M75 Sodium salicylate reduced transcript abundance of hypoxia-associated genes in MAC-T cells. C. M. Ylioja, T. H. Swartz, L. K. Mamedova*, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Hypoxia is an oxygen deficiency commonly found in growing tissues and is speculated to occur in the rapidly developing mammary gland in peripartum dairy cattle. Low oxygen concentrations can activate hypoxia-inducible factor-1 (HIF-1), which increases transcription of genes involved in angiogenesis (*VEGF*) and glucose transport (*GLUT1*). The mRNA stability of these genes is positively regulated by AUF1. In our previous research, postpartum administration of sodium salicylate (SS) increased whole lactation milk yield in multiparous cows but tended to reduce milk yield in primiparous. Because rapid mammary tissue development likely occurs in cows approaching first lactation, we hypothesized that SS inhibited the activation of HIF-1α and decreased transcription of downstream targets. MAC-T cells were treated with SS (100 μM) or control media before incubation under either hypoxic (1% O_2) or normoxic conditions for 12 h. Additionally, cells were transfected with either HIF1α siRNA or a scrambled siRNA negative control 48 h before hypoxia treatments. *HIF1α*, *GLUT1*, *VEGF*, and *AUF1* were quantified using the $2^{-\Delta Ct}$ method and normalized to the internal control gene *NENF*. Transcript abundance was assessed using a linear mixed model with the fixed effects of SS, hypoxia, and siRNA and all 2- and 3-way interaction terms, and the random effect of plate nested within hypoxia. SS tended to decrease *HIF1α* as compared with untreated cells ($P = 0.09$). For *GLUT1*, SS treatment interacted with hypoxia ($P = 0.05$), as SS reduced *GLUT1* when MAC-T cells were cultured in normoxic conditions ($P < 0.01$), however, no effect of SS was found in hypoxia-treated cells ($P = 0.39$). Regardless of oxygen status, SS reduced *VEGF* ($P = 0.04$) and *AUF1* ($P = 0.04$) relative to untreated cells. Hypoxia increased *GLUT1* ($P = 0.01$), yet no effect was identified on *VEGF* ($P = 0.45$) or *AUF1* ($P = 0.22$). siRNA knocked down *HIF1α* ($P < 0.01$), but no effect was found on *GLUT1* ($P = 0.98$), *VEGF* ($P = 0.99$), or *AUF1* ($P = 0.62$). In conclusion, SS reduced transcript abundance of genes involved with mammary gland development, but generally did not interact with oxygen status.

Key Words: hypoxia, NSAID, mammary gland development

M76 Circadian *PER2* gene silencing suppresses lipid synthesis partly via inhibition of *PPARG* and *SREBF1* in bovine mammary epithelial cells. Y. J. Jing[1], Y. F. Chen*[1], M. Z. Wang[1], L. Y. Hu[1], Q. Y. Xu[1], Z. N. Xi[1], and J. J. Loor[2], [1]*Yangzhou University, Yangzhou, Jiangsu, China*, [2]*University of Illinois, Urbana, IL.*

In non-ruminants it is well-established that biological rhythms play a profound role in coordinating whole-body metabolism. In dairy cows there is evidence that milk yield and milk fat content have rhythmic pattern thought to be regulated by circadian rhythms. The core circadian clock gene period 2 (*PER2*) is associated with mammary gland development and lipid synthesis in rodents, partly via regulating peroxisome proliferator-activated receptor gamma (PPARG). Whether such type of molecular link between circadian clock and lipid metabolism exists in bovine is unclear. We hypothesized that PER2 is associated with lipid metabolism in bovine mammary cells. To test this hypothesis, the bovine mammary tissue samples were obtained from 3 mid-lactation (averaged 110 d postpartum) cows and digested by collagenase to gain the primary bovine mammary epithelial cells (BMECs). Small interfering RNA (siRNA) technology was used to inhibit PER2 expression in primary BMECs. The primary BMECs were transfected with 3 siRNAs at 0, 12, 24, 36, 48, 60 h to screen out the best siRNA and its transfection time point. The lipid droplet was measured by red oil O staining, and the triacylglycerol (TAG) content of BMEC was determined with the tissue triglyceride assay kit (APPLYGEN, China). The lipid droplet and TAG content were determined at 36 h (36 h showed the greatest PER2 gene inhibitory effect of 84.7%) after the siRNA transfection. One-way ANOVA and Duncan's multiple comparison were used to conduct statistical analysis by SPSS software version 22.0 (statistical significance set at $P < 0.05$). Silencing of PER2 led to lower concentration of cellular lipid droplets and TAG levels in BMECs ($P < 0.05$). In addition, PER2 silencing downregulated mRNA of *ACACA*, *FASN*, *LPIN1* and *SCD* ($P < 0.05$), indicating an overall inhibition of lipogenesis and desaturation. The downregulation of PPARG and SREBF1 in response to PER2 silencing underscore the importance of circadian clock signaling and transcriptional regulation of lipogenesis. Therefore, data suggest that PER2 participates in the coordination of mammary lipid metabolism and may be a component of the control of lipid droplet and TAG synthesis in mammary cells.

Key Words: *PER2* silencing, mammary epithelial cell, lipid metabolism

M77 Milk fatty acid profiles of beef cows in response to a short feed restriction during lactation. I. Casasús*, J. R. Bertolín, K. Orquera, J. Ferrer, and M. Blanco, *Ctr Invest y Tecnol Agroal Aragon (CITA), IA2 (CITA-Universidad de Zaragoza), Zaragoza, Spain.*

The relationship between energy balance and the milk fatty acid (FA) profile is well established in dairy cows but has received little attention in beef cattle. We analyzed the milk fatty acid profile of 16 Parda de Montaña beef cows 2 mo post-calving in response to a 4-d (d) dietary restriction (55% of energy requirements, 6.2 kg dry matter (DM) hay/d), as compared with a previous basal and an 8-d refeeding period (100% of requirements; 7.0 kg DM/d hay + 2.7 kg DM/d concentrate). With d0 as the start of restriction, milk was sampled on days d-2 (basal), d1, d3 (restriction) and d5, d6, d8 (refeeding). Individual FA were identified by gas chromatography, and sums of FA were calculated (saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), cis-MUFA, trans-MUFA, C4-C15 de novo synthesis FA and C16-C24 mobilization FA). These sums and the 4 major FA (C16:0, C18:1–9c, C18:0, C14:0) were analyzed using mixed models, with day as fixed and cow as random effects. All the results presented here were significant at $P < 0.001$. The milk FA profile responded immediately to changes in the energy balance and/or the diet. On d1 of restriction, the concentrations of SFA decreased, mainly due to a reduction in the de novo synthesis FA and C16. A concomitant increase in MUFA (associated with that of C18:1–9c, predominant in body fat) was observed. These changes, along with the increments in C16-C24 FA, indicate an enhanced fat mobilization from the adipose tissue. During the restriction, C18:0 and trans-MUFA decreased while *cis*-MUFA and PUFA increased, as a result of both the mobilization and the change in diet composition. The opposite occurred in the refeeding phase. On d5, MUFA decreased (due to the reduction in C18:1–9c) and SFA increased because of the rise in the de novo synthesis FAs and C16:0, reflecting the reversion of fat mobilization. At the end of refeeding (d8), the individual FA returned to basal concentrations, but the sum of C16-C24 mobilization FAs was even lower and that of C4-C15 de novo synthesis FAs was higher than basal values, indicating a possible "rebound effect" after restriction and refeeding.

Key Words: beef cows, nutritional challenge, milk fatty acid profile

M78 Effects of glucose and acetate infusion on mammary uptakes of essential amino acids by lactating dairy cows. B. Li*[1], R. Laforest[1], L. Wright[1], J. Kim[1], P. Kedzierski[1], V. Osborne[1], J. Doelman[1,2], and J. Cant[1], [1]*University of Guelph, Guelph, ON, Canada*, [2]*Trouw nutrition, Putten, the Netherlands.*

Previous research suggests that glucogenic energy can stimulate milk protein yield of dairy cows while lipogenic energy does not. To explore differences in mammary essential amino acid (EAA) utilization between these types of energy, 5 rumen-fistulated cows were given additional glucose or acetic acid in a 5 × 5 Latin square design. Infusion treatments were: