W297 Regulatory effects of individual essential amino acids on casein synthesis rates in bovine mammary tissue slices. J. A. D. R. N. Appuhamy*, T. R. Wiles, and M. D. Hanigan, *Department of Dairy Science, Virginia Polytechnic Institute and State University*, *Blacksburg*.

In mammalian cells, amino acids are able to stimulate protein synthesis by phosphorylating mammalian target of rapamycin (mTOR) and ribosomal protein S6 (rpS6), and dephosphorylating eukaryotic elongation factor 2 (eEF2). Little work has explored the effects of these signals on milk protein synthesis in bovine mammary glands. The objective of this study was to investigate the effects of individual essential amino acid (EAA) deficiencies on cellular signals and protein synthesis rates in mammary tissue slices. Slices were prepared from the rear quarter of four lactating cows immediately after slaughter and incubated in serum free DMEM/F12 media containing all EAA (+EAA); media deprived of Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, or Val; or media deprived of all EAA (-EAA). After 1 h, 2D5-Phe was dosed into media and incubations continued for another 30 min. Cell lysates were subjected to Western immunoblotting analysis to determine total and phosphorylated mTOR (Ser2448), rpS6 (Ser235/236), and eEF2 (Thr56) and GC-MS analysis to determine 2D5-Phe enrichment (proportional to protein synthesis rates) in protein precipitated at pH=4.6 (enriched for casein). The –EAA treatment caused the phosphorylation state (PS) of mTOR and rpS6 to decrease by 46 and 76%, respectively, PS of eEF2 to increase by 136%, and protein synthesis to decrease by 46%. Phosphorylation of mTOR was positively correlated (r=0.80) with Phe enrichment. Deprivation of Leu resulted in the greatest reduction of PS for mTOR (46%) and rpS6 (51%) whereas Met deprivation was associated with the greatest PS increase for eEF2 (64%). The greatest reductions in protein synthesis were associated with Ile (58%), Leu (47%), and Met (45%) deprivations. Essential amino acids, in particular Leu and Met had substantial regulatory effects on protein synthesis efficiency in bovine mammary tissues, and a significant proportion of the signaling appeared to be mediated by mTOR.

Key Words: essential amino acid deprivation, casein synthesis rate, mTOR

W298 In vivo effects of insulin and dietary protein level on signaling proteins for protein synthesis in the mammary glands of lactating dairy cows. W. A. D. Nayananjalie*[1], A. G. Rius[1], D. Kirovski[2], J. A. D. R. N. Appuhamy[1], J. Escobar[1], and M. D. Hanigan[1], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg,* [2]*University of Belgrade*, *Serbia*.

Insulin and amino acids have been found to significantly enhance muscle protein synthesis by signaling through mammalian target of rapamycin (mTOR) and in turn ribosomal protein S6 (rpS6), eukaryotic initiation factor 4E binding protein 1 (4EBP1), and eukaryotic elongation factor 2 (eEF2). Insulin also stimulates mTOR through Akt. Increased phosphorylation (PhS) of Akt, mTOR, rpS6 and 4EBP1 and decreased PhS of eEF2 stimulates protein synthesis. We hypothesized that insulin and amino acid supply would also cause a change in the PhS of these proteins to promote protein synthesis in bovine mammary glands. The effects of infused insulin and varying dietary protein on total and phosphorylated forms of Akt, mTOR, rpS6, 4EBP1, and eEF2 in mammary tissues of lactating dairy cows were examined. Cows were fed two levels of dietary crude proteins (17.5 or 14.0% of dietary DM) and infused with two levels of insulin (0 or 1 μg/kg BW) under euglycemic conditions in a 2 × 2 factorial design. At the end of each treatment period mammary tissue biopsies were harvested. Tissue homogenates were subjected to Western immunoblotting analysis for total and phosphorylated forms of Akt (Ser473), mTOR (Ser2448), rpS6 (Ser235/236), 4EBP1 (Thr37/46), and eEF2 (Thr56). Increasing dietary protein level increased ($P = 0.02$) PhS of Akt. Neither dietary protein nor insulin had significant effects on PhS or total mTOR expression. However, increased dietary protein and insulin infusion significantly increased both the PhS ($P < 0.01$) and total 4EBP1 ($P = 0.03$) expression. Total rpS6 expression ($P = 0.02$) but not PhS was increased when insulin was infused. Increased dietary protein was associated with greater total eEF2 ($P = 0.04$) expression. Increased rpS6 expression and increased PhS of 4EBP1 should stimulate protein synthesis, but the lack of change in mTOR PhS would not be stimulatory.

Key Words: insulin, dietary protein, signaling protein

W299 A novel multiplex real-time PCR assay for bovine liver pyruvate carboxylase 5′ UTR variants during the transition to lactation. H. M. White*, S. L. Koser, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Pyruvate carboxylase (PC; EC 6.4.1.1) is a key enzyme in glucose and energy metabolism. The bovine PC gene contains three promoter sequences (P3, P2, and P1 from 5′ to 3′) and is regulated by physiological changes such as the onset of calving and feed restriction. Expression of P1 is glucogenic and lipogenic tissue specific and codes for 5′ UTR A, B, C, and F whereas P2 and P3 are expressed in several tissues and code for 5′ UTR E and D, respectively. The objective of this study was to develop a multiplex real-time RT-PCR assay for bovine PC 5′ UTR variants and to characterize their expression during the transition to lactation. The multiplex assay was designed to quantify the PC coding region, 5′ UTR D, E, and F mRNA, as proxy measures for their relative promoter activities. Combined expression of 5′ UTR A, B, and C mRNA was determined by difference. Liver biopsy samples were collected from eight multiparous Holstein cows at −28, +1, and +28 days relative to calving (DRTC). Expression of PC mRNA was increased ($P < 0.05$) by 6-fold at +1 DRTC compared to precalving levels. Expression of variants from P1 was greater ($P < 0.05$) than variants from P2 or P3. Expression of 5′ UTR F from P1 was decreased ($P < 0.05$) and the combined expression of 5′ UTR A, B, and C from P1 increased ($P < 0.05$) at +1 DRTC. There was no effect ($P \geq 0.05$) of DRTC on 5′ UTR D or E mRNA expression. Increased expression of PC mRNA at calving is due to an increase in activity of PC promoter 1 and a lack of change in activity of PC promoters 2 and 3. These data suggests that the onset of calving leads to activation of factors specific to targets on bovine PC promoter 1.

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Key Words: pyruvate carboxylase, multiplex qPCR, transition cow