Effects of nutrient restriction on mammary cell activity and hormonal statement in lactating dairy cows

Frederic Dessauge, Vanessa Lollivier, Erwan Cutullic, Jacques Portanguen, Sarah Barbey, Benjamin Ponchon, Marion Boutinaud

proteins following differentiation into mammary gland-like structures (gland ducts, lateral buds, and alveoli). Hollow fiber bioreactors have been used for large-scale mammalian cell culture to produce monoclonal antibodies or recombinant proteins. The hollow fibers provide a culture system with a high surface to volume ratio. The system allows extremely efficient exchange of nutrients and waste products across the fiber wall. Therefore, we attempted to culture PMEC transformed with recombinant DNA in these hollow fiber bioreactors to produce recombinant proteins. In the present study, we investigated the optimum conditions for culturing the PMEC onto polysulfone hollow fibers (PHF). The results showed that the seeded PMEC could attach, grow and form monolayers on the surface of PHF. The PMEC could differentiate into mammary gland-like structures when the cells were grown on PHF coated with Matrigel. The regulatory region of the milk gene, α lactalbumin, was inserted upstream of luciferase cDNA to generate a recombinant DNA, pAL-luc. The pAL-luc was transformed into PMEC and used to test the potential for recombinant protein production by PMEC cultured on PHF. Luciferase activity expressed from the PMEC grown on the PHF coated with 2.5 mg/ml and 5 mg/ml Matrigel were increased by 2.7-fold and 7.0-fold compared with that of the cells grown on the PHF without Matrigel (n = 3). Moreover, prolactin supplementation enhanced the luciferase expression. In this established PHF-cell culture approach, the PMEC could be continuously cultured for one week and potentially express recombinant protein. In conclusion, we have provided a potential PHF-cell culture approach in which PMEC can differentiate and express recombinant protein.

Key Words: mammary epithelial cells, polysulfone hollow fiber, recombinant protein

M146 Effects of nutrient restriction on mammary cell activity and hormonal statement in lactating dairy cows. F. Dessauge*[1,2], V. Lollivier[1,2], E. Cutullic[1,2], J. Portanguen[1,2], C. Disenhaus[1,2], S. Barbey[3], B. Ponchon[1,2], and M. Boutinaud[1,2], [1]*INRA UMR 1080 Dairy Production*, *35590, Saint Gilles, France*, [2]*Agrocampus UMR 1080 Dairy Production*, *35000, Rennes, France*, [3]*INRA UE 326 Domaine Experimental du Pin au Haras*, *61310, Le Pin au Haras, France*.

Feed restriction results in milk yield (MY) decrease. However the consequences on mammary activity and the involvement of prolactin (PRL) in the lower mammary synthesis are not known. The aim of the study was to investigate the effects of nutrient restriction on mammary epithelial cell activity in lactating dairy cows. We used 15 Holstein × Normande crossbred dairy cows, divided into 2 groups submitted to 2 feeding levels. From calving to wk 11 postpartum, the cows were fed a total mixed ration composed either of 55% maize silage, 15% dehydrated alfalfa and 30% concentrate (Basal diet-group as control, n = 7) or of 60% grass silage and 40% hay (Restricted diet-group, n = 8). Cows were milked twice daily. MY and composition were measured. Blood samples were harvested at wk 11 postpartum for the determination of PRL concentration. After 11 weeks of lactation, cows were slaughtered and mammary glands were removed and weighted. Expression of proteins involved in secretory activity was evaluated on mammary tissue by real-time qPCR and immunohistochemical staining was performed. Restricted diet-group cows had lower 11-week average daily MY (20.5 kg/d vs. 35.5, $P < 0.001$) and lower milk protein and lactose content from calving to slaughter than Basal diet-group cows. The size of the mammary acini were lower (−41%, $P < 0.01$) in the Restricted diet-group. Nutrient restriction decreased kappa-casein ($P < 0.01$) and α-lactalbumin ($P < 0.01$) mRNA levels in the mammary gland. The decrease in mean PRL concentration was not significant (−27%, $P = 0.15$) in Restricted diet-group compared with Basal diet-group. In conclusion, nutrient restriction resulted in a lower MY in lactating dairy cows. This was partly due to a modulation of mammary epithelial cells activity regulated at mRNA level.

Key Words: nutrient restriction, mammary epithelial cell, prolactin

M147 Effects of incremental sunflower seed supplementation on milk composition and mammary expression of genes regulating fatty acid uptake and lipogenesis. J. W. Møller, T. Bjørn, P. K. Theil, M. T. Sørensen, and K. Sejrsen*, *Faculty of Agricultural Science, Aarhus University*, *Tjele, Denmark*.

Dietary supplementation with unsaturated fatty acids is a well-established strategy for enhancing milk fat content of unsaturated fatty acids. The objective was to examine the effects of increased sunflower seed supplementation (SFS) on milk fat composition and mammary expression of genes regulating fatty acid uptake and lipogenesis. Twenty 4 lactating Holstein Friesian cows (186 ± 20 DIM; 25.3 ± 2.5 kg/d) were randomly assigned to 4 groups and fed a control diet or diets supplemented with 5%, 10%, or 15% sunflower seeds (% of DM) for a 5 week experimental period. DM intake and milk yield was reduced in cows fed 10% and 15% SFS when compared with control, whereas 5% SFS did not differ from control. All levels of SFS tended to increase milk fat content ($P = 0.08$). SFS decreased content of C4–14 ($P = 0,008$) and C16 fatty acids ($P = 0.014$). Content of ≥ C18 fatty acids was increased ($P = 0.015$). SFS increased the level of unsaturated fatty acids ($P < 0.001$) when compared with control and increased in a linear manner ($P < 0.001$) the content of rumenic acid (C18:2 c9t11) from 0.3% to 0,9%. Gene expression was analyzed by RT-PCR on RNA from mammary biopsies using the ΔΔCT method. SFS (5–15%) reduced mRNA abundance of SREBP-1 ($P = 0.034$), SCAP ($P = 0.0075$), FASN ($P = 0.035$), FADS1 ($P = 0.006$), and SCD ($P = 0.046$). mRNA abundance of ACC tended to be reduced ($P = 0.06$). SFS did not affect expression of the lipid uptake and transport genes LPL ($P = 0.189$), FABP3 ($P = 0.862$), and FAT ($P = 0.403$). In conclusion, our results show that dietary supplementation with high levels of unsaturated fatty acids in form of sunflower seeds leads to increased milk fat content in spite of decreased de novo milk fat synthesis as substantiated by a reduction in lipogenic genes. Furthermore, the expression of genes regulating fatty acid uptake and transport were unaffected although the amount of dietary fat present in milk was increased.

Key Words: dietary supplementation, mammary gene expression, conjugated linoleic acid

M148 Principal component analysis of milk fatty acid composition and the relationships between stearoyl CoA desaturase genotype and conjugated linoleic acid production in dairy cattle. J. Thomson*, L. Clark, M. Oba, and S. Moore, *University of Alberta*, *Edmonton, AB, Canada*.

The objectives of this study were to assess the relationships between individual milk fatty acids and conjugated linoleic acid (CLA) concentration in bovine milk fat and to assess the relationship between a single nucleotide polymorphism in the stearoyl CoA desaturase gene and CLA production using principal component analysis (PCA). 215 cows from an Alberta commercial dairy farm were genotyped and milk samples were collected for milk fatty acid analysis. Forty-three variables including milk production parameters, individual fatty acid concentrations, and indices of desaturation were analyzed. The first 3 principal components explained 47.61% of the total variance (PC 1, 24.13%; PC 2, 13.95%; and PC 3, 9.53%). The first PC had high loadings for most of the short chain fatty acids, the second PC had high loadings for the yield