LACTATION BIOLOGY II

1232 (W141) Daylength affects simultaneously mammary epithelium integrity and mammary epithelial cell exfoliation in milk. M. Boutinaud[*1], A. Bondon[1], P. Debournoux[1], J. Couedon[1], M. Johan[1], A. Narcy[2], and C. Hurtaud[1], *[1]INRA, Saint Gilles, France, [2]INRA, 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 hbefore 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 kg. 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 vs. 227 · 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. Argüello[1], M. A. Rivero[2], J. Capote[3], and N. Castro[1], *[1]Dep. of Animal Science, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain, [2]Dep. of Morphology, Universidad de Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain, [3]Canarian 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 phosphosibosyltransferase 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

1234 (W143) Immortalization of a primary bovine mammary epithelial cell line by the SV40 large T-antigen gene. H. Hu[1,2,3], N. Zheng[1,2,3], W. Dai[1,2,3], H. Gao[1,2,3], and J. Wang[*1,2,3], *[1]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture–Milk and Dairy Product Inspection Center, Beijing, China, [3]Ministry of Agriculture–Laboratory of Quality & Safety Risk Assessment for Dairy Products, Beijing, China.*

In this study, we report an immortalized cell line expression of SV40 large T-antigen established from a Chinese Holstein primary mammary epithelial cell (CMECs) cultured in vitro. The plasmid that carried the SV40 large T-antigen sequence was introduced into mammary epithelial cells by retroviral mediation. Following injection, cells were cultured under puromycin for selection and 10% resistant cells remained after two wk. Four immortalized bovine mammary epithelial cell colonies were obtained, but only a single colony was surpassed over 50-passage and was designated CMEC-H. The obtained clone was characterized with respect to their morphogenetic behavior, long-term proliferative potential, and differentiation characteristics. The immortalized mammary epithelial cells grew in close contact with each other and exhibited the typical "cobblestone" morphology characteristic with obvious boundary that were more homogeneous than in the primary mammary epithelial cells. And the homogenously polygonal of immortalized cell was maintained from passage 1 to 50. The population growth rate between immortalized cells at passage 5 or 50, doubled in number within 36 h, was not significantly different but was faster than in the primary cells. The typical and representative karyotype of CMEC-H was counted at 100 chromosomes, which was more than the normal diploid chromosome number 60. The telomerase expression of CMEC-H had consistently demonstrated the presence of telomerase activity as an immortalized cell line, but the cell line failed to induce tumor formation in nude mice. The immortalized epithelial cell expressed epithelial cell markers, including cytokeratins CK7, CK8, CK18, and CK19. The gene and protein expressions of caseins (α_{S1}-casein, β-casein, and κ-casein) indicated that the immortalized CMEC-H maintained the milk protein synthesis function of epithelial cells. We conclude that CMEC-H may become a valuable reagent for studying the secretion mechanism of mammary gland.

Key Words: bovine mammary epithelial cell, immortalization, SV-40 large T-antigen

1235 (W144) Color measurement as potential tool for determination of colostrum quality in primiparous and multiparous dairy cows. J. J. Gross[*], E. C. Kessler, and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty University of Bern, Switzerland*

Instruments for on-farm determination of colostrum quality like refractometers and densimeters are increasingly used in dairy farms. The colostrum color is also supposed to reflect its quality. A pale or mature milk like color is associated with a lower colostrum value compared to a more yellowish and darker color. The objective of this study was to elucidate the relationships between color measurements (CIE L* = from white to black, a* = from red to green, b* = from yellow to blue) and colostrum quality as assessed by two common on-farm instruments and composition in colostrum in cows and heifers. Thirteen primiparous and twelve multiparous cows were milked for the first time exactly 4 h post-calving. Colostrum was analyzed for total IgG by ELISA and for fat, protein and lactose by a FTS Infrared Milk Analyzer (Bentley Instruments Inc., Chaska, MN, USA) (Previously validated for use with colostrum). A Brix sugar refractometer (BRIX) and a Kruuse colostrum densimeter (DENS) were used to assess colostrum quality at 20°C. For color measurements of colostrum samples, a calibrated spectrophotometer (Microflash 200d, Data-color International) was used. In primiparous cows, the total IgG concentration was poorly correlated with L*, a*, and b* (r = -0.13, 0.02, and 0.12; $P > 0.05$), while in multiparous cows correlations were higher (-0.40, 0.32, and 0.06, resp., $P > 0.05$). While DENS did not correlate with color measurements, BRIX was closely correlated with L* (r = -0.68, $P < 0.01$), and b* (r = 0.55, $P < 0.0001$) in primiparous and for b* in multiparous cows (r = 0.52, $P < 0.001$). Milk fat concentration was correlated with a* (r = 0.42, $P < 0.001$, and r = 0.44, $P < 0.001$, for primi- and multiparous cows) and b* (r = 0.27, $P < 0.05$, and r = 0.43, $P < 0.01$), while milk protein concentration was more correlated to b* (r = 0.53, $P < 0.0001$, and r = 0.30, $P < 0.05$). Highest correlations were found between milk lactose percentage and b* in primiparous (r = -0.59, $P < 0.0001$) and multiparous cows (r = 0.56, $P < 0.0001$). In conclusion, the color measurements via spectrophotometer were closest correlated with milk fat, protein and lactose concentrations in colostrum but only to a lesser extent with total IgG concentration colostrum of primiparous cows. An implementation of color measuring devices in automatic milking systems might be a potential instrument also to access colostrum quality besides detecting abnormal milk.

Key Words: colostrum, color, quality

1236 (W145) Effect of milk yield genotype on gene expression in liver and adipose tissue from periparturient Holsteins. W. J. Weber[1], M. Carriquiry[2], S. C. Fahrenkrug[1], and B. A. Crooker[*1], *[1]University of Minnesota, St. Paul, [2]Universidad de la República, Montevideo, Uruguay.*

Multiparous cows from unselected (stable milk yield since 1964; UH; $n = 5$) and contemporary CH; $n = 6$) Holsteins that differed in milk yield (6200 and 11,100 kg milk/305 d) were fed the same diet ad lib, milked 2x/d, and exposed to the same management and environmental conditions. Liver and adipose biopsies were collected at -14, 3, 14, and 35 d in milk (DIM). RNA was extracted and expression of 38 genes (focused on the somatotropic axis, glucose and lipid metabolism) and 12 possible internal control genes determined by digital multiplexed analysis (nanoString nCounter). Expression was normalized to the positive control and the geometric mean of 5 internal control genes. Data were transformed (square root) and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Expression of 23 genes in liver and 20 in adipose was altered by DIM. Liver and adipose expression of 6 and 8 genes was greater in CH and of 8 and 7 genes was greater in UH, respectively. There were line by day interactions for IGFBP2 and IGF-ALS in liver and DGAT2, HNF4a, IGF2 in adipose. Hepatic GHRtot and GHR-1A were greater in UH than CH. GHR-1A decreased at 3 but recovered by 14 DIM. Adipose GHRtot was greater in CH. Hepatic IGFBP2 was greater in CH than UH, increased at 3 DIM and although decreasing, remained increased through 35 DIM. Hepatic IGF-ALS was greater in UH, decreased at 3 DIM in both lines and returned to prepartum values by 14 and 35 DIM in UH and CH, respectively. Hepatic FGF21 was greater in CH than UH and peaked at 3 DIM in both lines. FGF21 was not detected in adipose. DGAT1 was similar in UH and CH liver and greater in CH adipose. Liver DGAT1 increased at 3 DIM but was not altered by DIM in adipose. DGAT2 was similar in UH and CH liver and greater in UH adipose. Liver DGAT2 decreased through 35 DIM in both lines. Adipose DGAT2 decreased at 3 DIM and recovered by 35 DIM in UH but not in CH cows. Results are consistent with a prolonged postpartum reduction in hepatic sensitivity to somatotropin and in triglyceride synthesis from de novo fatty acids in adipose of contemporary Holsteins.

Key Words: gene expression, liver, adipose

1237 (W146) Comparative glycolysis and Krebs cycle metabolism of the bovine and murine mammary gland determined with [$^{13}C_6$]glucose and mass spectrometry. L. J. Juengst[*1], E. E. Connor[2], R. L. Baldwin, VI[2], and B. J. Bequette[1], *[1]Dep. of Animal and Avian Sciences, University of Maryland, College Park, [2]USDA-ARS, Bovine Functional Genomics Laboratory, Beltsville, MD.*

The compositions of bovine and murine milk differ significantly with respect to the proportions of lactose, protein, and fat. To better understand the metabolic origins of this difference, we interrogated the crossroads of glycolysis and the Krebs cycle in the mammary gland of cows and mice using a glucose stable isotope (^{13}C) tracer approach in vitro. Mammary tissue was collected from mid-lactation dairy cows ($n = 4$) and Day 15 of lactation mice ($n = 6$) then sliced to form explants (0.5 mm thick, 100 to 150 mg). Explants were incubated for 3 h (5% CO_2) at 37°C in DMEM containing a 50:50 mix of unlabeled and [$^{13}C_6$] labeled glucose at 2.5, 5, 7.5 or 10 mM concentrations. Following incubation, explants were rinsed in PBS and stored at −80°C. Intracellular metabolites were extracted and derivatized for determination of ^{13}C-isotopomer enrichments employing gas chromatography-mass spectrometry. Alanine, glutamate, and aspartate ^{13}C-isotopomer enrichments were monitored as representative surrogates of their Krebs cycle counterparts pyruvate, α-ketoglutarate, and oxaloacetate, respectively. These data provided the inputs to calculate glycolytic and Krebs cycle fluxes. In bovine mammary tissue, increasing media glucose concentration increased glycolytic flux as represented by an increasing contribution of glucose to pyruvate flux. However, the proportion of pyruvate derived from glucose catabolism reached a plateau (44 to 46%) at 7.5 mM glucose. Similarly, in murine mammary tissue, glycolytic rate increased with increasing media glucose concentration, though no plateau was attained and glucose contributed to 43% of pyruvate flux at the highest glucose concentration. Krebs cycle flux was assessed by the relative activities of pyruvate dehydrogenase (PDH) vs. pyruvate carboxylase (PC) based on [^{13}C] tracer kinetics. For bovine mammary explants, PDH flux activity increased to a maximum at 5.0 mM glucose whereas PDH vs. PC activities of murine mammary tissue was not responsive to glucose concentration. The current study suggests that the dairy cow mammary gland shifts from high anapleurotic flux rates into the Krebs cycle to energy-producing (oxidative) fluxes with increasing glucose concentration whereas the murine mammary gland maintains a more rigid metabolic balance, and thus is less adaptive to glucose availability.

Key Words: bovine, murine, mammary metabolism

1238 (W147) Is there a core microbiome in bovine milk samples from healthy quarters with somatic cell counts of less than 200,000 cells/mL?. S. L. Brooker*[1], J. E. Williams[1], S. M. Reynolds[1], K. M. Yahvah[1], L. K. Fox[2], and M. A. McGuire[1], *[1]University of Idaho, Moscow, [2]Washington State University, Pullman.*

Recent analysis has shown that healthy bovine milk contains a commensal bacterial community. Rigorous data are not available on the bacterial community structure in bovine milk, thus it is unknown whether there is a 'core' microbiome within healthy bovine milk. Quarter milk samples were collected from nine Holstein cows that had at least two low SCC quarters (< 200,000 cells/mL). Characterization of the microbial community was performed by culture independent 454 pyrosequencing of amplicons from the V1-V3 region of the 16S rRNA gene to determine relative abundance. Cows were selected only if milk from at least two quarters was below 200,000 SCC. The relative abundance data show that most healthy quarters have bacterial communities that include *Clostridium* spp. (5 to 75% relative abundance), *Pelomonas* spp. (10 to 35% relative abundance), *Duganella* spp. (< 5% relative abundance), *Turicibacter* spp. (0 to 10% relative abundance), and *Sporacetigenium* spp. (0 to 45% relative abundance), with all samples having a large influence from unclassified bacterial spp. (5 to 55% relative abundance). The Shannon and Chao diversity indices of the bacterial communities between cows were not different ($P > 0.1$) suggesting similar distribution of community membership. Analysis by PCoA using the Bray dissimilarity matrix showed strong clustering based on the relative abundance of *Clostridium* spp., which was a major contributor in all healthy quarter samples. No clustering based on SCC was apparent among the samples. Clustering of healthy quarters within cow was also not discernable suggesting a high variation in the bacterial community even between quarters of the same cow. These results propose a highly variable bacterial community exists in bovine milk even between healthy quarters within a cow. Though there appears to be no obvious 'core' set of bacterial members, the variation present could account for similar functional roles within quarter milk bacterial community. This work was supported by the Idaho Agricultural Experiment Station, NIH grants P20 RR15587 and P20 RR016454 and the Institute for Bioinformatics and Evolutionary Studies (IBEST) at the University of Idaho.

Key Words: milk, bacteria, microbiome

1239 (W148) Impact of machine milking on teat dimensions. J. F. Guarin*[1,2], D. J. Reinemann[3], and P. L. Ruegg[1], *[1]Dep. of Dairy Science, University of Wisconsin–Madison Madison, [2]Grupo de Investigación Biogénesis, Facultad de Ciencias Agrarias, Universidad de Antioquia, Medellin, Colombia, [3]Dep. of Biological Systems Engineering, University of Wisconsin–Madison, Madison.*

The objective of this study was to determine associations between machine milking and changes in teat dimensions. A total of 1751 teats from 445 cows from the University of Wisconsin dairy herd were measured pre and immediately post-milking. The difference in teat length (Length), barrel width (Barrel) and teat tip diameter (Tip) before (PRE) and after (POST) machine milking was determined. Absolute change (Delta) on teat dimensions was calculated as POST-PRE measurements. Relative change (DeltaMeasureREL) was calculated as POST-PRE/PRE and expressed as percentage. Additional covariates included: Milk flow peak (PeakFlow), and parity (1, 2 or $\geq$ 3). Descriptive statistics were determined using PROC UNIVARIATE, FREC, GLM, and LOGISTIC of SAS version 9.3. Means and SD for Pre-milking Length, Barrel, and Tip were 44.3 ± 8.3, 23.9 ± 3.7, and 19.6 ± 2.3 mm, respectively. Means for PRELength were different for parity ≥ 3 (46.6 mm) when compared with 1 (42.8 mm) and 2 (43.6 mm) ($P < .0001$). Means for PREBarrel were significantly different among parities ($P < .0001$). Means for PRETip increased significantly with parity and were 19.3, 19.6, and 20.0 mm for parities 1, 2, and ≥ 3, respectively ($P < 0.001$). A positive association was found between PREBarrel and PeakFlow ($P < .0001$) for primiparous cows only. Teat dimensions were affected by milking. During milking, teat length increased by 1.3 mm, teat barrel decreased by 2.2 mm and teat tip decreased by 0.7 mm. Changes in teat dimensions on length, barrel, and tip were not influenced by parity. Out of the 1751 teats, 163 (9.3%) became congested at the barrel. Out of the 1751 teats, 163 (9.3%) teat barrels became congested. Congestion was defined as PREBarrel measurements that were smaller than PostTeatBarrel measurements, implying congestion of the tissue during milking. There was a strong association between pre-milking teat barrel dimension and peak milk flow for primiparous cows. Further research on teat dimensions changes and its influence on mastitis is required.

Key Words: dairy cow, induced changes, teat dimension

Table 1239. Descriptive statistics for the impact of machine milking on teat dimensions

Teat dimension (n = 1751 quarters from 445 cows)	Mean ± SD	Range
Pre-milking (mm)		
PRELength	44.3 ± 8.3	26.0–80.0
PREBarrel	23.9 ± 3.7	14.0–50.0
PRETip	19.6 ± 2.3	10.0–30.0
Post-milking (mm)		
PostLength	45.6 ± 8.5	24.0–100.0
PostTeatBarrel	21.7 ± 2.8	12.0–40.0
PostTeatTip	19.0 ± 2.2	10.0–30.0
Absolute change[1] (mm)		
DeltaLength	1.3 ± 5.5	-20.0–26.0
DeltaBarrel	-2.2 ± 3.0	-20.0–10.0
DeltaTip	-0.7 ± 2.3	-10.0–8.0
Relative change[2] (%)		
DeltaLengthREL	5.5 ± 23.4	−83.3–118.2
DeltaBarrelREL	−8.3 ± 11.2	−50.0–50.0
DeltaTipREL	-2.5 ± 12.4	−50.0–66.7

[1] Postmilking value– premilking value.
[2] (Postmilking value– premilking value)/premilking value x 100.

1240 (W149) Comparison of ecological indices of bacterial communities in bovine milk varying in somatic cell count. J. E. Williams*[1], S. M. Reynolds[1], K. M. Yahvah[1], S. L. Brooker[1], L. K. Fox[2], B. Shafii[1], and M. A. McGuire[1], [1]*University of Idaho, Moscow,* [2]*Washington State University, Pullman.*

Somatic cell count (SCC) of milk is often used to determine the health status of the mammary gland. However, little is known about the bacterial community structure within milk of varying SCC. Next generation sequencing technology has provided researchers the opportunity to characterize the bacterial diversity and community structure within bovine milk. We hypothesized that the bacterial diversity and community structure would be different among milk with low (< 200,000 cells/mL), medium (200,000 to 400,000 cells/mL), and high (> 400,000 cells/mL) SCC. Utilizing ecological indices that describe bacterial diversity, we analyzed 16S rRNA (V1-V3 region) sequencing data from quarter milk samples collected from 15 Holstein cows. Comparisons among quarter milk samples with different SCC were performed using analysis of variance and mixed model procedures of SAS (v9.3) and significance was declared at $p \leq 0.05$. Additionally effects of SCC status were compared using predetermined contrasts of milk with low versus medium and high, as well as medium versus high levels of SCC. Based on richness estimators (Chao1 and abundance-based coverage estimators [ACE]), milk with high SCC had a bacterial community less rich and diverse than milk with low or medium SCC. Also, according to Shannon's and Simpson's diversity indices when SCC is medium and high, there is a decrease in number of bacterial genera present as well as a decrease in the evenness of the bacterial community membership compared to milk with low SCC. While milk categorized as high SCC is different from milk with low and medium SCC, the bacterial diversity and community structure in low SCC milk is not different from milk with medium SCC. Future studies are needed to explore how bacterial community membership among milk samples differs with varying SCC. *This work was supported by the Idaho Agricultural Experiment Station, NIH grants P20 RR15587 and P20 RR016454 and the Institute for Bioinformatics and Evolutionary Studies (IBEST) at the University of Idaho.*

Key Words: milk, microbiome, diversity

1241 (W150) Effects of arginase inhibition on casein expression and proliferation of bovine mammary epithelial cells. *L. Ding[1], M. Wang*[1], L. Chen[1], H. Wang[1], and J. J. Loor[2], [1]Yangzhou University, China, [2]University of Illinois, Urbana-Champaign, Urbana.*

Reviews of the ruminant literature concluded that the uptake of Arg by the mammary gland greatly exceeds its output in milk. Furthermore, milk protein yield was increased by post-ruminal infusion of Arg compared with a control treatment that included infusion of water and a mixture of essential amino acids (AA) excluding Arg. Those results indicated that Arg might have an important role on casein synthesis regulation. Our previous research subsequently revealed that excess Arg has a regulatory function on the casein synthesis via effects on transcription of the casein gene. Whether Arg regulates the synthesis of casein by the metabolism of enzymes is not very clear. The specific objective of this work was to elucidate the effect of arginase on the regulation of casein expression using an arginase inhibitor in vitro. Primary bovine mammary epithelial cells (The mammary epithelial cell were isolated from three healthy multiparous dairy cow at lactation stage) were cultured with different concentrations (0, 0.5, 1, 2 μmol/L) of the arginase inhibitor nor-NOHA. The casein expression and the proliferation of mammary epithelial cells were determined after 24-h of in vitro culture triplicate/treatment). Statistical analysis was done using the OneWay Analysis of Variance (ANOVA) with multiple comparison test of Duncan using SPSS v16.0. And the P values less than 0.05 were declared to be significant ($P < 0.05$). The results showed that the concentration of arginase-1 in different groups dropped ($P < 0.05$) when the concentrations of nor-NOHA increased, and was quite small in the cells incubated with 1 and 2 μmol nor-NOHA/L. The different concentrations of nor-NOHA had no effect ($P > 0.05$) on the proliferation of mammary epithelial cells. Compared with the control group (0 μmol/L), the expression of casein was lower ($P < 0.05$) in other groups and was lowest in the group with the nor-NOHA concentration of 2 μmol/L. Additionally, in cells incubated with 0.5 μmol/L nor-NOHA the expression

level of OTC and ODC was significantly lower ($P < 0.05$) than other treatments. The expression of eNOS was lowest ($P < 0.05$) in the group with the nor-NOHA concentration of 1 μmol/L. In conclusion, the activity of arginase can be inhibited by nor-NOHA. Furthermore, nor-NOHA affected expression of casein and some arginine metabolism-related enzymes. With the increase of nor-NOHA concentration, the inhibitory effects on casein increased.

Key Words: arginine, casei expression