (P = 0.03). NEFA concentrations gradually increased in both treatments (3-fold, relative to baseline; P ≤ 0.04), but peaked (43%) higher in CONIL compared with LPSIL cows (P = 0.01). Circulating BHB decreased in both treatments for the first 8h of P2, after which it gradually increased. Infusing IL increased (36%) liver TG content in CONIL cows at 16 h relative to baseline (P = 0.05), but it had no effect on lipid content of LPSIL cows. No treatment differences in liver lipid content were observed at 48h. Relative to CONIL, circulating LPS-binding protein in LPSIL cows increased 2-fold at 8h postbolus then markedly decreased (5-fold; P < 0.01). Serum amyloid A concentrations progressively increased in LPSIL cows during P2 (3-fold, relative to CONIL; P < 0.01). In summary, IL infusion altered the characteristic patterns of insulin and LBP in response to LPS, but did not cause fatty liver.

Key Words: lipid infusion, LPS

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Objectives were to evaluate the relationship between fecal pH, milk yield (MY) and components, DMI, and blood parameters during the transition period. From d −21 to 28 relative to calving, DMI and MY were recorded daily, whereas fecal pH and milk composition were determined weekly from 52 multiparous cows. Based on antepartum pH, cows were retrospectively categorized into 1 of 2 groups: 1) High (AH; top 1/3 of cows, pH >7.3; n = 17) or 2) Low (AL; bottom 1/3 cows, pH <7.1; n = 17); likewise for postpartum pH (PH, pH >6.8 or PL, pH <6.5), and pH change pre- to postpartum (ΔPH or ΔL). Data were analyzed using the MIXED and CORR procedures of SAS. Although magnitude of ΔpH did not affect MY or DMI, these response variables from PL cows were less than those of PH cows 1 wk postpartum (P ≤ 0.03). Decreased antepartum DMI was associated with lower postpartum fecal pH (r = −0.28; P = 0.05). A large fecal pH change from wk-1 to 1 was associated with decreased DMI on wk 3 postpartum (r = −0.33; P = 0.05). Antepartum fecal pH was negatively associated with the rate of MY increase from wk 1–2 (r = −0.31; P = 0.03). The rate of change in fecal pH from wk 1–2 was positively correlated with the rate of increase in MY from wk 1–4 (r = 0.38; P = 0.03). Fecal pH was negatively associated with wk 1–4 SCC (r = −0.28; P = 0.05). Relative to PH cows, those with lower fecal pH postpartum had decreased ECM and concentration of milk fat and lactose 1 wk postpartum (P ≤ 0.04). Postpartum fecal pH was positively correlated with contents of milk fat, lactose, MUN, and ECM for wk 3 postpartum (r = 0.33, 0.39, 0.29, and 0.28, respectively; P ≤0.07). Fecal ΔpH from wk 1 to 1 was positively associated with the slopes of circulating LBP and Hp concentrations from wk 1–postpartum (r = 0.73 and 0.55, respectively; P ≤ 0.03). Change in pH from wk −1 to 1 was positively correlated with NEFA and BHB concentrations postpartum (r = 0.48 and 0.51, respectively; P ≤ 0.04). In summary, fecal pH appears to be moderately associated with production parameters, metabolism and inflammation during the transition period in dairy cows.

Key Words: hindgut acidosis, inflammation


Study objectives were to evaluate effects of hindgut acidosis in cows acclimated to a low-starch diet. Ten rumen-cannulated Holstein cows (243 ± 20 DIM; 663 ± 18 kg BW) acclimated to a low-starch diet (15% starch) for 17d were enrolled in a study with 2 experimental periods (P). During P1 (4d), baseline data were collected. During P2 (7d) cows were randomly assigned to 1 of 2 treatments: 1) control (CON; 1.5 L H2O/infusion) or 2) 4 kg/d of starch (ST; 1 kg corn starch + 1.5 L H2O/infusion) and abomasally infused every 6 h. Daily, milk, feces, and blood samples were collected daily. Effects of treatment, time, and treatment by time interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Starch infusions markedly reduced fecal pH compared with CON (5.8 vs. 7.2; P < 0.01). Rectal temperature of ST cows progressively increased from d 1–3 of P2 (P = 0.04). Milk yield and DMI were unaffected by treatment. Milk protein content of ST cows gradually increased from d 2–5 of P2, while it remained unchanged in CON (P > 0.01). No treatment differences were observed in milk fat, although, it decreased in ST cows compared with P1 (6%; P = 0.10). Milk urea nitrogen decreased in ST cows from d 1–4 of P2 and then plateaued (P > 0.01). No overall treatment differences were observed for SCC during P2, but it decreased in ST cows relative to P1 (29%; P = 0.04). During P2, circulating insulin increased (47%; P = 0.08) in ST relative to CON, however, glucose and BHb concentrations were unaffected by treatment. Starch infusions decreased BUN concentrations from d 1–5 of P2 (38%; P < 0.01) relative to CON. Similarly, NEFA decreased in ST cows (44%; P = 0.07) while they were unchanged in CON cows. Relative to CON, ST infusions increased circulating monocytes on d 3 (77%; P = 0.04) and decreased basophils on d 0.5 and 7 (both 31%; P = 0.01) of P2. Circulating serum amyloid A and lipopolysaccharide binding protein concentrations were unaffected by starch infusion. In summary, although abomasal ST infusion reduced fecal pH and altered energetic metabolism, little to no effects were observed on inflammation and production in cows consuming a low-starch diet.

Key Words: endotoxin, LPS, immune system

477 Oxidative stress pathway components in adipose tissue of Holstein cows during the periparturient period differ by body condition score. Y. Liang1, E. Trevisi2, and J. Loor1, 1University of Illinois, Urbana-Champaign, Urbana, IL, 2Università Cattolica del Sacro Cuore, Milan, Italy.

The periparturient period is characterized by increased oxidative stress status in dairy cows. In non-ruminants, a chronic state of excessive fat deposition is accompanied by inflammation and oxidative stress. The objective was to investigate if prepartal body condition score (BCS) is associated with plasma and adipose tissue biomarkers of oxidative stress in Holstein cows. Twenty 2 multiparous Holstein cows were divided by BCS before parturition (~30 d prepartum) into a BCS ≤ 3.25 (LoBCS, n = 11) or BCS ≥ 3.50 (HiBCS, n = 11) group. Blood sampled from the coccygeal vessel at −30, −15, 7, 10, and 30 d relative to calving date was used for oxidative stress and inflammation biomarkers analysis. Adipose tissue obtained from each group on d −15, 7 and 30 relative to calving date was used for RT-PCR analysis. The statistical model in SAS included the fixed effect of treatment, time and its interaction. There was no difference in prepartal DMI and milk yield between groups (P > 0.05). A treatment × time interaction (P < 0.05) was observed for postpartal DMI due to greater responses in LoBCS cows. Although there was no overall treatment or interaction effect (P > 0.05) for plasma myeloperoxidase and haptoglobin, the fact that ferric-reducing ability (antioxidant capacity) increased between −10 and 7 d (interaction P = 0.01) and reactive oxygen metabolites between −10 and 15 to 30 d (interaction P < 0.01) in HiBCS cows suggested a chronic inflammatory and oxidative stress state. The greater (P < 0.05) overall concentration of carotene in LoBCS cows suggested they had better antioxidant status. Cows with HiBCS had greater abundance of cullin 3 (CUL3) (P < 0.05) and lower overall abundance of factor erythropoietin 2-like 2 (NFE2L2). However, HiBCS cows had greater (P < 0.05) abundance of genes associated with glutathione metabolism including glutathione peroxidase 1 (GPX1) and glutathione reductase (GSR). Regardless of BCS, mRNA abundance of NFE2L2 decreased and KEAP1 increased from 7 to 30 d after parturition. Overall, the data suggest a more pronounced systemic and localized oxidative stress status in cows with prepartal BCS ≥ 3.50.

Key Words: body condition score, dairy cow, oxidative stress