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Mammary gland transcriptome and proteome modifications by nutrient restriction in early lactation Holstein cows challenged with intramammary lipopolysaccharide

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an intramammary LPS challenge.

Key Words: inflammation, liver transcriptome, undernutrition

0130 Growth and transcriptional profile analysis following oral probiotic supplementation in dairy cows. M. Worku*, S. Adjei-Fremah, K. Ekwemalor, E. Asiamah, and H. Ismail, *North Carolina Agricultural and Technical State University, Greensboro.*

The objective of this study was to assess the impact of probiotic administration on growth and global gene expression profile in dairy cow. Use of probiotic supplements is a nonchemical approach to promote animal health. Understanding the mechanism of action of probiotics in cows may aid in sustainable dairy production. Lactating Holstein-Friesian cows ($n = 10$) received daily oral doses (50ml) of a commercial probiotic FASTtrak microbial pack (Conklin Company, Kansas City, MO) (containing *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Aspergillus oryza*, and *Bacillus subtilis*) over a 60-d period. Body weight was recorded weekly. Whole blood was collected at the beginning (d 0) and end of the study (d 60). Blood samples were analyzed for total and viable cell count, packed cell volume (PCV), white blood cell differential counts (WBC), and total protein concentration in plasma. Daily supplementation of probiotics had no effect on BW, PCV, and total protein concentration in plasma at the end of the study ($P > 0.05$). Percentage lymphocyte count increased ($P < 0.05$), and percentage neutrophil count ($P < 0.05$) decreased in probiotic-treated animals. Gene expression analysis identified 10,859 differentially expressed genes, 1168 up-regulated and 9691 down-regulated genes respectively following probiotic administration. Pathway analysis identified 87 bovine pathways impacted by probiotic treatment. These pathways included the Toll-like receptor signaling pathway, inflammation response and Wnt signaling pathways. Oral administration of probiotic to dairy cows has a systemic effect on global gene expression, including genes involved in immunity and homeostasis (Wnt). The results of this study show that the utilization of probiotics in animal agriculture impacts genes important to dairy cow health and production. Further definition of the interaction between the pathways involved may aid in the design of the most effective probiotics for optimum dairy production and health.

Key Words: dairy cows, innate immunity, microarray, probiotic

0131 Mammary gland transcriptome and proteome modifications by nutrient restriction in early lactation Holstein cows challenged with intramammary lipopolysaccharide.

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The objective was to evaluate the effect of nutrient restriction and intramammary lipopolysaccharide (LPS) challenge on mammary gland (MG) gene expression in early lactation cows. At 24 ± 3 d in milk, multiparous cows were either allowed to continue ad libitum intake of a lactation diet (CON, $n = 6$), or the ration was diluted with barley straw (48% DM) for 4 d (RES, $n = 6$). On d 3, one healthy rear mammary quarter was infused with 50 μ g of LPS. Mammary biopsies were performed 24 h after LPS challenge. RNA and proteins analyzed using bovine 44 K microarrays (Agilent Technologies) and micro-LC-MS/MS, respectively. Transcriptomic data were analyzed using GeneSpring (moderated- t test with Westfall-Young correction, $P < 0.05$). Proteins were analyzed with Proteogenis LC-MS software v.4.1 (Nonlinear Dynamics). Production and energy balance did not differ before diet change. Negative energy balance was aggravated in RES (41 vs. $97 \pm 15\%$ of requirements, mean \pm SD; $P < 0.001$). A total of 87 differentially expressed genes (DEG) were highlighted through the comparison of RES vs. CON group. Among the 33 DEG identified in the transcriptomic analyses, 11 and 22 were down- and up-regulated by restriction, respectively. Among the up-regulated DEG, there were *PKD4* and *CPT1A* which are involved in the regulation of fatty acid, ketone, and glucose metabolism. *CPT1A* is the key enzyme in the carnitine dependent fatty acid transport, promoting fatty acid oxidation. Genes involved in immune response such as *PG-LYRP3* and *TRIB2* were up-regulated, suggesting a higher inflammatory response in RES than CON. Proteomic analysis identified 54 proteins with 14 up- and 40 down-regulated in RES cows. Up-regulated proteins were mostly involved in gene expression mechanisms such as translation, RNA splicing and cellular protein modification. The down-regulated proteins (e.g., EIF3H, RS27A, RS15) take part in protein metabolism. This is coherent with transcriptomic results, namely the down-regulation of *RPL 37A*, a component of ribosomal complex, which catalyzes protein synthesis and may partially explain the lower milk protein yield in RES (834 vs. 1163 g/d; $P = 0.02$). Proteins involved in antigen processing and presentation were down-regulated in RES compared with CON, suggesting an impaired ability to counteract inflammation in RES MG. Preliminary transcriptomics and proteomics analyses show that undernutrition may influence the MG response

to inflammation at each level of gene expression.

Key Words: mammary omics, undernutrition, inflammation

0132 Methionine supplementation modulates the inflammatory response of dairy cow blood neutrophils in response to lipopolysaccharide.

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Methionine (Met) is among the two most-limiting amino acids for milk production in dairy cow diets. The accepted optimal ratio when formulating diets is a Lys:Met of 3:1. However, blood from cows fed corn silage-based diets without supplemental rumen-protected Met averages ~3.6:1. Our recent in vivo research on immunonutrition revealed the immune system could benefit from additional Met. To study the effect of different Lys:Met ratios, blood neutrophils were isolated from five mid-lactating pluriparous Holstein cows (238 ± 20 DIM, 33.8 ± 3.9 kg/d average milk production) to obtain a homogenous pool. Neutrophils were then incubated at a concentration of 6 × 10⁶ cells/mL for 2 h in a sterile incubator at 37°C and 5% atmospheric CO₂. A 3 × 2 factorial arrangement of treatments including three Lys:Met ratios (3.6:1, 2.9:1, 2.4:1) and two levels of lipopolysaccharide (LPS, 0 and 50 µg/mL) were evaluated in triplicate. After incubation, cellular RNA was used to measure expression of genes related to immune function and oxidative stress. Data were log₂ normalized and subjected to ANOVA using the Proc MIXED procedure of SAS. As expected, LPS increased (*P* < 0.05) the expression of pro- and noninflammatory cytokines (*IL1B*, *IL10*, *IL6*, *TNF*) and immune-related nuclear receptors (*NFKB1*, *NR3C1*). However, LPS decreased (*P* < 0.05) the expression of chemokine *CXCR1* and antimicrobial enzyme *LYZ*, the latter only when cells were incubated with higher Met (2.9:1 or 2.4:1), and had no effect (*P* < 0.05) on other pathogen killing mechanisms (*MPO*, *SOD1*). Among genes related to Met metabolism, LPS increased (*P* < 0.05) expression of *MAT1A*, while reducing expression of *GPX1* and *GSR*, suggesting a greater use of Met and a reduced antioxidant system during the inflammatory response. Compared with the lowest level of supplemental Met (3.6:1 Lys:Met) the highest level (2.4:1 Lys:Met) decreased (*P* < 0.05) expression of *NFKB1*, *NR3C1*, and *GSR*, while it increased (*P* < 0.05) *IL6* independently of the LPS level. Furthermore, expression of the noninflammatory cytokine *IL10* was greatest (*P* < 0.05) at 2.4:1 Lys:Met and in non-LPS challenged cells, indicating that supplemental Met improved the oxidative status and the non-inflammatory conditions of neutrophils. Overall, data support the idea that Met supplementation could improve the inflammatory and oxidative status of bovine neutrophils.

Key Words: LPS, methionine, neutrophils

0133 Feasibility and safety of nitric oxide releasing solution as a treatment for bovine mastitis.

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Nitric oxide releasing solution (NORS) is a liquid formulation that releases nitric oxide (NO). NO is a broad, non-species-specific nonmicrobial nanomolecule, endogenously produced during the innate response in mammals. The objective of this study was to explore the feasibility of NORS as a potential treatment for bovine mastitis (BM). Two common pathogens found in BM (10⁶ CFU/mL of *Escherichia coli* and *Staphylococcus aureus*) were added to raw milk from healthy cows, as an in vitro model, to determine the antimicrobial efficacy of NORS at different concentrations (100–400 mM) and at two different NORS:milk ratios (2:1 and 1:1). Next, 10 ex vivo samples of milk from dairy cows presenting with clinical mastitis were obtained and treated with NORS to confirm efficacy. A dose escalating safety study was then performed using three dairy cows, where 40 mL of increasing concentrations of NORS (50–400 mM) were infused into the teat. Nitrite and methemoglobin levels were measured 5, 30, and 460 min posttreatment and nitrites in milk were measured 8 and 24 h posttreatment. Nitrite was measured by chemiluminescence, while methemoglobin was measured using a CO-Oximeter. Results show that NORS could eradicate, in vitro, both bacteria, and was dilution and time dependent. In the 2:1 ratio, NORS significantly (*P* < 0.05) reduced bacterial concentration in milk both in vitro and ex vivo within 2 min, and had no detectable bacteria after 5 min of exposure. In the 1:1 ratio, a significant bacterial load reduction (*P* < 0.01) occurred within 10 min and no detectable bacteria within 20 to 30 min. In the safety study we found an increase in blood nitrites (*P* < 0.05) within 5 min of the NORS treatment at all concentrations. After 8 h, the blood values at all concentrations returned to baseline (*P* = 0.27). Blood methemoglobin changes were nominally increased in the 5 and 30 min samples post 400 mM NORS and no detectable change was seen after 8 h. Eight hours posttreatment, before the evening milking, milk nitrites were 18 times higher than baseline, while 24 h posttreatment nitrites returned to baseline level (*P* = 0.36). NORS was found to eradicate bacteria in milk and, clinically, the treatment was well tolerated. This suggests that NORS has a potential to be a safe and effective nonmicrobial treatment for BM and may allow salable milk during antimicrobial treatment of mastitis. Further, it would provide an alternative to antibiotics, thus contributing to the reduction of antibiotic drug resistance. Further studies are justified.

Key Words: mastitis, nitric oxide, safety study, treatment