430 Glucose activates translation factors in muscle but not in mammary glands of lactating dairy cows when essential amino acids are in excess supply. Kelly Nichols*[1], Michelle Carson[2], Julie J. M. Kim[1], John A. Metcalf[2], John P. Cant[1], and John Doelman[2,1], *[1]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada, [2]Nutreco Canada Agresearch, Guelph, ON, Canada.*

To determine how glucose modulates protein synthesis when essential amino acids (EAA) are supplied in excess, 5 early-lactation Holstein cows (78 ± 13 DIM) were abomasally infused for 5 d with EAA and glucose in a 5 × 5 Latin square design, and abundances and phosphorylation states of ribosomal S6 kinase 1 (S6K1), eukaryotic initiation factor 4E binding protein-1 (4EBP1) and eukaryotic initiation factor 2α (eIF2α) in mammary tissue and longissimus dorsi were assayed. Treatments were saline, 844 g/d EAA in the profile of casein, 1126 g/d EAA, 844 g/d EAA + 1000 g/d glucose, or 1126 g/d EAA + 1000 g/d glucose. Cows were fed a diet containing 6.96 MJ/kg NE_L and 12% crude protein. Measurements were subjected to ANOVA using PROC MIXED in SAS, where cow was a random effect. While no differences were observed between levels of EAA, compared with saline, infusion of EAA increased arterial concentrations of EAA 3- to 4-fold ($P < 0.01$), increased mammary uptake of EAA from plasma 66% ($P < 0.01$), and led to 256 g/d higher milk protein yield ($P < 0.01$). The addition of glucose to EAA infusions decreased Ile, Leu and Val concentrations by 29% ($P < 0.01$), but did not affect mammary uptake of any amino acids ($P > 0.40$) or milk protein yield ($P = 0.32$). Infusion of EAA increased the mammary abundance of S6K1 ($P = 0.01$) and tended to increase phosphorylated S6K1 abundance ($P = 0.09$), indicating activation of mRNA translation. Infusion of glucose tended to increase the mammary abundance of phosphorylated eIF2α ($P = 0.09$) and decrease the total abundance of eIF2α ($P = 0.07$), both of which are inhibitory to mRNA translation. In muscle tissue, EAA infusion increased the phosphorylation state of 4EBP1 ($P = 0.02$), which is stimulatory to mRNA translation. When glucose was added, the phosphorylation state of 4EBP1 increased ($P = 0.11$), and total S6K1 abundance tended to increase ($P = 0.11$). Thus, EAA activated regulators of mRNA translation in both mammary glands and skeletal muscle, while the addition of glucose activated pathways of mRNA translation in muscle tissue but not in the mammary glands.

Key Words: protein synthesis, mammary, muscle

431 Early postnatal plane of nutrition of Holstein calves has an effect on milk production and feed intake during their first lactation. Steffi Wiedemann*[1], Patricia Holz[2], Hans-Juergen Kunz[3], and Martin Kaske[4], *[1]Animal Health, Institute of Animal Breeding and Husbandry, Kiel University, Kiel, Germany, [2]Clinic for Cattle, University of Veterinary Medicine Hannover Foundation, Hannover, Germany, [3]Chamber of Agriculture of Schleswig-Holstein, Blekendorf, Germany, [4]Department for Farm Animals, University of Zurich, Zurich, Switzerland.*

The objective of the study was to assess the effect of 2 different feeding strategies during the very early postnatal life of Holstein calves on subsequent first lactation milk production and feed intake. During the first 4 wk of life, calves were fed either ad libitum (AdL; ad libitum feeding of whole milk during wk 1 and milk replacer ([MR], 160 g/L) from d 8–28; n = 38) or according to a restrictive feeding protocol (RES; 4 L milk/d during wk 1, 6 L MR (120 g/L) from d 8–28; n = 30). Feeding was similar in both groups after the 4th wk of life. All animals were kept individually during the first wk of life and in groups thereafter. The feed intakes were analyzed during the first 10 wk of life in all animals and during first lactation in 37 animals. Daily milk yield and monthly milk composition were recorded. Total energy intake during the first 4 wk was higher in AdL-calves compared with RES-calves (16.6 MJ ME/d vs. 10.2 MJ ME/d, respectively; $P < 0.01$). Thereafter, no difference in energy intake was observed until the 10th wk of life. In AdL-calves the average daily gain was higher compared with RES-calves during the first 4 wk of life (0.72 vs. 0.45 kg/d; $P < 0.001$), while age at first calving did not differ (765 vs. 777 d; $P = 0.30$). In the AdL- and RES-group, 21 and 26 animals remained on the farm for a full 305-d first lactation, respectively. The FCM yield was higher in AdL-animals compared with RES-animals (29.2 ± 0.4 vs. 28.0 ± 0.4 kg FCM/d; $P < 0.05$); but milk composition did not differ. The higher FCM yield was accompanied by a higher feed intake in AdL-animals (19.3 kg DM/d vs. 18.8 kg DM/d; $P < 0.01$). The results indicate that an increased feeding intensity during early life has positive long-term effects on the milk production potential in the first lactation, which could be the result of an improved girth or height or of an enhanced development of mammary parenchyma.

Key Words: calf feeding, feed intake, milk production

432 Interrelation of somatic cell count, lactate dehydrogenase, and immunoglobulin G during mastitis caused by different pathogens. Lorenzo E. Hernández-Castellano*[1], Samantha K. Wall[1], Roger Stephan[2], and Rupert Bruckmaier[1], *[1]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zürich, Switzerland.*

Somatic cell count (SCC) is the most widely used mastitis indicator. However, immunoglobulin G (IgG), part of the specific immune system, is transferred from blood to milk, and the extent of this transfer appears to be pathogen specific. As IgG measurement is not available for farmers, milk lactate dehydrogenase (LDH) activity has been considered a suitable mastitis indicator and a marker for the presence of IgG in milk. This study aims to analyze the correlation and linear regression of the variables SCC, LDH and IgG in mastitis produced by different bacteria, and to determine if LDH can be used as an indicator for elevated IgG. Four quarters of 38 cows on 2 dairy farms with automatic milking systems were sampled. Selection criteria was based on the composite milk SCC (>100,000 cell/mL) from each cow. Samples were measured for SCC, LDH, IgG and cultured for bacteriology. SCC, LDH and IgG data were analyzed using the CORR and GLM procedures of SAS. When milk samples were not grouped by bacterial population, IgG and LDH were the highest correlated variables (r = 0.49) compared with IgG and SCC (r = 0.41) or LDH and SCC (r = 0.41). Prediction equations for all variables had an R-square ≤0.24. After quarter milk samples were classified by bacteria (control, *S. aureus*, *C. bovis*, *E. coli*, other *Staphylococcus* spp. and *S. uberis*), different correlations and regression patterns were observed. Control and infected quarters showed a positive correlation between LDH and SCC, with the exception of those infected with *E. coli* and *S. uberis*. A positive correlation between SCC and IgG was observed in quarters where *C. bovis* was identified. LDH/SCC regression slopes differed from the control group. Several differences between LDH/SCC and LDH/IgG regression slopes were observed among bacterial infections. In conclusion, LDH appears to be a good indicator for some bacterial infections (namely *C. bovis*); however the use of both SCC and

LDH may increase the mastitis detection rate, particularly infections caused by *C. bovis*, other *Staphylococcus* spp. and *S. aureus*.

Key Words: mastitis, lactate dehydrogenase, SCC

433 The innate immune response of bovine mammary epithelial cells to live or heat-inactivated *Mycoplasma bovis*. Christina Zbinden*[1,3], Paola Pilo[2], Joachim Frey[2], Rupert M. Bruckmaier[1], and Olga Wellnitz[1], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Institute for Veterinary Bacteriology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [3]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.*

Although *Mycoplasma bovis,* an emerging etiological agent of bovine mastitis, lack classical virulence factors, they induce an immune reaction and inflammation in the host. Because effects on the bovine mammary immune system are not yet well characterized, this study aimed to investigate the immunogenic effects of *M. bovis* on the mammary gland in an established primary bovine mammary epithelial cell (bMEC) culture system. Primary bMEC in 4th passage of 4 different cows were challenged with live or heat-inactivated *M. bovis* strain JF4278 isolated from acute bovine mastitis in Switzerland with a multiplicity of infection (MOI) of 108, as well as with the type strain PG45 with a MOI of 30. The immune response of bMEC was evaluated after a co-incubation with mycoplasmas for 6, and 24 h at 37°C by measuring the relative mRNA expression of important immune factors by quantitative PCR. Live JF4278 *M. bovis* triggered a considerable immune response in bMEC ($P < 0.05$), reflected by the upregulation of relative mRNA expression (ΔΔCT) of tumor necrosis factor (TNF)-α (6 h: 4.5 ± 0.9; 24 h: 4.9 ± 0.4 threshold cycles [CT]), interleukin (IL)-1β (6 h: 7.9 ± 1.0; 24 h: 8.1 ± 0.7 CT), IL-6 (24h: 2.1 ± 0.4), IL-8 (6 h: 5.6 ± 0.9; 24 h: 7.1 ± 1.1 CT), lactoferrin (after 24 h: 4.4 ± 1.0 CT), Toll-like receptor (TLR)-2 (6 h: 1.9 ± 0.3 CT), and serum amyloid A (SAA; 24 h: 8.5 ± 0.7 CT). For live type strain PG45, very similar results were obtained; that is, a significant induction of TNF-α (6 h: 4.9 ± 0.4; 24 h: 4.4 0.5 CT), IL-1β (6 h: 7.9 ± 1.1; 24 h: 9.2 ± 0.5 CT), IL-8 (6 h: 5.6 ± 0.3; 24 h: 7.6 ± 1.0 CT), lactoferrin (6 h: 1.1 ± 0.2; 24 h: 4.0 ± 1.4 CT), TLR-2 (24 h: 2.3 ± 0.7 CT), and SAA (24 h: 8.5 ± 0.7 CT). Interestingly, this cellular reaction was only observed in response to live, but not to heat-inactivated *M. bovis*. This study provides evidence that bMEC exhibit a strong inflammatory reaction in response to live *M. bovis*. The lack of a cellular response to heat-inactivated *M. bovis* may indicate that its intracellular localization or secondary metabolites are involved in *M. bovis* pathogenesis.

Key Words: bovine mastitis, *Mycoplasma bovis*, innate immune response

434 Heat stress and amino acid supplementation affected dramatically the expression of genes related to mammary cell activity and number. A. A. K. Salama*[1], M. Duque[2], K. Shahzad[3], and J. J. Loor[3], *[1]Grup de Recerca en Remugants (G2R), Departament de Ciència Animal i dels Aliments, Universitat Autónoma de Barcelona, Bellaterra, Spain, [2]Grupo de Investigación Biogénesis and GRICA. Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellín, Colombia, [3]Department of Animal Sciences, University of Illinois, Urbana; IL.*

Heat stress (HS) causes reductions in milk yield and content of fat and protein. It is not clear whether these losses are due to reduced number or activity (or both) of mammary epithelial cells. To test mechanisms by which mammary metabolism is impaired by HS, MAC-T cells were incubated in different ambient temperature conditions: thermo-neutral (TN; 37°C) and heat stress (HS; 42°C). In both conditions, 3 mediums varying in amino acid (AA) concentrations were used. These media were: optimal amino acid profile as control (Con), control plus methionine (Met), and control plus arginine (Arg). Consequently, there were 6 treatment combinations: TN-Con, TN-Met, TN-Arg, HS-Con, HS-Met and HS-Arg. After incubation, cells were harvested and RNA was extracted for the study of gene expression by quantitative RT-PCR. Both HS and AA increased ($P < 0.01$) the expression of heat shock proteins (HSP70A1A), transcription and translation factors (RPS6KB1, JAK2, AKT2), and AA transporters (SLC1A5, SLC7A1). The expression of PPARG (fat transcription regulation), FASN (de novo fatty acids synthesis), BCL2L1 (anti-apoptotic), and AKT1 (cell survival) decreased ($P < 0.05$) with HS, but increased ($P < 0.05$) with AA. Furthermore, HS downregulated ACACA (de novo fatty acids synthesis) and upregulated BAX (apoptotic). Supplementation with Met or Arg upregulated ($P < 0.05$) genes related to transcription and translation (MAPK1, MTOR, SREBF1), cell proliferation (MKI67), and insulin signaling (IRS1). The expression of EIF4EBP1 (inhibitor of protein synthesis) was upregulated by HS, but downregulated by Met. Results suggest that heat stress exerts its negative effect on milk production at least in part by inhibiting mammary synthetic capacity as well as increasing apoptosis of mammary cells without affecting cell proliferation. Supplementation with AA (especially Met) increased mammary synthetic activity and had a positive effect on cell number by increasing proliferation and decreasing apoptosis. This raises the possibility that supplemental rumen-protected Met during heat stress might have a positive effect on mammary metabolism.

Key Words: mammary cells, gene expression, heat stress

435 Effects of different lysine/methionine pattern and glucose level on expression of the key genes involved in milk protein transcription and translation in bovine mammary epithelial cells. F. Wang[1], J. Q. Wang[1], D. P. Bu*[1,2], X. M. Nan[1,3], and S. Lian[1], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]CAAS-ICRAF Joint Laboratory of Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, [3]Synergetic Innovation Center of Food Safety and Nutrition, Harbin, China.*

The synthesis of milk protein requires the availability of amino acids and a large supply of energy. Lysine, methionine and glucose regulate milk protein production of lactating dairy cows in vivo. Moreover, the content and ratio of lysine to methionine can regulate milk protein synthesis via cellular signaling pathways involving JAK2-STAT5 and mammalian target of rapamycin (mTOR). This study was conducted to investigate the effects of different lysine/methionine pattern and glucose level on milk protein synthesis in vitro, mainly focused on genes related to JAK2-STAT5 and mTOR. Primary bovine mammary epithelial cells (BMEC) were obtained from Holstein dairy cows and cultured in Dulbecco's modified Eagle's medium-F12 medium containing 10% fetal bovine serum. BMEC were subjected to 4 treatments arranged in a 2 × 2 factorial design with lysine/methionine ratio (3: 1 vs. 2.3: 1, namely balanced vs. unbalanced) and glucose level (17.5 m*M* vs. 2.5m*M*, namely high vs. low) as 2 factors. In this experiment, total casein content, cell proliferation and genes expression related to JAK2-STAT5 and mTOR pathways were measured. Compared with low level of glucose groups, casein content and cell proliferation increased in groups with high level of glucose ($P < 0.05$). Casein content was also higher in lysine/methionine balanced groups than in unbalanced groups

($P < 0.01$). Expression of *CSN1S2, CSN2, LALBA, STAT5, ELF5, mTOR* ($P < 0.01$) and *CSN1S1* ($P < 0.05$) were upregulated in groups with high level of glucose compared with low level groups. The upregulation of *CSN1S2, CSN2, LALBA, JAK2, STAT5, ELF5, mTOR* ($P < 0.01$) and *CSN1S1, CSN3* ($P < 0.05$) were also observed in Lysine/Methionine balanced groups compared with unbalanced groups, while *EIF4EBP1* was downregulated ($P < 0.05$). In conclusion, proper ratio of Lysine to methionine and high level of glucose may directly accelerate the BMEC proliferation and regulate the expression of genes related to milk protein transcription and translation, which can increase milk protein synthesis.

Key Words: mammary epithelial cell, glucose, amino acid

436 *Trans*-10,*cis*-12 CLA regulates SREBP1 activation in bovine mammary epithelial cells through proteasomal degradation of Insig1. Liang Chen*, Andrea Lengi, and Benjamin Corl, *Virginia Tech, Blacksburg, VA.*

trans-10,*cis*-12 conjugated linoleic acid (t10,c12-CLA) was linked to milk fat depression in dairy cows; transcription factor sterol response element binding protein-1 (SREBP1) regulates fatty acid synthesis. SREBP1 activation and migration to the nucleus requires the removal of Insig1, a protein that anchors SREBP1 in the endoplasmic reticulum membrane. The molecular basis orchestrating the effect of t10,c12-CLA on bovine SREBP1 activation has not yet been elucidated. We hypothesize that t10,c12-CLA reduces SREBP1 activation through delay of Insig1 degradation. In the present study, we employed a bovine mammary epithelial cell line (Mac-T) and found that, mRNA and protein levels of SREBP1 declined over 56% when cells were treated with 60 μ*M* or greater t10,c12-CLA for 24 h ($P < 0.05$). Similar dose effects were observed in the mRNA expression of SREBP1-regulated genes including FAS, SCD1, and Insig1. Compared with 0 μ*M* t10,c12-CLA, 60 μ*M* or higher CLA increased Insig1 protein expression over 2-fold in cells transfected with FLAG-tagged Insig1 ($P < 0.05$). The effect was greater with t10, c12-CLA than other fatty acids including cis9, trans11-CLA, linoleic acid, or oleic acid when cells were treated with 75 μ*M* for 6 h. Further investigation revealed that increased FLAG-Insig1 was due to the inhibitory effect of t10,c12-CLA on the proteasomal degradation of Insig1. Cells treated with 75 μ*M* t10,c12-CLA or 10 μ*M* MG132, a proteasome inhibitor, for 6 h had 2.5-fold greater accumulation of FLAG-Insig1 compared with 0 μ*M* t10,c12-CLA ($P < 0.05$). The degradation of FLAG-Insig1 was delayed when cells were treated with 75 μ*M* t10,c12-CLA for 6 h. These findings suggest that t10,c12-CLA plays a role in regulating SREBP1 activation by reducing proteasomal degradation of Insig1. We conclude that stabilized Insig1 retains SREBP1 in the ER preventing activation and migration to nucleus, thus reducing lipogenic gene transcription.

Key Words: conjugated linoleic acid, SREBP1, Insig1