Effect of methionine in membrane traffic for milk secretion in the goat's mammary epithelial cell

Marion Boutinaud, Antoine Leduc, Sophie Lemosquet, L Balhoul

1) 10 L/d ruminal water (CTL), 2) 1.1 kg/d (LoG) or 3) 2.2 kg/d (HiG) abomasal glucose, and 4) 1.25 kg/d (LoA) or 5) 2.5 kg/d (HiA) ruminal acetic acid. Acetic acid and glucose infusion rates were isocaloric at low and high levels, respectively. Milk yields were recorded daily and milk samples were collected on the last 3 d of each 7-d infusion period. Tail and mammary venous samples were collected on d 7 to estimate mammary uptakes. Plasma samples for each cow were pooled over time by period. Plasma AA concentrations for each time point were analyzed using Ultra Performance Liquid Chromatography in conjunction with Empower Chromatography Data Software (Waters Corporation, Milford, MA). Linear contrasts of glucose and acetic acid dose were estimated by ANOVA assuming fixed effects of period and treatment, and random effects of cow. Glucose infusion had no effect on DMI ($P = 0.97$) or milk protein yield ($P = 0.15$) but increased lactose yield ($P = 0.03$) and tended to increase milk yield ($P = 0.07$). Acetic acid infusion dramatically decreased DMI from 18.8 kg/d on CTL to 13.8 and 14.9 kg/d on LoA and HiA respectively. Milk yield decreased 5.4 kg/d, protein yield decreased 201 g/d and lactose yield decreased 224 g/d on HiA compared with CTL ($P \leq 0.01$) due to the decrease of DMI. Glucose infusion decreased arterial concentrations of all EAA ($P < 0.01$) except Met and Thr, but increased mammary plasma flow (MPF) rate ($P < 0.01$), so that mammary uptakes of EAA were not affected ($P > 0.16$). In contrast, acetic acid infusion increased concentrations of Ile, Leu, and Val ($P < 0.08$) without affecting other EAA concentrations ($P > 0.20$), had no effect on MPF ($P = 0.70$), and decreased mammary uptakes of Arg, Ile, Leu, and Phe ($P < 0.10$). Findings suggest that exogenous glucose encouraged milk protein production despite reduced plasma concentrations of some EAA, while exogenous acetic acid discouraged milk protein yield thereby increasing concentrations of some EAA.

Key Words: cow, amino acid, milk protein

M79 Effect of methionine in membrane traffic for milk secretion in the goat's mammary epithelial cell. M. Boutinaud*[1], A. Leduc[1], S. Lemosquet[1], and L. Bahloul[2], [1]*INRAE, Agrocampus Ouest, PEGASE*, *Saint-Gilles, France*, [2]*Centre of Expertise and Research in Nutrition, Adisseo France S.A.S*, *Commentry, France*.

Methionine (Met) supplementation increases milk, protein and fat yields in cow. We investigated whether this could be partly explained by an increasing flow of milk components in the secretory pathways of mammary epithelial cells. Multiparous Alpine goats at mid lactation (n = 48), grouped by levels of expression of the *CSN1S1*, were assigned to 4 treatments in a randomized complete block design. Goats were fed a fixed amount of hay and a low (LE, 1.47 Mcal/kg DM) or adequate (AE, 1.54 Mcal/kg DM) energy concentrate combined with 2 levels of metabolizable Met: unbalanced vs. balanced using isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (HMBi 0.24% concentrate DM) to cover 100% of Met requirement, based on cow requirement (INRA, 2007). Treatments were: LE, LEMet (LE, balanced Met), AE and AEMet (AE, balanced Met) for 5 weeks. Goats (23) were slaughtered and mammary tissue was processed for Western blotting using secretory compartment specific markers of membrane traffic. Milk protein yield ($P = 0.01$) and casein content ($P = 0.01$) increased in goats fed the Met balanced diets. The amount of the endoplasmic reticulum (ER) markers, Cnx and ERLIN2, decreased (20%, $P \leq 0.05$) in goats fed the LE diet. Met balanced diets had the opposite effect on both markers (20%, $P \leq 0.05$), as well as on protein disulfide isomerase (45%, $P \leq 0.05$). These observations are in agreement with a positive effect of Met on the activity of the ER, the site where protein and lipid are synthesized. On the other hand, a specific marker of the exit site of the Golgi apparatus and secretory vesicles formation (AP1) decreased with the LE diet (25%, $P \leq 0.05$) and its highest level was found in goats fed Met balanced diet at AE supply. The higher ßCOP ($P = 0.01$), a marker of intra Golgi transport, clearly reflected a decrease in membrane transport of LE diets. These data strongly suggest that energy level has a direct impact on membrane traffic in the secretory pathway of mammary epithelial cell while Met improve ER activity and has the tendency to further promote intracellular transport of milk components and, ultimately, their secretion.

Key Words: amino acid supplementation, dairy goats

M80 Effect of heat stress during the dry period on estradiol and prolactin interactions in mammary gland gene expression of Holstein cows. J. A. Negrao*[1,2], V. Ouellet[2], M. Marrero-Perez[2], T. F. Fabris[2], J. Laporta[2], and G. E. Dahl[2], [1]*University of Sao Paulo*, *Pirassununga, SP, Brazil*, [2]*University of Florida*, *Gainesville, FL*.

The dry period, a 6 to 8-week nonlactating state between lactations, is essential for maximal mammary development and lactation in dairy cows. Although late-gestation heat stress decreases estrogen (E) and increases prolactin (PRL) concentrations in blood, those impacts on mammary development remain unclear. The objective of this study was to determine how late gestation heat stress-induced E and PRL alterations affect the expression of their receptors and signaling in the mammary gland at different stages of the dry period. Fourteen cows were either exposed to in vivo heat stress (HT, n = 7) or active cooling by fans and soakers (CL, n = 7) for the entire dry period (~45 d). Mammary gland biopsies were performed on d 3 (i.e., involution) and 35 (i.e., proliferation) relative to dry off and equally divided in 3 explants, that were incubated in vitro for 24h in 1 of the 3 mediums: 1- Basal (Bm no PRL or E); 2- CL mimic (Cm: basal + 20ng/mL PRL + 5.8ng/mL E; and 3- HT mimic (Hm: basal + 40 ng/mL PRL + 2.9ng/mL E). Gene expression of *PRLR-SF*, *PRLR-LF*, *ESR1* and *ESR2* were measured using Real Time qPCR. An ANOVA using the mixed procedure of SAS was performed to assess the impacts of in vivo (HT, CL), in vitro (Bm, Cm, Hm) treatments and their interaction on relative transcript expression. Dry cows subjected to HT had increased rectal temperature and respiration rate relative to cows subjected to CL (39.1 vs 38.8 ± 0.01°C and 65.2 vs 55.4 ± 1.2 breaths/min, respectively), which confirms cooling conditions are effective and necessary for the thermal equilibrium of the CL cows. *In vivo* HT increased the expression of *PRLR-LF* relative to CL. However, Hm in vitro treatment decreased the expression of *PRLR-SF, ESR1* and *ESR2* relative to Bm treatment. These results suggest that E and PRL alterations caused by heat stress exposure can modulate the expression of receptors in the mammary gland, with potential implications for normal mammary development during the dry period.

Key Words: heat stress, mammary explants, culture

M82 Evaluation of breed and udder characteristics on somatic cell count and udder pathogens in lactating Holstein and Jersey cows. B. M. Brown, M. W. Hollis*, and J. G. Carter, *Middle Tennessee State University*, *Murfreesboro, TN*.

The objective of this study was to evaluate the impact of physical udder characteristics and breed on hygiene scores (HS), milk yield (MY), conductivity (COND), SCC, and bacterial cultures (BC) in lactating Holstein and Jersey dairy cows housed in a compost-bedded pack barn. Lactating Holstein and Jersey (n = 10 each) cows were evaluated during a 6-wk period. Milk samples were collected as a sterile composite from all 4 quarters during one milking/wk and SCC was determined using the DeLaval Cell Counter DCC. If SCC ≥350,000 cells/mL, the sample was cultured to determine bacterial species. Milk samples were cultured using a Tri-plate agar including Factor, MacConkey, and Focus media (University of Minnesota Easy Culture). Cows were evaluated once/wk using a multi-zone hygiene scoring system for udder cleanliness (1 = very clean to 4 = very dirty; Cook, 2002). Udder measurements were taken during wk 1 and included udder depth and circumference, and teat length. Milk yield and COND were measured daily and averaged by wk using the AfiMilk parlor system (Afimilk, Kibbutz Afikim, Israel). Statistical analysis of MY, COND, and udder measurements were conducted using the MIXED procedure in SAS (v9.4). Analysis of HS incidence and BC species counts were evaluated using the FREQ procedure in SAS (v9.4). No differences in BC, SCC, or physical udder characteristics were observed among breeds. Holstein