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Methionine supplementation impacts casein gene expression and cell death in the mammary tissue of lactating dairy goats fed low and adequate net energy supplies

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nitudes of normalized RNA-seq read counts as a relative indicator of their importance in supporting milk synthesis. Forty-five mL of milk was collected from multiparous cows ($n = 6$; DIM 68 ± 3 d) following 2 treatment periods of a post-ruminal lysine infusion of 0 and 63 g/d for 10 d, which was part of a larger study. Total RNA was isolated ($n = 12$ samples), libraries prepared, and paired end reads were sequenced on an Illumina HiSeq2500 platform. Of the total reads ($236M \pm 39M$), 79% mapped to the ENSEMBL bovine genome. EBseq analysis found no significant effect of lysine infusion on the transcriptome, therefore, reads were averaged across both treatments. Gene transcripts (12,730) with a normalized read count mean ≥ 5 were divided into 9 categories by magnitude of read counts (Table 1) for functional annotation analysis. The 13 most abundant transcripts were casein and whey proteins, regulators of milk fat synthesis and secretion, a ubiquitinating protein, and a tRNA transporter and together accounted for 67% of the 23M coding reads. Ingenuity pathway analysis revealed mTOR, JAK/STAT, PPAR α , and ubiquitin proteasome pathways were enriched with reads ≥ 200 . Genes with < 200 reads corresponded to tissue homeostasis and immune response. Some of the most abundant genes corresponded to maintenance of translation, protein turnover, and amino acid recycling suggesting their importance in supporting mammary function and milk synthesis.

55 Elevated circulating serotonin alters calcium metabolism in mid-late lactation dairy cows. M. K. Connelly*, H. P. Fricke, J. Kuehn, M. Klister, and L. L. Hernandez, *University of Wisconsin-Madison, Madison, WI.*

Serotonin is an evolutionary conserved monoamine that modulates a variety of physiological functions. Of interest in the dairy cow is serotonin's action on calcium homeostasis due to the increased calcium demand that occurs during lactation. Understanding serotonin's mechanism, specifically at the level of the mammary gland, is critical to understanding the role of serotonin during lactation. Therefore, the objective of this study was to determine how intravenous infusion of 5-hydroxytryptophan (5-HTP) in mid-late lactation dairy cows altered calcium metabolism. Twelve multiparous Holstein cows were blocked by parity in a randomized complete block design. Cows were intravenously infused daily at a constant rate with (1) 1.5 mg/kg 5-HTP ($n = 6$) or (2) 0.9% saline ($n = 6$) for 3 consecutive days with sampling through 24h post termination of final infusion. Baseline mammary biopsies, blood and milk samples were taken one day before initiation of treatment. Additional mammary biopsies and blood samples were collected at 0, 8, and 24h post final intravenous treatment. Mammary tissues were analyzed via qPCR to evaluate calcium metabolism genes. Milk samples were taken each morning before infusion and the morning following final infusion. Circulating serotonin increased in 5-HTP infused cows ($P = 0.03$), while circulating calcium concentrations were decreased ($P = 0.03$) relative to control cows. Relative expression of plasma membrane calcium ATPase 2 and calcium release-activated channel protein 1 were upregulated in 5-HTP treated cows when compared with control cows ($P = 0.118$ and $P = 0.116$, respectively). Cows infused

with 5-HTP had increased ($P = 0.01$) milk serotonin concentrations on d 4 and increased milk calcium concentration ($P = 0.03$) on d 3 of the experiment. Eight hours after final intravenous treatment, relative expression of parathyroid hormone-related peptide was increased ($P = 0.04$) and mammary gland serotonin content tended to be elevated ($P = 0.08$) relative to control. This supports previous rodent and cow data from our lab on serotonin's regulation of mammary gland physiology and calcium trafficking.

Key Words: serotonin, calcium

56 Methionine supplementation impacts casein gene expression and cell death in the mammary tissue of lactating dairy goats fed low and adequate net energy supplies. M. Boutinaud*¹, E. Chanat¹, A. Leduc¹, S. Wiart¹, P. Debournoux¹, L. Balhou², and S. Lemosquet¹, ¹*INRAE Agrocampus Ouest, PEGASE, Saint Gilles, France*, ²*Centre of Expertise and Research in Nutrition, Adisseo France S.A.S, Commeny, France.*

Methionine supplementation is known to modulate milk and protein yields in dairy ruminants. However, the mechanism involved in this phenomenon is still unknown. The cellular and molecular responses in the mammary tissue to methionine (Met) supplementation through HMBi (MetaSmart, Adisseo) at low (LE) and adequate levels (AE) of NEL were investigated according to a 2×2 factorial arrangement on 48 multiparous Alpine goats at mid lactation, assigned to a randomized block design in 4 groups during 5 weeks. Each goat was genotyped for CSN1S1 gene to balance each group according to the high or low genotypes for CSN1S1 gene. A fixed amount of hay was distributed per group (1.17 Mcal/kg DM) and a fixed amount of concentrates was distributed individually (1.72 vs. 1.83 Mcal/kg DM in LE and AE, respectively). Metabolizable methionine were 1.95 vs. 2.45% of metabolizable protein in unbalanced (LE and AE) vs. balanced (LEMET and AEMET) diets, respectively. Goats (23) were slaughtered after 5 weeks of treatment. Mammary tissues were collected for the analyses of milk protein mRNA levels by real time RT-PCR and apoptosis by immunohistochemistry using TUNEL assay. A variance analysis was performed with genotype, E, Met and E x Met, E x genotype, Met x genotype interactions as fixed effects and for milk, pre-treatment data yields was used as a covariate. Met supplementation increased milk protein yield (+6.5 g/d; $P = 0.03$). The treatments did not modify LALBA whereas an interaction E x Met ($P = 0.001$) were observed for both CSN3 and CSN1S1 mRNA levels showing 2-fold increases with Met supplementation in goats fed AE diet. The supplementation of Met reduced by 51% apoptosis rates in the mammary tissue ($P < 0.01$). Apoptosis rate was correlated with milk yield (-0.48 ; $P < 0.05$). These results suggest that Met increased casein gene expression at AE level and reduced cell death which could partly explain the positive effect of Met supplementation on milk synthesis in the goat mammary tissue.

Key Words: dairy goat, mammary tissue, amino acid supplementation