11 Modeling the interaction of milking frequency and nutrition in lactation. I. Vetharaniam and S. R. Davis, *AgResearch Limited, Hamilton, New Zealand*.

A model of lactation, parameterized for primiparous New Zealand cows grazing pasture was used to understand and quantify how milking frequency interacts with nutrition. In a simulation, cows were given one of two intakes over a lactation of 270 days: a low allowance (LA) reflecting actual pasture intake patterns, and a higher (on average 20%) allowance (HA) designed to counter deficit periods. Milking frequencies were varied from 1 to 4 times per day.

Once daily milking (1DM) compared with twice daily (2DM) resulted in a production losses of 29% on LA and 32% on HA. 3 and 4 milkings per day (3DM and 4DM) increased production compared with twice daily milking by 9% and 12% respectively on LA, and 11% and 17% on HA. At the end of the lactation, 1DM resulted in 44% less mammary tissue than with 2DM, while 3DM and 4DM respectively gave mammary tissue increases of 22% and 40% over 2DM. Increasing the solids' content of milk by 20% reduced the loss associated with 1DM by 4%. Increasing cistern capacity by 20% only reduced this loss by 1%.

Temporary 1DM for the first 3 weeks of lactation resulted in a production loss of 19%, compared with 2DM, on HA. With LA, this effect was only 9%. After 3 weeks, there was a long term loss in mammary tissue of 4%, and a loss in production of the same amount for either allowance. A 20% increase in cistern capacity of the udder reduced production loss in the first three weeks by 3% for HA.

The model shows that mammary gland size over time is modulated by milking frequency, and determined the production potential of the udder, but actual production is strongly influenced by nutrition affecting secretion rates of alveoli. The response to milking frequency varied considerably with nutrition. A significant portion of the loss associated with 1DM is due to udder fill effects inhibiting secretion as opposed to loss of mammary tissue. The model reflects the underlying biology and its behavior is in good agreement with experiment. It demonstrates that higher milking frequencies need to be coupled with higher nutrition to obtain the potential benefits, and thus would be a useful teaching or research tool.

Key Words: Milking frequency, Nutrition, Lactation

12 Changes in cisternal and alveolar milk throughout lactation in dairy sheep. M. Rovai*, X. Such, G. Caja, and J. Piedrafita, *Universitat Autonoma de Barcelona, Bellaterra, Spain*.

With the aim to study the changes in the cisternal traits of the udder of dairy ewes, a total of 212 primiparous and multiparous dairy ewes (Manchega, **MN**; n= 133; and, Lacaune, **LC**; n= 79) were used during suckling (wk 0 to 5) and milking (wk 6 to 20) periods. Udder evaluation was done 8 h after the a.m. machine milking (0800 h) at 30, 60 and 105 d of lactation. Milk yield, machine fractioning and main udder traits (depth, length and teat distance) were also measured throughout the milking period. Cisternal scans were obtained by using a portable ultrasound scanner with a 5 MHz and 80° sectorial transducer and their area measured. Cisternal milk was measured after drainage by using a teat cannula, and alveolar milk was machine milked after an oxytocin i.v. injection (4 IU/ewe). Milk yield varied according to breed (MN, 0.86 l/d; and LC, 1.69 l/d; P<0.001) and lactation stage (P<0.001). Machine milking fractioning (machine milk: stripping and residual milk) was 62:38 and 79:21 for MN and LC (P<0.001), respectively. Cisternal area (MN, 14 cm^2; and LC, 24 cm^2) and cisternal milk (MN, 149 ml; and LC, 275 ml) varied according to breed (P<0.001) and tended to increase with parity (P<0.10). Moreover, both cisternal area and cisternal milk, decreased in both breeds through lactation (P<0.001). Values for cisternal area were: MN (15.9, 13.7 and 12.3 cm^2) and LC (24.6, 24.0 and 22.2 cm^2); and for cisternal milk were: MN (178, 161 and 109 ml) and LC (335, 263 and 228 ml), for 30, 60 and 105 d, respectively. Alveolar milk decreased with lactation stage in both breeds (P<0.001) but only showed a tendency between breeds (MN, 160 ml; and LC, 194 ml; P= 0.060). Cisternal area and cisternal milk were correlated (r= 0.76; P<0.001) in both breeds, as well as milk yield during the milking period (r= 0.42 to 0.60; P<0.01) and udder size traits (r= 0.21 to 0.51; P<0.05). Positive correlations of cisternal area with machine milking fractions (r= 0.47 to 0.55) were also observed. Results indicate that cisternal scanning is an efficient method to evaluate the cistern size and the productive capacity of the ovine udder.

Key Words: Cisternal Milk, Alveolar Milk, Udder Scans

13 Insulin response to amino acid infusions in Holstein cows. C. A. Toerien* and J. P. Cant, *University of Guelph, Guelph, Canada*.

Despite reported positive effects of insulin (INS) on milk precursor availability, milk protein production and milk yield, few studies have focussed on the effect of individual amino acids on insulin release in dairy cows. In Experiment 1, our objective was to investigate the insulin response in non-pregnant cows in early lactation (EL; mean ± SEM: 32 ± 1 kg milk/d) to pulse i.v. infusions of phenylalanine (Phe), arginine, glycine, histidine or lysine (at 14 mg/kg BW). Baseline INS values were similar across treatments, and all amino acids stimulated similar total INS release (as area under the curve; AUC). Peak response above baseline (ng/mL) was the highest for arginine (3.4 ± 0.6) and differed from that of glycine and histidine (1.6 ± 0.5 and 1.3 ± 0.6; P < 0.05). In Experiment 2, we compared INS release to various levels of Phe, between non-pregnant lactating (Lact; 16 ± 0.5 kg milk/d) and dry (Dry) cows. Treatments were pulse i.v. doses of Phe at 7, 14, 76, and 112 mg/kg BW. Baseline INS was higher in Dry cows across treatments. Contrary to responses in Dry cows, high levels of Phe failed to elicit an appreciable response in Lact cows in AUC or peak INS. Results indicate that physiological state plays an important role in regulation of INS release in dairy cows. Because INS stimulates protein synthesis in various tissues, caution should be used when applying the flooding dose Phe technique to measure protein synthesis in cows at different physiological stages.

		Treatments (mg Phe/kg BW)				
	Group	7	14	76	112	Trt*Gr
AUC[1]						
($ng.mL^{-1}.min^{-1}$)	Dry	17.9^a	9.2^a	95.9^b	103^b	P<0.001
	Lact	-9.1^a	-7.4^a	1.21^a	0.9^a	
Peak INS[1]						
(ng/mL)	Dry	1.9^a	1.8^a	7.7^b	6.2^c	P<0.001
	Lact	0.8^d	0.9^d	1.7^a	2.0^a	

[1] Differences within and between groups in AUC and Peak INS reported at P < 0.05.

Key Words: Insulin response, Amino acids, Dairy cows

14 Involvement of Oct-1 in transcriptional regulation of beta-casein gene expression in mouse mammary gland. Feng-Qi Zhao*[1] and Takami Oka[2], [1]*University of Vermont, Burlington, Vermont,* [2]*National Institute of Health, Bethesda, Maryland*.

Mouse beta-Casein gene promoter contains a region termed block C which is crucial for its gene transcription induced by lactogenic hormones. Nuclear extracts from mouse mammary glands contain at least two binding complexes (DS1 and DS2) which specifically bind to double-stranded block C region DNA. The binding sequence of these complexes was identified to be 5'- AAATTAGCATGT -3' which contains a sequence element related to the consensus octamer motif's complement ATTTGCAT. In the present study, we demonstrate that this sequence element indeed is the binding site for octamer-binding transcription factor (Octs) and Octs represent the double-stranded DNA binding proteins specifically binding to the block C region. Formation of the specific double-stranded binding complexes can be completely blocked by Oct binding motif oligonucleotides and anti-rOct1 antiserum. We also show that Oct-1B represents at least partial, if not all, double-stranded binding protein, DS1, in mammary nuclear extract. Oct-1B may functions as a transcriptional activator on casein gene promoter. The Oct binding activity to beta-casein gene promoter in the mammary gland is affected under influence of hormones both in vitro and in vivo. The DS1 binding activity can be induced by the combination of insulin, hydrocortisone

and prolactin in virgin mouse mammary gland organ culture and induced by injection of progesterone or the combination of progesterone and estradiol in virgin mice.

Key Words: Transcriptional regulation, beta-Casein gene, Oct-1

15 Synthesis of insulin-like growth factor binding proteins by a bovine mammary cell line. F Cheli*[1], A Baldi[1], L Rossi[1], M Vestergaard[2], and S Purup[2], [1]*Dept. VSA, University of Milan/I,* [2]*Danish Institute of Agricultural Sciences/DK.*

The insulin-like growth factor binding proteins (IGFBPs) are a family of locally-produced growth regulators involved in mammary gland development. The production of IGFBPs within the mammary gland is species specific and depends on the stage of mammary gland differentiation. The aim of this work was to investigate whether bovine mammary epithelial cells (BME-UV1) produce IGFBPs and whether retinoic acid (RA) modulates the production in vitro. BME-UV1 cells were kept cultured in either control medium or in media supplemented with either, insulin (1μg/ml), all-trans-retinoic acid (1μM), or insulin+RA. Cell proliferation was evaluated at 48 and 72 h. At the same time samples of the medium were collected. Concentration of IGFBPs was evaluated by Western ligand blotting. Autoradiographs from the blots were exposed for 14 days and were evaluated by desktop scanning densitometry. RA inhibited ($P<0.05$) proliferation of both control and insulin-stimulated BME-UV1 cells by 30 and 26%, respectively. IGFBP-2 and IGFBP-3 were detected in BME-UV1 culture medium. RA affected the relative distribution of the two IGFBPs in the culture medium. RA significantly ($P<0.01$) increased IGFBP-2 content in both control and insulin-stimulated cells. In conclusion, BME-UV1 cells produce IGFBP-2 and IGFBP-3 and the production seems to be regulated by RA. Results also indicate that RA-induced inhibition of BME-UV1 cell proliferation is related to an increase in IGFBP-2 in the culture medium.

Key Words: bovine mammary cells, retinoic acid, IGFBP

16 Influence of dietary starch and of phase of lactation on haematological markers of oxidative stress in early lactation. G. Stradaioli[1], G. Gabai[2], and B. Stefanon*[1], [1]*Dipartimento di Scienze della Produzione Animale - Universita' di Udine (Italy),* [2]*Dipartimento di Scienze Sperimentali Veterinarie - Universita' di Padova (Italy).*

Ten Friesian heifers were randomly assigned to two groups and fed until 30 DIM a basal TMR. At 35 DIM the control group (CTR, 24% starch/DM) continued to receive the same ration and the experimental group (EXP) was allotted to the experimental diets, which consisted in a stair step compensated starch regimen. Experimental diets were designed isoenergetic in order to have a reduction of starch (LSD, 21% starch/DM) followed to an increase (HSD, 28% starch/DM) of starch contents, with a final return to the basal diet. Blood was sampled at 37, 50, 60, 70, 80 and 94 DIM and analysed for glutathione peroxidase activity (GPx), glutathione (GSH), malondialdheyde (MDA), glucose (GLU), beta-hydroxy butyrate (b-OHB) and free fatty acids (FFA) concentrations. The stair step compensated starch regimen did not significantly affected milk yield and FCM between the groups during the experiment, the average milk yield for the EXP group being 25.88 kg/d, lower than the 27.42 kg/d of the CTR group. Plasma GLU was significantly lower with LSD diet and higher with HSD compared to mean values at 37 DIM. Plasma b-OHB significantly decreased in the EXP group at 70 DIM, when the LSD was replaced by HSD, but no variations were observed for plasma FFA concentrations. GPx activity decreased considerably after HSD administration to the EXP group (DIM 70 and 80), and recovered to initial mean value after return to basal diet(94 DIM). GSH concentrations, a measure used to identified antioxidant pool depletion, were not statistically affected from dietary variations of starch, although numerically lower mean values were observed during the LSD and HSD administration to the EXP group. Plasma MDA was significantly higher ($P<0.001$) for CTR group compared to EXP group, as was the interaction "dietary treatment" X "DIM" at 60 and 80 DIM. The results indicated that a moderate starch variation in the diet can contribute to enhance specific scavenger enzymatic activity, i.e. GPx, but did not substantially cause a reduction of blood antioxidant pool or an enhancement of MDA. The variations of plasma MDA were positively related to milk yield, indicating that milk production per se is a factor potentially affecting the level of oxidative stress during the early phase of lactation in dairy cows.

Key Words: Oxidative stress, Milk yield, Dairy cows

17 Effect of milking and a suckling/milking combination on oxytocin and prolactin release and on milk yield in crossbred Gir x Holstein cows. J. A. Negrao*[1] and P. G. Marnet[2], [1]*USP/FZEA, FAPESP, Pirassununga/SP, Brazil,* [2]*UMR INRA/ENSAR, Production de lait, Rennes, French.*

Unspecialized cattle farmers in Brazil have used crossbred Gir x Holstein cows to produce both calves and milk during spring and summer in extensive systems. Traditionally, these crossbred cows are reputed not to be well-adapted to machine milking and are milked with their calves. However, this type of management increases the labour of milkers and also milking time. For these reasons, 10 Gir x Holstein cows (F2) were used to evaluate the effect of different milking methods on oxytocin (OT) and prolactin (PRL) release and on milk yield. All experimental cows were milked twice/day: 5 cows were suckled by their calves, immediately before and after milking (SM group) and the other 5 cows were separated from their calves and submitted to exclusive milking (M group). Milk yield was recorded throughout lactation. Blood samples were taken on days 60, 61, 62 and 63 of lactation, before and after udder stimulation. Plasma concentrations of OT and PRL were measured by EIA and RIA method, respectively. Highest OT levels were observed during suckling, however there were no significant differences between peak levels of OT measured during suckling and milking in the SM group. At the same time, both groups had similar levels of OT during machine milking, however, the hormone profiles were different because OT increased more rapidly in the SM group (2 min after the beginning of milking) than in the M group (5 min). In general, the SM group showed higher PRL levels than the M group and during suckling, PRL levels were significantly higher in the SM than in the M group. Despite these results, SM cows produced more milk (milking plus suckling, 18.4 $\pm$ 1.2 L/day) than M cows (15.0 $\pm$ 0.4 L/day). Our results indicate that both type of management were effective to induce OT and PRL release in crossbred Gir x Holstein cows.

Key Words: Milking/suckling, Oxytocin, Prolactin

18 Milk emission during machine milking in dairy sheep. M. Rovai*, X. Such, G. Caja, and J. Piedrafita, *Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The aim of this work was to compare the milkability of Manchega (MN, 1.03 L/d, n=133) and Lacaune (LC, 1.71 L/d, n=79) dairy ewes. The kinetic parameters of milk emission during machine milking were measured at 6, 10 and 14 weeks post-partum. Data were recorded by the manual method in two successive days for individual udder halves during the evening milking. The curves were classified into three groups: 1 peak (1P), 2 peaks (2P) and in plateau (IP). The last type refers to ewes with larger emission curves and did not show clear differences between peaks (1 and 2). The frequency of different curve types (1P:2P:IP) was 25:66:9 and 5:60:35 for MN and LC, respectively, which means a greater milk ejection reflex in LC ewes (95% vs 75%). Milk production varied according to curve type in both breeds (0.82,1.03,1.16 L/d in MN and 0.93,1.68,1.82 L/d in LC for 1P, 2P and IP, respectively). The IP as compared with 2P, showed greater total emission volume (0.30 vs 0.24 L) and total time of emission (49 vs 46 s). As compared to MN ewes, LC showed greater ($P<0.001$) flow rate (0.39 vs 0.29 L/min), milk volumes (0.28 vs 0.16 L) and emission time (44 vs 39 s) for all curve types. All parameters of milk emission kinetics increased with age in LC ewes ($P<0.01$), whereas in MN ewes the differences were observed only for some parameters which may be due to low variation in milk yield with age in MN ewes in this experiment. The frequency of milk emission type curves varied according to the parity number, showing an increase in the ejection reflex with age. Throughout lactation, all kinetic parameters decreased ($P<0.001$) except time of latency (28 s) and second peak flow (37 s) that remained constant in 2P/ewes, suggesting that the time of milk ejection reflex did not change throughout lactation. Moreover, the percentage of ewes presenting the ejection reflex decreased throughout lactation. In conclusion, herds selected for high milk production (e.g. LC ewes) tended to have a better milk ejection reflex (2P and IP) and milkability.

Key Words: Dairy Ewes, Milking Kinetics, Milk Ejection

19 Induction of milk yield decrease and mammary gland involution in lactating Holstein cows and female rats. L. Delbecchi*, N. Miller, D. Petitclerc, and P. Lacasse, *AAFC-Dairy and Swine R&D Centre, Lennoxville, Quebec, Canada.*

Increasing amounts of estrogens in the blood of pregnant lactating cows may be one factor inducing the progressive involution of the mammary gland after the peak of lactation. In a first experiment, non-pregnant, mid-late lactation cows received sc. injections of either 17-estradiol (15 mg/cow/day; treated group, n=4) or 95% ethanol (control group, n=4) from d 0 to d 8. Measurement of milk production (d -10 to d 20) and evaluation of milk composition (before, during and after estradiol injections) showed that treated cows presented signs of mammary gland involution. Milk production was reduced ($P < 0.01$) in treated versus control cows by 14.8% on d 3, 37.2% on d 6, 76.5% on d 8, and 81.6% on d 11. Between d 0 and d 7, in treated cows, milk fat content and lactose concentration decreased ($P<0.05$) by 37.6% and 15.9%, respectively, while milk protein content increased by 61.9% ($P<0.05$). Control cows showed no significant variation in these parameters during the same period. Quantitative RT-PCR was performed on RNA extracted from mammary biopsies taken at d 0, d 1, d 2, and d 4. Levels of b-casein mRNA were reduced ($P<0.05$) by estradiol but those of bax and bcl-2 were not significantly affected, suggesting a lack of short-term effect of estradiol on regulation of apoptosis in the mammary gland. In a second experiment, three potential inhibitors of estradiol, i.e. trans-retinoic acid (tRA), melatonin, and mimosine were tested in lactating female rats. Milk production was reduced ($P<0.05$) in rats injected with estradiol as compared to controls. Melatonin and mimosine showed no significant effect ($P>0.1$) on this reduction, while tRA enhanced it ($P<0.01$). Analysis of gene expression is currently performed on mammary biopsies taken at the end of treatments. These results support the hypothesis that estrogens produced by the fetal-placental unit induce a gradual decline in milk production in pregnant lactating cows, but the molecular bases for this effect remain unclear. Work supported by Dairy Farmers of Ontario and Agriculture and Agri-food Canada.

Key Words: Involution, Mammary gland, Estrogens

20 Cloning lactoferrin gene in a novel expression vector and its expression/secretion in bovine mammary cells. N Bissonnette*, P Lacasse, and D Petitclerc, *Agriculture and Agri-Food Canada, Dairy and Swine Development and Research Center.*

Milk is a source of nutrients and a carrier of various forms of specific factors influencing bacterial growth, which may have significant benefit for the health of the suckling neonates. Milk proteins, such as lactoferrin (LF), are part of the innate immune system with antimicrobial properties and are an important component of this line of defence of the mammary gland. LF is a glycoprotein naturally produced by mammary cells and is found in milk of human (1-6 g/L) and cows (0.01-0.1 g/L). The aim of this study was to develop a molecular tool that permits the cloning and the expression of a bactericidal gene without killing the bacteria when the DNA vector is produced but allowing its full bactericidal expression when secreted by transfected eukaryotic cells. As conventional eukaryotic vectors with commonly used promoters (CMV, RSV or SV40) permit a basal expression in bacteria, others and we did not manage to clone lactoferrin gene in bacteria using such expression vectors. Expression systems reported so far to produce recombinant LF protein have used molds or yeasts. As glycans on mammalian glycoproteins influence their functions in many different ways, the major drawback of these lower eukaryotic systems is their inappropriate post-translational modifications. Hence, a method of inhibiting, in bacteria, LF gene expression by using a single eukaryotic expression vector without any repressor/activator molecule was developed. This system allowed the cloning of the bactericidal lactoferrin gene in an expression vector using bacteria as a host and permitted the production of high amount of vector by bacteria. Subsequently, this vector allowed the secretion of 200 ug/L of LF using our eukaryotic expression vector when transfected in bovine mammary cells. Therefore, DNA expression vector could be quantitatively produced by bacteria; thereby, large quantity of a bactericidal therapeutic gene, therein lactoferrin, could be used with the aim of being expressed as antimicrobial protein in transfected or transgenic eukaryotic cells.

Key Words: lactoferrin, expression vector, gene therapy

21 Effect of milking interval on milk yield and quality and the rate of recovery during subsequent frequent milking. K. Stelwagen*, V.C. Farr, and S.R. Davis, *AgResearch Ltd., Hamilton, New Zealand.*

Forty multiparous cows (DIM: 118 18) on an all-pasture diet were used in a completely randomised block design to examine the effects of milking interval (MI) on milk yield and quality, and its recovery during subsequent frequent milking. Following 2 d of normal twice-daily milking cows were not milked for either 6, 12, 18, 24 or 30 h, after which they were milked every 6 h for 24 h. Means shown are for, respectively, the 6, 12, 18, 24 and 30-h MI. Milk yield increased with increasing MI, but plateaued after 24 h of milk accumulation (4.7^a *vs.* 8.2^b *vs.* 11.5^c *vs.* 15.2^d *vs.* $16.4^d \pm 0.6$ kg, $^{abcd}P<0.01$). Although, yield recovered to at least pre-MI yields for all groups, the rate of recovery was slowest for the highest MI (regression coefficients: 0.72^a *vs.* 0.70^{ab} *vs.* 0.68^b *vs.* 0.67^{bc} *vs.* $0.64^c \pm 0.02$ kg/h, $^{abc}P<0.05$). The decrease in milk secretion after 18 h of milk accumulation coincided with an increase in mammary tight junction permeability, based on plasma lactose levels (27^a *vs.* 29^a *vs.* 47^a *vs.* 255^b *vs.* $413^c \pm 40$ μM, $^{abc}P<0.001$). Increased permeability increased the concentration of serum albumin in milk (184^{ab} *vs.* 146^b *vs.* 211^{bc} *vs.* 235^c *vs.* $234^c \pm 19$ μg/ml, $^{abc}P<0.07$), and differences remained during the first three subsequent 6-hourly milkings. SCC(*1000/ml, ln-transformed) were not different at the end of each MI, but were significantly elevated in the milk from cows in the 24-h and 30-h groups during the subsequent frequent milking period (4th 6-hourly milking: 4.0^a *vs.* 4.1^a *vs.* 4.4^a *vs.* 5.0^b *vs.* $5.1^b \pm 0.2$, $^{ab}P<0.05$). This is consistent with earlier data on once-daily milking, showing an increase in SCC after a 24-h lag phase. In conclusion, the rate of milk secretion begins to decrease with MIs excess of 18 h, which may be, at least partly, related to increased mammary tight junction permeability, and leads to poorer milk quality. The adverse effects (except SCC) can be reversed if the long MIs are followed by a 24-h period of frequent milking.

Key Words: Milking interval, Milk quality, Tight junction

22 Leptin variations in dry and lactating periods of dairy cows with different genetic merit. R. Lombardelli[1], P. Bani[1], C. Delavaud[2], Y. Chilliard[2], and G. Bertoni*[1], [1]*UCSC, Facolta di Agraria, Piacenza, Italy,* [2]*INRA-UHRH, Theix, France.*

Even though leptin is a quite recently discovered hormone, it has been intensively studied suggesting that it operates both directly and indirectly to orchestrate complex pathophysiological processes. In a previous paper we did demonstrate that, in the early lactation, dairy cows of different genetic merit mobilise their protein and fat reserves with a different intensity. To ascertain a possible role of leptin, 11 dairy cows of low (LG), medium (MG) and high (HG) genetic merit, 50 days before to 300 days after calving were checked: daily for milk yield and dry matter intake, twice a week for blood metabolites and hormones (including leptin) and fortnightly for live weight (LW) and BCS (on a 0-5 scale). The 300 days' mean milk yield was: 20.6 (LG), 27.2 (MG) and 32.8 kg/d (HG). Maximum post-partum LW loss was lower in LG (6%) than in MG (14.6%) and HG (13.8%). The level of leptin was not strictly related to the genetic merit, but 5 animals showed constantly low values (1.5 - 3.5 ng/ml) during dry and lactation stages; 5 more animals showed quite high values during dry period (7 - 11 ng/ml), a sharp decline after calving (1.5 - 3.5 ng/ml) and a partial recovery afterwards (2.5 - 4.0 ng/ml). The last one showed constantly high values during the whole experiment. Among the main data to be discussed there is the positive correlation between leptin and BCS found in LG cows only. Moreover, glucagon is related to leptin but in a positive way for LG cows whereas the reverse is true for the HG ones. Other important data are the higher live weight (but not BCS) and lower GH values recorded in the cows with higher leptin during late pregnancy. Also different, but not significantly, are milk yield, DMI and insulin, all higher in the latter cows. (Supported by RAISA- CNR).

Key Words: Leptin, Genetic merit, Dairy cow

23 Comparison of milk yield and of oxytocin and cortisol release during machine milking in Gir, Gir/Holstein and Holstein cows. J. A. Negrao*[1] and P. G. Marnet[2], [1]*USP/FZEA, FAPESP, Pirassununga/SP, Brazil,* [2]*UMR INRA/ENSAR, Production de lait, Rennes, French.*

Gir cows (adapted to tropical conditions) and Holstein bulls (more productive) have been crossbred in Brazil to improve dairy production. In general, this crossbreeding program was a success and at present, many specialized farmers have Holstein cows with varying percentages of Gir blood. Although Gir cows and Gir/Holstein cows are reputed to be easily stressed and not well-adapted to machine milking, adaptation to exclusive machine milking has not been described in the literature for these cows. Taking this into account, 6 Gir cows (group G), 6 Gir x Holstein cows (F3; group GH) and 6 Holstein cows (group H) were used to evaluate the effect of exclusive machine milking on oxytocin (OT) and cortisol (CORT) release and on milk yield. Milk yield was recorded throughout lactation. Blood samples were taken on days 45, 48, and 51 of lactation, before and after milking. Plasma concentration of OT was measured by EIA method and CORT was measured by commercial EIA Kit (dslab Inc). As expected, milk yield was significantly higher in the H group (25.6 $\pm$ 3.3 L/day) than in the GH group (20.2 $\pm$ 1.7 L/day), and the GH group produced more milk than the G group (13.1 $\pm$ 1.0 L/day). In contrast, all groups exhibited similar levels of OT, although OT increased more rapidly during milking of the H and GH groups (1 min) than of the G group (3 min). Simultaneously, CORT levels were significantly different for groups, the G group presented highest levels of CORT than GH and H groups. Our results indicate that G, G/H and H groups presented similar release of OT during exclusive milking, however CORT levels were inversely related to milk production.

Key Words: Milking, Oxytocin, Cortisol

24 Mixed linear model analysis of factors affecting the evolution of milk electrical conductivity along lactation in dairy cattle. N.P.P. Macciotta[1], M. Mele*[2], A. Cappio-Borlino[1], and P. Secchiari[2], [1]*Dipartimento di Scienze Zootecniche - Universit degli Studi di Sassari, Italy,* [2]*D.A.G.A. Settore Scienze Zootecniche - Universit di Pisa, Italy.*

Electrical conductivity (EC) of cow milk is affected by the health status of the mammary gland but also by other factors that usually affect milk yield such as variations among cows, Test date, stage of lactation and parity. In order to evaluate the effects of all these factors on EC, Test Day (TD) records of an index of EC obtained by means of a computerized milk meter and milk yields of 138 Holstein Friesian cattle affected by clinical mastitis were analysed by the following mixed linear model: Y= H + TD + PA + DIM + MAST + DIM(MAST) + LATT + E where H is the effect of the herd (2 levels), PA is the effect of parity (1, 2, 7), TD is the effect of test date (1020 levels), DIM indicates the effect of lactation stage (30 levels of 10d each), MAST is the effect of the period of mastitis occurrence (3 levels: MAST1=<100d, MAST2=99< and <200, MAST3=>199), LATT is the random effect of individual lactation, E is the random residual. EC and milk yield curves for different classes of period of mastitis occurrence were constricted by plotting DIM(MAST) estimates against days in milking. EC tends to increase along the lactation, with a different behaviour in the different classes of period of mastitis occurrence and with a variance among animals equal to about 50% of the total random variability. EC in MAST1 cows was higher than in MAST2 and MAST3 cows at the beginning of lactation (mS 12.07 vs. 11.43 and 11.55 respectively) but curves of MAST1 and MAST2 were quite similar from about 150 DIM. The EC curve of MAST3 cows showed an increasing rate markedly lower than the other two classes during the whole lactation. Parity affected EC, with first calving cows having lowest values. Milk yield was affected by all fixed factors considered in the analysis except from MAST class; however, lactation curves separated for this last effect show a low peak for cows having mastitis >100 DIM and a higher persistency for those that were affected by the disease in the last part of lactation.

Key Words: Electrical conductivity, mastitis, milk

25 Effect of stimulation intensity on oxytocin release before and after milking. D. Weiss*, A. Dzidic, and R.M. Bruckmaier, *Institute of Physiology, Techn. Univ. Munich-Weihenstephan.*

Oxytocin (OT) released from the pituitary causes myoepithelial contraction and milk ejection. Elevated concentrations of OT are necessary throughout the whole milking process to ensure complete milk ejection. The objective of this study was to test the effect of teat stimulation intensity on the level of OT released. Six Brown Swiss cows were machine milked at 5 a.m. and 4 p.m. and blood samples were taken during milking at 1-min intervals for OT analysis. Milk flow was recordered on a quarter level. Control milking (CM) corresponded to daily milking routine and included forestripping, dry paper cleaning and a 1-min vibration stimulation before the start of milking. In addition, vacuum in liner closed position without pulsation was applied, for 5 min, either before the start of milking (LCBM) or after the end of milking (LCAM). In a third treatment a 1-min vibration stimulation was applied to one quarter before the end of milking (STIMO). Stripping was performed in all treatments if total milk flow declined below 0.3 kg/min. Milk yield, milking time and average milk flow rate did not differ between treatments. During liner closed phase before milking (LCBM) OT concentrations were significantly ($P \leq 0.05$) higher compared to the period before teat cleaning in CM. Area under curve (AUC)/min during this period was 7.5 $\pm$ 1.0 pg/ml and 5.2 $\pm$ 0.9 pg/ml, respectively. The slightly increased OT concentrations in LCBM were sufficient to induce the alveolar milk ejection as indicated by the absence of bimodal milk flow curves in LCBM. LCAM had no obvious stimulatory effect on OT release, the decline in OT concentrations after the end of milking was similar in LCAM and CM. In STIMO, no effect of the additional stimulation on OT levels could be observed as compared to CM. In conclusion, different degrees of stimulation by the milking machine release different amounts of OT. However, only slightly elevated OT levels induce milk ejection at the start of milking.

Key Words: oxytocin, cow, milking

26 Factors affecting level and post-feeding behaviour of insulin in dairy cows. G. Bertoni*, E. Trevisi, R. Lombardelli, and F. Piccioli Cappelli, *UCSC, Facolta di Agraria, Piacenza, Italy.*

It is well-known that blood insulin level is reduced after calving and particularly in high yielding dairy cows although the data regarding the insulin release factors and/or its receptors sensitivity seem contradictory. To contribute to clarify some of these aspects we have studied the post-feeding behaviour of blood insulin in cows with different parity (trial 1) or genetic merit (trial 2) during the last month of pregnancy and the first 3 months of lactation. In the 1st trial 4 cows were considered in their 1st (L1) and 2nd lactation (L2); while 8 multiparous cows, 4 of high (HG) and 4 of average (AG) genetic merit, were used in the 2nd trial. Blood samples were taken every week before the morning meal and 1, 2, 3, 4, 5 and 6 hours after it, for metabolic profile and insulin determination. The environment conditions and diets were kept constant during the trials, while feed intake, milk yield and BCS were recorded. It is confirmed that insulin in dairy cows is strongly reduced before calving and rises again 1-2 weeks later, reaching the levels of dry period after 10 weeks. Furthermore, the after-meal insulin increase is stronger in dry period (+30-50%) than in the first 2 months of lactation (+10-30%). L1 and L2 of the same cows seem to have similar insulin values and behaviour after meal, particularly from 2 weeks before to 8 weeks after calving. HG showed lower levels of insulin before and after calving ($P<0.05$ vs AG). As regards the after-meal behaviour, HG had a similar but more prolonged rise in dry period, whereas the increase was less marked after calving. These variations were related to the glucose levels (positively), but particularly in AG cows and around calving; a negative correlation has been observed with βOHB, NEFA and BCS, particularly for HG cows after calving. In general, insulin changes do not seem strictly related to the energy balance indices. It appears that insulin level and after-meal behaviour are strongly affected by stage of lactation and that genetic merit and/or parity could contribute to explain them.

Key Words: Insulin, Post-feeding behaviour, Dairy cow

27 Evidence for the presence of the cationic amino acid transporter CAT-1 in porcine mammary gland during lactation. J. Perez Laspiur*, J.L. Burton, P.S.D. Weber, and N.L. Trottier, *Michigan State University*.

Biomedical literature suggests that mammary epithelial cell expression of the CAT-1 cationic amino acid transporter is important in determining cell activity. It is specific for transporting lysine, and thus may be involved in regulating milk protein synthesis. Manipulation of CAT-1 expression may help producers control the volume and quality of milk for neonatal animals. The objective of the current study was to determine if CAT-1 is expressed in porcine lactating mammary tissue. A multiparous sow was sacrificed on day 19 of lactation for this work, and the udder removed immediately following cessation of heartbeat. Four anterior mammary glands (two from each lateral side) were isolated, the parenchymal tissue collected and cut into approximately 1.0-g pieces and frozen in liquid nitrogen. Total RNA from mammary tissue was isolated using the TRIzol Reagent method. Samples (10-μg) of total RNA were run in duplicate on a Northern blot to assess CAT-1 gene expression. Expression of CAT-2 (another member of the CAT family of amino acid transporters) was used as a negative control and β-actin expression as a RNA loading control. Duplicate liver RNA samples from a prepubertal gilt were used as the positive control for CAT-2 expression. Human CAT-1 and CAT-2 cDNA probes (donated by Dr. E. I. Closs, Johannes Gutenberg University, Germany) and a rat β-actin cDNA were ^{32}P-labeled and hybridized sequentially to the Northern blot with complete stripping of the probes between hybridizations. Resulting autoradiographs revealed low level expression of CAT-1 in day 19 of lactation mammary tissue with no detectable expression in liver. As expected, CAT-2 was highly expressed in liver but not in mammary parenchyma. These preliminary results are the first to show that CAT-1 is expressed during lactation in porcine mammary tissue.

Key Words: cationic amino acid transporter, lactating mammary gland, porcine

28 Oxytocin release and milk ejection induced by teat cleaning in a single stall automatic milking system. A. Dzidic, D. Weiss, and R.M. Bruckmaier*, *Institute of Physiology, Techn. Univ. Munich - Weihenstephan, Freising, Germany*.

Oxytocin (OT) is released in response to tactile teat stimulation and causes alveolar milk ejection. The objective of this study was to evaluate the effect of teat cleaning by two rolling brushes on OT release and milk ejection during milking in a single stall automatic milking system (AMS, Merlin, Lemmer-Fullwood). Forty-eight German Fleckvieh cows were investigated during their voluntary milkings. Five treatments B0 (no brushing), B1 (1 brushing cycle for 16 s, 4 s per teat), B2 (2 brushing cycles), B4 (4 brushing cycles) and B6 (6 brushing cycles) were performed for 2 days each and quarter milk flow was recorded. In addition blood samples were taken from 10 cows during milking at 1-min intervals for OT determination in treatments B0, B2, B4 and B6. Basal OT concentrations were similar (2.7 to 3.8 pg/ml) in all treatments. At the start of milking, OT concentration was lower ($P<0.05$) in B0 as compared to all other treatments. One min after the start of milking OT concentrations did not differ between treatments (20.8 to 26.8 pg/ml). OT concentrations throughout milking (AUC/min) were 26.5±4.1, 25.6±4.9, 20.2±2.7 and 19.8±3.1 pg/ml in B0, B2, B4 and B6 respectively. The portion of bimodal curves decreased ($P<0.01$) with increasing number of brushing cycles (46, 25, 11, 1 and 0 % in B0, B1, B2, B4 and B6 respectively). Time until milk ejection occurred, recorded in B0, decreased ($P<0.05$) with increasing degree of udder filling (defined as a percentage of actual milk yield from maximum milk yield in month two of the current lactation). At low degree of udder filling (i.e. after short interval from previous milking) cows needed a longer pre-stimulation for well-timed induction of milk ejection and to reduce number of bimodal curves. In conclusion, the teat cleaning device in the used AMS was suitable to induce milk ejection in cows before the start of milking. Duration of teat cleaning needs to be adapted to the actual degree of udder filling.

Key Words: Oxytocin release, Milk ejection, AMS

29 mRNA expression of immunologically important factors and milk proteins in mammary tissue of dairy cows during LPS-induced mastitis. S Schmitz, MW Pfaffl, HHD Meyer, and RM Bruckmaier*, *Institute of Physiology, Techn. Univ. Munich-Weihenstephan, Freising, Germany*.

Inflammatory factors are known to increase during mastitis. This study was conducted to determine changes of mRNA expression of various immunologically important factors in mammary tissue during the first 12 h of lipopolysaccharide (LPS) induced mastitis. Five healthy lactating cows were injected in one quarter with 100 g E.coli-LPS (O26:B6) and the contralateral quarter with saline (9 g/l) serving as control. mRNA expression in mammary biopsy samples of various factors at 0, 3, 6, 9 and 12 h after LPS administration was quantified by real-time RT-PCR. Blood samples were taken following the same time course and rectal temperature was measured at 1-h intervals. Temperature increased until 5 h ($P<0.05$) after LPS administration and decreased to pretreatment levels within 24 h after LPS-challenge. Blood leukocyte number decreased ($P<0.05$) from 0 to 3 h from 7.71.1 x 10^9/l to 5.71.0 x 10^9/l and thereafter recovered to pretreatment levels until 12 h after LPS-challenge. In LPS-challenged quarters tumor necrosis factor α and cyclooxygenase-2 mRNA expression increased to highest values ($P<0.05$) at 3 h after LPS-challenge. Lactoferrin, lysozyme, inducible nitric oxide synthase mRNA expression increased ($P<0.05$) and peaked at 6 h after challenge, while platelet-activating factor acetylhydrolase mRNA increased only numerically. mRNA expression of the investigated factors did not change in control quarters. mRNA expression of insulin-like growth factor-1, 5-lipoxygenase and of αS1-casein (CN), αS2-CN, β-CN and β-lactoglobulin did not change significantly, whereas mRNA expression of α-lactalbumin decreased ($P<0.05$) in LPS-treated and control quarters and that of κ-CN only in the LPS-treated quarters. In conclusion, mRNA expression of most inflammatory factors changed within hours, whereas that of most milk proteins remained unchanged.

Key Words: LPS-challenge, Mastitis, Inflammatory factors

30 Body lipid change in lactation: consequences for the prediction of energy requirements. N. C. Friggens*, K. L. Ingvartsen, and G. C. Emmans, *Danish Institute of Agricultural Sciences, Foulum, Denmark*.

The size of the body lipid reserves (L) of the dam changes in a characteristic and repeatable manner through lactation in virtually all mammals. This is an evolutionary adaptation designed to support the changing reproductive priorities of the dam that is largely independent of current feed availability. Pregnancy leads to an increase in L to help meet the nutritional demands of the following lactation. Lactation is characterised by an initial decline in L followed by a return to the pre-pregnancy level. These patterns of changing L persist even under conditions that can reasonably be assumed to be nutritionally non-limiting. Thus there is a genetically driven, and therefore predictable, cycle of body energy mobilisation and deposition. Prediction of the cow#s energy requirements can be substantially improved, particularly in early lactation, by incorporating genetically driven body energy mobilisation. With very few exceptions, existing prediction systems do not account for this. This paper presents in detail a method to quantify the genetically driven rate of change of L (dL/dt) at any given timepoint in lactation. The method requires assumptions about target levels of L at calving and in the pre-pregnant state, and about the time taken from calving to return to the pre-pregnant state. These assumptions are discussed and experimental results presented concerning the effects of breed and parity on the parameter estimates. The method requires input estimates of actual L at calving and time from calving to subsequent conception. A method to estimate L from body condition score and liveweight is described. In addition to being a practical means to improve prediction of energy requirements, this method provides a useful basis for exploring genetic variation in body lipid mobilisation and characterising the consequences of genetic selection on the lactational cycle in body lipid reserves. These issues are discussed.

Key Words: Lipid mobilisation, Lactation, Energy

31 Serum insulin-like growth factor 1 and placental lactogen profiles in Holstein nulliparous and multiparous cows in early gestation. W. J. Weber*[1], C. R. Wallace[2], H. Chester-Jones[1], and B. A. Crooker[1], [1]*University of Minnesota, St. Paul,* [2]*University of Maine, Orono.*

A positive relationship between serum insulin-like growth factor (IGF-1) and placental lactogen (bPL) in dairy cattle suggests that the increase in bPL may contribute to an increase in IGF-1 during gestation. However, this relationship is not well established. Objectives of this study were to examine the relationship between serum bPL and IGF-1 in nulliparous and multiparous Holstein cows in early gestation. Blood samples from nulliparous (n=17) and multiparous (n=15) cows were collected (± 3d) at 56, 70, 84, 98, 112, 140 and 168 d of gestation. Multiparous cows were less than 200 days in milk when sampling was initiated. Serum samples were analyzed for IGF-1 and bPL by RIA. Data were analyzed as repeated measures using PROC MIXED and results reported as least squares means. Means were considered different when $P < 0.05$. Serum bPL was less in nulliparous than multiparous cows (0.16, 0.31 ± 0.03 ng/ml) and increased from d 56 to 168 of gestation (0.11^a, 0.15^b, 0.19^{bc}, 0.20^c, 0.26^d, 0.31^e, 0.43^f ± 0.03 ng/ml). The rate (2.7 $\mu g \cdot ml^{-1} \cdot d^{-1}$) and overall (0.32 ng/ml) increase in bPL was similar for both parities from d 56 to 168 of gestation. Serum IGF-1 was greater in nulliparous than multiparous cows (231, 115 ± 5.0 ng/ml) and increased from d 56 to 168 of gestation (164^a, 167^a, 168^a, 165^a, 174^{ab}, 182^{bc}, 191^c ± 5.2 ng/ml). Although there was no interaction, the increase in IGF-1 in multiparous cows (35 ng/ml, 33%) was greater than in nulliparous cows (20 ng/ml, 9%) during this 122 d interval. From 56 to 98 d of gestation, serum bPL and IGF-1 in multiparous cows were relatively stable but both began to increase by d 112 and continued to increase through d 168 of gestation. The greater IGF-1 concentration in nulliparous cows made this relationship less apparent. The strong relationship between bPL and IGF-1 during early gestation supports the concept that bPL may play a role in regulating serum IGF-1.

Key Words: Gestation, IGF-1, bPL

32 Detecting beta-casein and beta-lactoglobulin variants using real-time PCR taking advantage of single nucleotide polymorphisms in milk cell DNA. Ralf Einspanier*[1], Andreas Klotz[1], Johann Buchberger[2], and Ingolf Krause[2], [1]*Institute of Physiology TU Munich Germany,* [2]*Institute of Chemistry TU Munich Germany.*

In cattle several genetic variants for the beta-casein and the beta-lactoglobulin locus have been described. With regards to a possible selection of genotypes being favorable to cheese making, we have applied a new technique (real-time-PCR) to determine the main genotypes of bovine beta-LG and beta-CN variants. The aim of this study was to rapidly detect genetic variants of beta-casein (beta-CN A1, A2, B) and beta-lactoglobulin (beta-LG A, B, C, D) directly from milk. Through introducing non-invasive and faster methods it appears advantageous to use milk cells instead of other DNA sources like blood. After the initial characterization of distinct mutations in the genome using PCR amplification, deduced proteins were verified by isoelectric focusing of corresponding milk samples. Furthermore, a partial nucleotide sequence of the beta-LG-gene D, containing allele-specific point mutations, could be determined. For beta-CN allel-specific mutations occur at amino acid residue 67 and 122, whereas for the beta-LG variants specific mutations occur at amino acid residues 45, 59, 64 + 118. Based on specific PCR fragments generated from milk cell DNA, genotyping of alleles of beta-CN and beta-LG or admixtures becomes efficient and simultaneous. Hence, a real-time PCR approach (LightCycler) was established specifically distinguishing three important beta-CN milk protein variants with remarkable benefits when compared to other DNA-based mutation detection systems. As a consequence, genotyping of cattle will become more easily and faster through introducing this new technique.

33 Effect of contact time between calves and cows on IgG transfer, cortisol release, milk yield and residual milk. F. A. Paiva, A. R. Bueno, A. Saran-Neto, M. S. Freiria, and J. A. Negrao*, *USP/FZEA, FAPESP, Pirassununga/SP, Brazil.*

At parturition, cows and calves remain in contact for several hours and this period is essential for adequate absorption of IgG and for survival of neonates. During extended contact time, cows become selective and take longer to adapt to milking. Our objective was to verify if contact time between cows and calves could influence IgG transfer and milking adaptation. Thus, 18 Holstein cows and their calves were divided into 3 groups: SC group (short contact); cows and calves remained 6 h in contact, PC group (periodic contact); cows and calves were brought together twice/day for 30 min and LC group (long contact); cows and calves remained in contact for 3 days. Following, cows and calves of the SC and LC groups were definitively separated. After separation, all calves received 4L of colostrum/day. All groups were submitted to 2 milkings/day, without calves. Blood samples were taken once a day from cows and calves, from parturition to 4 days post-parturition. Milk yield and residual milk were measured on days 4, 15 and 30 of lactation. Cortisol (CORT) level was measured using EIA kit (dslabs, Inc) and IgG level was determined by radial immunodiffusion. After parturition, all cows had similar levels of IgG. Before separation, LC calves presented lower CORT levels than other calves but after separation, LC calves had a higher increase in CORT levels than PC calves. CORT profiles of cows were similar, however LC cows exhibited higher CORT levels after separation than PC and SC cows. During first milkings, SC and PC cows produced more milk (19.5 ± 1.1 L and 19.2 ± 0.5 L, respectively) than LC cows (16.5 ± 5.6 L). On day 4, residual milk was higher for LC and PC cows (6.53 ± 0.7 L and (5.6 ± 0.4 L, respectively) than for SC cows (2.0 ± 0.2 L). On days 15 and 30, milk yield and residual milk were similar for all groups. Our results indicate that IgG transfer was adequate to all calves, and that adaptation to milking was not influenced by contact time between cows and calves.

Key Words: Milk ejection, Milk residual, Cortisol

34 Prolactin receptor expression responds to photoperiod similarly in multiple tissues in dairy cattle. T. L. Auchtung*[1], B.C. Pollard[1], P.E. Kendall[1], T.B. McFadden[2], and G.E. Dahl[1], [1]*University of Illinois, Urbana, IL,* [2]*University of Vermont, Burlington, VT.*

Photoperiod (PP) influences circulating prolactin (PRL) concentrations in cattle. Prolactin exerts its effects through its receptor, which has two isoforms in the bovine. Therefore, it is likely that PP also has an effect on PRL receptors (PRLR), which are present in many tissues of the body. The objective of this experiment was to identify the effect of photoperiod on PRLR expression in multiple tissues of dairy cattle. Holstein steers (n = 10) were maintained on either long day photoperiod (LDPP; 16 h light: 8 h darkness) or short day photoperiod (SDPP; 8 h light: 16 h darkness) for 9 wk, then photoperiod treatments were reversed for four weeks. Liver tissue was obtained via biopsy at 2-wk intervals throughout the 13 wk experiment. Lymphocytes were isolated from blood collected on heparin at four times during the experiment. Density gradient centrifugation of the buffy coat through Histopaque-1077 was followed by washing of cells with RPMI-1640 cell growth media. Mammary tissue was collected via biopsy at the end of the experiment. Isolation of RNA from all tissues was performed using Trizol reagent and RNA was converted to cDNA prior to real-time PCR. Concentrations of plasma PRL, measured by RIA, were greater ($P < 0.05$) in LDPP than SDPP animals. Compared to LDPP, SDPP increased ($P < 0.01$) expression of PRLR mRNA in liver at Week 5, and responses to photoperiod at Week 13, four weeks after the treatment reversal, were similar to those at Week 5 ($P < 0.05$). Lymphocyte responses were similar, with SDPP increasing PRLR mRNA expression significantly ($P < 0.05$) over LDPP, both before and after the treatment reversal. Expression of PRLR mRNA in mammary tissue was also increased ($P < 0.01$) in SDPP animals relative to LDPP. In summary, PRLR mRNA expression in liver, mammary, and lymphocytes is increased in animals on SDPP treatment as compared with LDPP. Expression of PRLR in lymphocytes provides a minimally invasive method to monitor PRLR expression in multiple tissues.

Key Words: Cattle, Prolactin Receptor, Photoperiod

35 Possible role of enterolactone on mammary development and lactation in cattle. S Purup, M Vestergaard, MR Weisbjerg, T Hvelplund, and K Sejrsen*, *Danish Institute of Agricultural Sciences, Foulum.*

The lignan enterolactone (Enl) is produced by microbial fermentation of the phyto-estrogens secoisolariciresinol (Seco) and matairesinol (Mata) in the gastro-intestinal tract. Seco and Mata occur as glycosides in wholegrain cereals, seeds, nuts, vegetables and berries. The objective of the present study was to measure the concentration and bioactivity of

Enl in milk and blood and to investigate the effect of Enl on proliferation of mammary epithelial cells in culture. Blood and milk was collected from 35 dairy cows fed diets either with grass-clover silage or whole-crop barley silage as the main roughage. Concentrations of Enl in whey and serum was measured by TR-IFMA. Bioactivity of whey and serum was studied in mammary epithelial cells isolated from prepubertal heifers and cultured in collagen gels for 5d. Proliferation of epithelial cells was determined during the final 24 h of culture using [methyl-^{3}H]thymidine incorporation as a measure of DNA synthesis. The effect of Enl on mammary epithelial cell proliferation was investigated by addition of Enl in concentrations of 10-100,000 ng/ml. Concentrations of Enl were 1.84 and 2.40 ng/ml (P<0.10) in whey and 177 and 249 ng/ml (P<0.01) in serum from dairy cows fed diets based on grass-clover silage and whole-crop silage, respectively. Whey or serum added to mammary epithelial cells in concentrations of 0.5-10% in culture medium showed no significant differences in cell proliferation due to silage type. The effect of Enl added to cell culture medium on mammary epithelial proliferation was biphasic. Enl at low concentrations (10 and 100 ng/ml) stimulated proliferation slightly (approximately 15%; P<0.06 and P<0.05, respectively), whereas higher concentrations (>10,000 ng/ml) strongly inhibited (P<0.01) cell proliferation. Maximal inhibition at 100,000 ng/ml corresponded to a 97% inhibition (P<0.001) of mammary cell proliferation. It is suggested that phyto-estrogens such as Enl may have a role in mammary development and lactation in cattle.

Key Words: Enterolactone, Mammary Cells, Cattle

36 Effects of omitting one milking per week on milk yield, milk composition and udder health of dairy cows. M. Ayadi[1], G. Caja*[1], X. Such[1], E. Albanell[1], M. Ben M'Rad[2], and R. Casals[1], [1]*Universitat Autonoma de Barcelona, Spain,* [2]*Institut National Agronomique de Tunisie, Tunisia.*

Five Holstein dairy cows (milk yield: 21.0 ± 3.4 l/d; 227 ± 67 DIM) were used for 10 weeks to study the effect of omitting one milking per week (Sunday afternoon) throughout lactation on milk yield, milk composition and udder health. Cows were milked twice a day (8.00 and 18.00 h) but on Sunday one milking only was performed at 12.00 h. Milk yield from each milking was recorded. Milk samples were taken individually from each milking to analyze milk composition and somatic cell count (SCC). Average milk yield and composition for Friday and Saturday were used as reference values to evaluate the effect of changing the milking frequency. Milk yield and milk composition did not vary (P > 0.14) during the experimental weeks, but SCC increased with lactation stage. On Sundays, milk yield (15.6 l/d), fat content (3.38%) and log SCC (2.59) decreased by 29, 21 and 27% (P < 0.05), respectively, as a result of omitting one milking. On Mondays, milk yield (23.9 l/d), fat content (4.84%) and log SCC (3.02) increased by 9, 14 and 100% (P < 0.05), respectively. The raise in SCC was dependent on the previous levels. All values reached the average level by Wednesday. Milk protein (3.47%) increased by 2% and lactose (4.37%) decreased by 2% (P < 0.05) by Saturday. Compared with estimated values for 14 milkings/week, omitting one milking per week decreased the weekly yields of milk (3%), fat (4%), protein (5%) and lactose (5%), but milk SCC increased by 25%. Milk yield loss varied according to the cow#s yield but not to lactation stage. Clinical mastitis was not observed in any cow at any time. We conclude that omitting one milking per week could be an adequate strategy to reduce farm labor (7%) without important losses in milk yield in farms with low milk SCC values. Official milk recording should be conducted in the middle of the week to avoid residual effects from the milking omission. An improvement in the farmer#s quality of life is also expected.

Key Words: Milking Frequency, Milking Suppression, Milk Composition

37 Effects of conjugated linoleic acid (CLA) on milk fatty acid profiles and activities of lipogenic enzymes in the mammary gland, liver and adipose tissue of lactating rats. A. A. Hayashi*[1], S. R. Medeiros[2], and D.P.D. Lanna[1], [1]*ESALQ/ USP/ SP, Brazil,* [2]*Embrapa /Gado de Corte/ MS, Brazil.*

The objective of the present study was to evaluate the effects of feeding a mixture of CLA isomers on milk fatty acid profiles and the activities of lipogenic enzymes in lactating rats. Dams were fed either a control diet or a diet supplemented with 2.5% of calcium salts of CLA-60 from parturition to the 15^{th} day post-partum. The CLA-60, (Church & Dwight, Princeton, NJ) contained different isomers of CLA (24% c/t 9,11; 35% t,c 10,12; 15% c,t 8,10; 17% t,t 11,13 and 9% others). On the 15^{th} day post-partum, the rats were anesthesized, milked and killed by exsanguination. Mammary gland, liver and adipose tissues were immediately freeze-clamped for subsequent assays of activities of enzymes involve in lipid synthesis. Pups growth were decreased by CLA (P< 0.01) and concentration of 12:0 to 16:0 fatty acids in the milk of CLA-fed rats were lower compared to the control. The Fatty acid synthase (FAS) activity was decreased by CLA in the mammary gland, adipose tissue and liver (by 43%, P<0.01, 56%, P<0.01 and 68%, P<0.01 respectively). The activities of Glucose-6 phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) were decreased in all three tissues, by 35%, P<0.01; 36%, P<0.05 and 65%, P<0.05 for G6PDH, and by 28%, P<0.01, 22%, P=0.10 and 53%, P<0.01 for 6PGDH, for mammary, adipose and liver tissues respectively. In contrast, NADP malate dehydrogenase enzyme activities were unchanged by CLA supplementation to the diet in all tissues. Thus, CLA altered processes associated with de novo fatty acid synthesis. Furthermore, the reduction in the activities of these enzymes, with CLA treatment, was consistent with changes in milk fatty acid profiles, and similar to observations of feeding calcium salts of CLA-60 to lactating cows.

Key Words: Conjugated linoleic acid, Lactation, Lipogenesis

Forages and Pastures
The J. W. Thomas Forage Symposium: A Discussion on Silage Fermentation Issues

38 Microbiology of silage. Thomas Rehberger*[1], [1]*Agtech Products, Inc, Waukesha, WI.*

Silage is one of the largest microbial fermentation products with an estimated 102 million tons of corn silage alone made in the United States annually. Silage is a natural process-utilizing native, and in some instances inoculated, lactic acid bacteria for the preservation of crops. The microbiology of the dynamic process of ensiling will be discussed in context of the four phases of the ensiling process: aerobic, fermentation, stable and feedout. Emphasis will be placed on how management practices impact the microbial ecology of silage. The stability of silage during feedout depends on the surviving microorganisms during the aerobic, fermentation and stable phases and their production of organic acids from the plant carbohydrates during these phases. The importance of the homofermentative and heterofermentative lactic acid bacteria in controlling the major spoilage organisms for each of the major silage crops will be discussed. New plant varieties and crop processing techniques impact the availability of plant nutrients and offer new challenges for maintaining quality silage. Recent advances in molecular biological techniques utilizing PCR amplification of regions within the 16s rDNA gene will provide a better understanding of the complex microbial ecosystem of silage and provide new insights into producing quality silage.

Key Words: silage, microbiology, lactic acid bacteria

39 The history and future of silage inoculants. Limin Kung, Jr.*[1], [1]*The University of Delaware.*

Silage fermentation is a result of many interactions between microorganisms. Adding microorganisms in hopes of improving the fermentation process was practiced in the early 1900s. Intensive research began in the 1960s and widespread commercialization followed in the late 1970s. Homolactic acid bacteria (e.g. *Lactobacillus plantarum*) were the primary organisms of choice because of their high theoretical efficiency of fermenting sugars to lactic acid. Early research yielded variable results because of low applications rates and poor shelf life. In addition, not all of these organisms were rapid growers. The evolution of inoculants with other homolactic bacteria (e.g. Enterococcus or Pediococcus sp.) followed with marked improvements in application rates (minimum of 100,000 cfu/g of fresh forage) and manufacturing (e.g. fermentation, freeze drying, packaging and moisture scavengers). One draw back of