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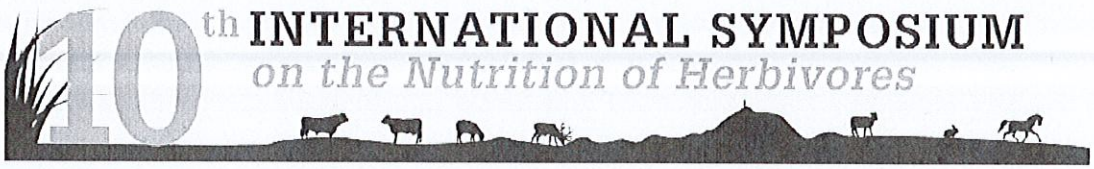

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Variations of global DNA methylation in Peripheral Blood Mononuclear Cells, in milk leukocytes and in milk epithelial cells in dairy cows: effects of micro-nutrient supplemented diet.

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Take home message Nutrition changes could induce global epigenetic effects in different cell types.

Introduction Postpartum period is a critical period in dairy cows. This crucial time is marked by major physiological and metabolic changes that affect milk production, immune response and fertility. The nutrition is considered as one lever to provide a control of negative energy balance and a better general health status of the dairy cows (Drackley *et al.*, 2014). Epigenetics provides molecular mechanisms (DNA methylation, post translational histone modifications, short and long non-coding RNAs) that affect genome activity and gene expression without DNA sequence modification (Schübeler, 2015). Moreover, epigenetic imprinting can also be triggered by environmental factors (pathogenic, nutritional, chemical and physical factors). This study aimed to evaluate the phenotypic effects and epigenetic imprinting of a micro-nutrient supplemented diet, distributed before and after calving in order to provide the metabolic statue and health in dairy cows.

Materials & methods Multiparous (n=17) and Primiparous (n=7) dairy cows were used in this study and randomly allocated to one of two dietary treatments (n=12 for each group): control diet (CTRL group) and diet with commercial nutrient supplementation (Genial®; SUPPL. group) for 4 weeks before calving and for 8 weeks afterwards. Before calving, the cows were fed ad libitum according to INRA guidelines (INRA 2007). Before calving, the diet contains 84.5% corn silage, 9% soybean meal, 2.5% straw, on a dry matter basis. After calving, the diet contains, on a dry matter basis, 52.5% and 64.7% corn silage, 24.7 and 15.5% non-mineral supplement, 2.5% straw for only the first 15 days of lactation and 8% soya oilcake, 10% alfalfa silage and 0.8% urea. The CTLR and SUPPL cows received after calving, 260 and 200 g classic mineral supplement for multiparous and primiparous cows respectively. The SUPPL cows received in addition 160 g for 4 weeks before calving and 250 g of Genial® during the first 60 days of lactation. Genial® is a nutrient supplementation enriched in organic selenium from yeast, in trace elements (zinc and copper), in a cocktail of minerals and in extracts from plants and microalgae containing □ carotene, vitamin A, D3, E and natural Superoxide dismutase, currently commercialized by PILARDIERE group (PILARDIERE, Saint-Mars-la-Réorthe, France). Milk production and composition (fat, protein and lactose contents, somatic cell count (SCC)), body weight (BW), body condition score (BCS), dry matter intake and health (calving score, metritis, mastitis) were recorded over the trial period. At D15 and at D60 post calving, blood and milk samples were collected from cows. From the milk samples, different components were analyzed. Mammary Epithelial Cells (mMEC) and milk leukocytes were purified and counted. The viability of both cell types was estimated. From blood, the Peripheral Mononuclear Blood Cells (PBMC) were purified and counted. As new phenotypic parameter, the global DNA methylation of the three different cell types (PBMC, mMEC and milk leukocytes) was measured by Luminometric Methylation Assay (LUMA).Statistical analysis: Zootechnical data were analyzed by ANOVA using the SAS (SAS Institute, 1999) MIXED procedure with the repeated statement using D15 and D60 of lactation as repeated effect and cows as subject. The effects of parity, diet, stages, and the interaction parity x stage, parity x diet, diet x stage and parity x diet x stage were tested.

Results & discussion Genial® provided an enhancement of BCS and BW without modification of dry matter intake and milk yield and composition. The supplement also improved calving conditions and reduced delay between calving and first observation of the cow heat (first service).

Global DNA methylation was measured by LUMA that provides the methylation level of pan genomic MspI/HpaII cleavage sites (Me-CCGG). Using 35 PBMC samples and matched 35 milk leukocytes and mMEC samples, Me-CCGG was found different between cell types (with median value of 65.4 % in mMEC, 68.3 % in milk Leukocytes and 74.6 % in PBMC, $P < 0.0001$). In PBMC, Me-CCGG was different between stages of lactation ($P = 0.021$) and modified by commercial nutriment supplementation for primiparous cows (LMS 72.4 % ± 0.9 and 75.4 % ± 0.8). In milk leukocytes, a parity x diet effect was observed on Me-CCGG ($P = 0.0054$). In mMEC, Me-CCGG was found not significantly different between parity, stage and diet groups. However, a stronger variability of Me-CCGG was observed in mMEC than in milk leukocytes and PBMC ($cv_{(mMEC)}$=11.8 %, $cv_{(milk\ leukocytes)}$= 5.9 % and $cv_{(PBMC)}$= 2.2 %).

Conclusion Our study shown that the cell specificity Me-CCGG was highly significant, suggesting the importance of global methylation level in regulation of cell differentiation. Moreover, the variations of Me-CCGG in response of different conditions analyzed (parity, lactation stages, diet) were also specific of the considered cell type.

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