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LACTATION BIOLOGY II

1232 (W141) Daylength affects simultaneously mammary epithelium integrity and mammary epithelial cell exfoliation in milk. M. Boutinaud^{1*}, A. Bondon¹, P. Debournoux¹, J. Couedon¹, M. Johan¹, A. Narcy², and C. Hurtaud¹, ¹INRA, Saint Gilles, France, ²INRA, Nouzilly, France.

Some of the mammary epithelial cells (MEC) responsible for milk synthesis are exfoliated into milk during the lactation process. MEC exfoliation into milk could play a role in mammary epithelium integrity. A trial was performed to study the effects of daylength and type of diet on milk calcium content. Calcium is known to play a role of cement for tight junction closure between epithelial cells. The aim of this study was to identify a potential effect of daylength and dietary anion-cation differences on mammary epithelium integrity and MEC exfoliation in milk. A trial was performed according to a Latin square design using 8 dairy cows averaging 103 ± 44 DIM with two treatments in a factorial arrangement with 4 periods of 14 d. The cows received 2 levels of dietary anion-cation differences (DCAD; 0 mEq/kg DM for D0 and 400 mEq/kg for D400) and 2 d lengths (8 h of light/d for short days and 16 h/d for long days). The cows were only exposed to solarium lights providing UVA and UVB. Once per period, milk was collected to purify MEC from milk after centrifugation and immunocytochemical sorting. MEC exfoliation was evaluated using the determination of MEC concentration in milk. The percentage of apoptotic MEC was determined by flow cytometry after TUNEL labeling. Epithelium integrity was monitored using the determination of blood lactose sampled 1 h before morning milking, and the ratio Na:K in milk. Blood prolactin concentrations from samples collected at 0700 h and 1400 h were determined by RIA. Data were analyzed using PROC MIXED. There was no significant interaction between daylength and DCAD level. Milk yield did not vary with any treatments averaging $32.7 \text{ kg} \cdot \text{d}^{-1}$. DCAD treatment did not affect any of the parameters. Blood lactose and Na:K ratio were higher with short compared with long days ($P < 0.05$) indicating that mammary epithelium integrity was more disrupted with short days. More MEC and more apoptotic MEC were exfoliated in milk with short days compared to long days ($338 \text{ vs. } 227 \cdot 10^6$ exfoliated MEC per day, for respectively short and long days; $P < 0.05$). As expected blood prolactin concentration was lower with short days ($P < 0.05$). Taken together these results suggest that MEC exfoliation could be induced by low prolactin concentration during short days.

Key Words: mammary epithelial cell, mammary epithelium integrity, photoperiod, feeding

1233 (W142) Serotonin receptors expression in caprine and ovine mammary gland by real time PCR-RT. A. Suárez-Trujillo^{1*}, A. Argüello¹, M. A. Rivero², J. Capote³, and N. Castro¹, ¹Dep. of Animal Science, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain, ²Dep. of Morphology, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain, ³Canarian Agronomic Science Institute, La Laguna, Tenerife, Spain.

The role of serotonin (5-HT) as a feedback inhibitor of lactation in humans, mice and cows has been previously reported. This action is mediated by 5-HT receptors, which are expressed in the mammary tissue. The 5-HTR subtype 7 is expressed in the three quoted species, but in bovine, subtypes 1B, 2A, 2B and 4 have been also found. The aim of this study was to identify the 5-HTR (subtypes 1A, 1B, 1D, 1E, 1F, 2A, 2B, 2C, 3A, 4, 5a, 6 and 7) expression in the goat and sheep mammary gland using Real Time PCR-RT. Lactating Holstein cows, Majorera dairy goats and Canarian dairy ewes were used to obtain hypothalamus and mammary gland samples. The sampling was performed immediately after slaughter. Samples were kept in RNA later, and subsequent RNA extraction was performed using a combination between TriPure reagent (Roche, Barcelona, Spain) and E.N.Z.A. total RNA kit (Omega, Nordic Naturals, inc) protocols. cDNA synthesis was conducted using iScript cDNA synthesis kit (BioRad, Madrid, Spain). The primers used in this study were obtained from published data (1B, 1F, 2A, 2B, 2C, 4, 5a and 7) or were designed using bovine CD sequences from GeneBank (1A, 1D, 1E, 3A and 6). Hypoxanthine phosphoribosyltransferase I (HPTR1), Ribosomal Protein (S18), β -Actin and Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) genes were utilized as internal control. Hypothalamus samples were used as positive control to evaluate the primers in the three studied species. Real Time PCR was performed at 57.5°C of annealing temperature for all primers. The receptors found in bovine mammary gland were in agreement with previous studies. Receptors 1B, 1E, 2A, 2B, 4 and 7 were found in the three species. Additionally, receptors 1D and 5a were observed in goats and sheep. Moreover, 1A and 1F subtypes were only detected in ovine mammary gland. Nevertheless, subtypes 2C, 3A and 6 were found neither bovine, nor caprine or ovine mammary gland. In conclusion, the same 5-HTR subtypes previously described in cows are expressed in caprine and ovine mammary gland. In addition, in caprine mammary gland were identified two more (5-HTR_{1D} and 5-HTR_{5a}), and the most number of 5-HTR were found in ovine mammary gland. Further studies will be necessary to study more in deep the role of serotonin as inhibitor of lactation in small ruminants.

Key Words: serotonin receptors, goat, sheep