



Characterization of the non-genetic causes of variation of bovine milk calcium concentrations on French farms

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This work provided some gene-associated insights to facilitate further investigation of the mechanisms underlying lactation in dairy cows.

Key Words: lactation, bovine mammary gland, transcriptomics

494 Understanding the regulatory mechanisms of milk production using integrative transcriptomic and proteomic analyses: Reducing inefficient utilization of crop by-products as forage in dairy industry.

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Milk from dairy cows is an essential nutrient for the young and human as well. Forage plays a vital role in dairy husbandry via affecting milk quality and quantity. However, the differences in mammary metabolism of dairy cows fed different forages remains elucidated. In this study, we utilized transcriptomic RNA-seq and iTRAQ proteomic techniques to investigate and integrate the differences of molecular pathways and biological processes in the mammary gland of dairy cows fed differing forages. Bovine mammary tissues were obtained from 6 healthy multiparous lactating dairy cows fed with corn stover (CS, low-quality; n = 6) and alfalfa hay (AH, high-quality; n = 6), respectively. A total of 1631 differentially expressed transcripts (DETs; 1046 upregulated and 585 downregulated) and 346 differentially expressed proteins (DEPs; 138 increased and 208 decreased) were detected in the mammary glands between the CS- and AH-fed animals. Expression patterns of 33 DEPs (18 increased and 15 decreased) were consistent with the expression of their mRNAs. The gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) analyses of the DETs and DEPs indicated that the decreased energy metabolism, increased fatty-acid oxidation, attenuated protein synthesis, enhanced protein degradation, and the lower mammary cell growth may be the prime factors contributing to the lower milk production in the CS-fed cows compared with the AH-fed cows. Moreover, 19 milk-synthesis-related genes were quantitated by real-time RT-PCR to examine the transcriptional profile and validate the proteins identified by LC-MS/MS between CS-fed and AH-fed bovine mammary gland. Four DEPs were further verified by Western blot analysis. These results provide the biological understanding of insights into mammary metabolism alterations affected by differing foraged and will be beneficial in developing highly efficient strategies for utilization of low-quality forages.

Key Words: dairy cow, mammary gland, forage

495 Characterization of the non-genetic causes of variation of bovine milk calcium concentrations on French farms. P. Gaignon^{*1,2}, M. Gele³, C. Hurtaud¹, and A. Boudon¹, ¹*PEGASE, INRA, Agrocampus Ouest, Saint-Gilles, France*, ²*CMI, 18 avenue F. Roosevelt, Saint-Malo, France*, ³*Institut de l'élevage, Angers, France*.

Calcium concentration (CaC) in bovine milk has often been described as independent of feeding strategy and mainly dependent on cow genetics and lactation stage. However, isolated experiments showed that variations in milk CaC could be linked to the diet of cows. Our objective was to identify and quantify non-genetic factors of variation in CaC in milk samples collected from about a thousand French dairy farms with contrasting feeding strategies and cow breeds. This study was based on the PhénoFinlait program that consisted of a survey performed between 2009 and 2010 in 924 dairy farms located in the major French milk production areas. The breeds used in the investigated farms were Holstein,

Normande and Montbeliarde. Each farm was visited on average 4 to 6 times during the year. Each time, information about cow diets and production were gathered and individual milk samples were collected to extract their mid-infrared (MIR) spectra. More than 200,000 MIR spectra were measured. Nearly 10,000 milk samples were also frozen and stored in a bank for further analyses. We estimated CaC in milk samples from their MIR spectra using a predictive equation. This equation was established from 300 milk samples extracted from the bank and chosen to represent the diversity of investigated dairy systems. From the composition of the cow diets collected at each survey, we characterized 7 feeding strategies using multiple factorial analyses across 3 periods: winter, early and late summer. For each breed, the variations in milk CaC were quantified by ANOVA with a model including the effects of feeding strategies, stage of lactation, parity, and calendar month as fixed effects and the cow as random effect. The feeding strategy affected milk CaC with the constant fact that the diets based on fresh or conserved grass induced lower milk CaC whatever the month of the year ($P < 0.05$). The difference in CaC can be up to 100 mg/kg between 2 extreme diets at a given month, which is as important as the drop in CaC observed at the beginning of lactation. This study reinforces the idea that the diet of cows has an influence on milk CaC.

Key Words: calcium, milk, feeding strategies

496 Milk fat globule size is regulated by phosphatidylethanolamine-dependent fusion: In vitro model. N. Argov-Argaman^{*1}, B.-C. Cohen¹, and A. Shamay², ¹*Hebrew University, Rehovot, Israel*, ²*The Volcani Center, The Ministry of Agriculture, Rehovot, Israel*.

Milk fat is secreted in a unique structure, termed milk fat globule (MFG) which consists of a triglyceride core covered with 3 layers of phospholipids (MFG membrane; MFGM). MFG are secreted in a wide range of sizes; from the nanometer length scale to over 15 μ m, and their size is tightly associated with their lipid composition. Particularly, higher MFGM content is found in small compared with large globules. MFG size is determined by the size of its precursors — the intracellular lipid droplets (LD) which are produced and secreted by the mammary epithelial cells (MEC). Fusion is one of the suggested mechanisms controlling LD size. Nevertheless, what controls the extent of fusion and how dominant this mechanism is in controlling LD size is still illusive, especially in mammalian cells. We hypothesized that LD fusion is controlled by the stability of their membrane, which is modulated by the content and mass ratio between 2 main phospholipids - phosphatidylethanolamine (PE) and phosphatidylcholine (PC). We used primary MEC culture, treated with oleic or palmitic acid, to study the role of membrane stability in determining LD size. Results show that 22% of MEC treated with oleic acid had large LD ($>2.5 \mu$ m) compared with only 4% of the cells treated with palmitic acid. The increased LD size in the oleic acid treatment was associated with 63% increase in PE, and 7 fold increase in LD fusion. Adding $\text{NaN}_3 + \text{NaF}$ to the oleic acid treatment decreased PE content by 19%, concomitantly with 8 fold decrease in the number of large LD. Interestingly, the addition of $\text{NaN}_3 + \text{NaF}$ to oleic acid treatment did not change the cellular triglyceride content. In contrast, adding 3-deazaadenosine to palmitic acid treatment tended to increase PE content by 29%, and consequently increased the number of large LD by 3 fold, relative to cells treated with palmitic acid alone. Our findings have uncovered a defining role for LD fusion in determining their size in MEC, which is independent of triglycerides content of the cells. Understanding the mechanisms controlling LD size in mammalian cells is of great importance, especially in MEC due to the effect of LD size on milk composition.

Key Words: milk fat globule, size, fusion