

Deep RNA-Seq reveals genetics and nutritional regulation of miRNomes in mammary gland of lactating Holstein and Montbéliarde cows

Pierre-Alexis Billa, Yannick Faulconnier, T. Ye, Sébastien Bes, José Pires,

Christine Leroux

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Pierre-Alexis Billa, Yannick Faulconnier, T. Ye, Sébastien Bes, José Pires, et al.. Deep RNA-Seq reveals genetics and nutritional regulation of miRNomes in mammary gland of lactating Holstein and Montbéliarde cows. American Dairy Science Association Annual Meeting, 2019, Cincinnati, Ohio, United States. Volume 102, Supplement 1, 2019, Journal of Dairy Science. hal-02788559

HAL Id: hal-02788559 https://hal.inrae.fr/hal-02788559

Submitted on 5 Jun2020

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Abstracts of the 2019 American Dairy Science Association[®] Annual Meeting

June 23–26, 2019 Cincinnati, Ohio

Journal of Dairy Science[®] Volume 102, Supplement 1



120 mL to colostrum would linearly decrease OS, increase n-3 byproducts of FA metabolism, and increase plasma n-3 FA during the first week of life. Twenty-four Holstein calves were randomly assigned to receive 0 (Con), 30, 60, or 120 mL of a 1:1 mix of fish and flax oils (FFtrt 30, 60, 120) supplemented in colostrum. All calves received 3 L colostrum within 6 h of birth. Blood was sampled before colostrum feeding and on 1, 2, 4, 7, and 14 d of age to assess plasma FA, phospholipid FA, oxidant status, and oxylipid concentrations. Plasma FA and oxylipids were determined with liquid chromatography-mass spectrometry, and phospholipid FA were determined with gas-liquid chromatography. Health indicators were observed daily. Data were analyzed with a mixed procedure in SAS version 9.4 including treatment, sex, and day as fixed effects and calf and block as random effects. FFtrt 30, 60, and 120 increased n-3 free FA concentrations by 23 to 90% in the first week of life (P < 0.01). Compared with control, FFtrt linearly increased n-3 FA of plasma phospholipids (Con: 4.3, FFtrt 30: 4.7, FFtrt 60: 5.7, FFtrt 120: 6.2 g/100g ; P < 0.01) and some n-3 FA derived oxylipids such as 14,15-dihydroxy-eicosa-tetraenoic acid (14,15-Di-HETE) ($P \le P$ 0.01) and 19,20-dihydroxy-docosapentaenoic acid (19,20-Di-HDPA) (P = 0.01), but did not change oxidant status (P = 0.35). Treatments did not alter calf health or growth (P > 0.22). All variables returned to control values by d 14. In conclusion, a colostrum supplement of n-3 FA administered in volumes of 30, 60, and 120 mL linearly increased plasma concentrations of n-3 FA and n-3 FA metabolites, but did not alter overall oxidant status.

Key Words: omega-3, oxidative stress, oxylipids

359 Extracellular vesicles modulate pro-inflammatory signaling in bovine macrophages. C. M. Ylioja*, M. Garcia, L. K. Mamedova, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Exosomes are extracellular vesicles that are released into circulation to facilitate communication between cells. These vesicles transport a variety of cargo, including cytokines, bioactive lipids, and regulatory RNA, that can modulate immune function. Immune suppression exhibited by dairy cows during early lactation may involve exosome-mediated communication between immune cells. We sought to determine whether fatty acids that are elevated in circulation of dairy cows during early lactation can alter exosome-mediated inflammatory signaling. Specifically, we studied the ability of bovine exosomes to alter immune responses of primary bovine monocyte-derived macrophages (MDM). Circulating monocytes were isolated from 6 healthy mid-lactation Holstein cows. Cells were incubated for 7-10 d to allow for differentiation into MDM. Cells were treated with either fatty-acid-free bovine serum albumin (BSA; control) or palmitic acid (PA; 100 μ M) plus BSA carrier for 6 h before exosomes were isolated from culture media. Untreated MDM were incubated for 12 h with either no treatment or exosomes from PA or BSA treatment, equalized by protein concentration of the isolated exosomes. Cells were then incubated for 6 h with or without LPS (100 ng/mL) before media was harvested for cytokine analysis. Treatment with exosomes from either control or PA-exposed MDM increased TNF α concentrations independently of LPS treatment (P < 0.001 compared with untreated cells), and in fact, PA exosome treatment resulted in greater TNF α concentrations than LPS treatment (P = 0.03). Surprisingly. PA exosomes also attenuated the TNFa response to LPS compared with control exosomes (P = 0.04). These results suggest that PA-treated exosomes caused an increase in basal inflammatory state of MDM but made them refractory to further inflammatory stimuli. Alterations in circulating metabolites may have both direct and indirect effects on inflammatory signaling, and further investigation of exosome signaling may contribute to our understanding of immune function during times of stress.

Key Words: exosome, immune function, transition cow

360 Deep RNA-Seq reveals genetics and nutritional regulation of miRNomes in mammary gland of lactating Holstein and Montbéliarde cows, P.-A. Billa*¹, Y. Faulconnier¹, T. Ye^{2,3}, S. Bes¹, J. Pires¹, and C. Leroux^{1,4}, ¹Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, Saint-Genès-Champanelle, Auvergne-Rhône-Alpes, France, ²Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, Grand Est, France, ³Centre National de la Recherche Scientifique, Illkirch, Grand Est, France, ⁴Department of Food Science and Technology, University of California Davis, Davis, CA.

The mammary gland (MG) is a complex secretory organ synthetizing milk, the production and composition of which vary under genetic and nutrition regulations. The mechanisms underlying the variations are not totally understood. MicroRNAs (miRNAs, small non-coding RNAs) regulate the expression of genes involved in many cellular processes. including in MG. The objective was to identify the effects of feed restriction and dairy breed on bovine MG miRNomes. Five Holstein and 6 Montbéliarde multiparous cows in midlactation (165 ± 21 DIM) underwent 6 d of feed restriction (meeting 50% of NE₁ requirements calculated before the challenge). Milk, fat, protein and lactose yield were measured before and during restriction. MiRNomes were analyzed by RNA-Seq using Illumina HiSeq 4000 from MG biopsies performed 1 d before (control) and on d 6 of feed restriction. Statistical analyses for milk production and composition and for miRNAs were performed using mixed models of SAS and the DESeq2 package of R, respectively. Significance was considered at $P_{adj} = 0.05$. As expected, milk, fat, protein, and lactose yields were lower in Montbéliarde than in Holstein cows and decreased by feed restriction. RNA-Seq analyses revealed 623 distinctly expressed miRNAs, among which 596 are known and 27 predicted. Breed influenced the expression of 19 miRNAs during the control period. The restriction modified the expression of 33 miRNAs in MG of Holstein cows, but only 2 miRNAs tended (padi = 0.10) to change in MG of Montbéliarde cows. Three miRNAs (miR-25, miR-2898, and miR-500) were commons between those modified by restriction and by breed. MiR-25 presented a high expression in lactating MG (over 4000 counts) and is known to repress triacylglycerol synthesis and lipid accumulation in mammary epithelial cells. The expression of miR-25 was higher whereas milk fat were lower in Montbéliarde and after restriction. In conclusion, we showed genetics and nutrition regulation of MG miRNomes, which suggest a potential role of miRNAs MG function and may be related to milk production and composition.

Key Words: microRNA, mammary gland, energy balance

361 Genome-wide association study in colostrum reveals QTL for natural antibodies in Swedish dairy cattle. J. M. Cordero-Solórzano*^{1,2}, J. J. Wensman¹, M. Tråvén¹, J. A. J. Arts², H. K. Parmentier², H. Bovenhuis², and D. J. de Koning¹, ¹Swedish University of Agricultural Sciences, Uppsala, Sweden, ²Wageningen University and Research, Wageningen, the Netherlands.

Colostrum with sufficient antibodies is essential for the newborn calf, as it requires this passive immunity to survive until weaning. High variation in the amount of colostrum antibodies in Swedish dairy cows has been reported, with a large proportion having low antibody levels. Natural antibodies (NAb) are produced without any antigenic stimulation and target self-antigens and pathogen-associated molecular patterns