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

using only homofermentative bacteria is that they often make aerobic stability worse because the production of antifungal end products is usually suppressed. Species of propionibacteria were introduced as silage inoculants in the late 1980s in hopes of producing propionic acid and alleviating this problem but have met with limited success. Over the last 15-20 y, researchers have studied the application of killer yeasts to improve aerobic stability, bacteriophages to inhibit clostridia, and incorporation of cellulase genes into lactobacilli for improved fiber digestion in silage, but these applications have not been commercialized. Recently, identification of *L. buchneri* by Muck (1996) has led to a new paradigm to improve the aerobic stability of silages. This organism, is a heterolactic bacterium that can anaerobically convert lactic acid to acetic acid, 1,2 propanediol and ethanol and has made us reevaluate the theory of only using homolactic acid bacteria as silage inoculants. The dynamic nature of silage fermentation provides an open canvas for future research which may include identification of organisms that produce bacteriocins and antifungal compounds or that may have direct-fed microbial activity in the rumen.

Key Words: Silage, Inoculant, Fermentation

40 The end products of silage fermentation and their relationships to animal performance. Richard Muck*[1] and Limin Kung, Jr.[2], [1]*USDA, ARS, US Dairy Forage Research Center,* [2]*University of Delaware.*

Analysis of silages for fermentation products is now possible on a routine basis, but how useful are these analyses as predictors of animal performance? The commonly reported silage fermentation end products are lactic acid, the volatile fatty acids (VFA; acetic, propionic and butyric), and ethanol. All but butyric acid can be produced by least two groups of common fermentative silage microorganisms (lactic acid bacteria, enterobacteria, clostridia, yeasts). This multiplicity of sources for a given product can complicate the interpretation of a fermentation profile for a given silage. Fermentation by any of these microorganisms usually results in an increase in the energy density of the silage compared with the fresh crop because dry matter losses from fermentation generally are greater than the accompanying energy losses. However, ensiling does not improve animal performance compared with the fresh crop. The reduction in performance has been attributed principally to two factors: reduced rumen microbial fermentation and reduced intake. The VFAs in silages are a source of energy for the cow but not for rumen microorganisms. Ethanol is poorly fermented in the rumen. Thus a ration high in VFA might be expected to produce less microbial protein than one low in VFA. Studies of silage inoculants where fermentation has been shifted from VFA production to lactic acid do show trends toward improved nitrogen efficiency, but effects are small. Reduced intake has been observed in silages high in fermentation products, particularly butyric acid, acetic acid and ethanol. However, addition of these acids to good silages has not reduced intake or performance. Also inoculating silage with heterofermentative lactic acid bacteria has not reduced animal performance even when the resulting silages had acetic acid concentrations above 5% dry matter. These results suggest that the dominant microorganisms in a silage affect animal performance through mechanisms other than the major end products of fermentation. Thus, fermentation product analyses are not necessarily accurate forecasters of animal performance but can be very useful in both troubleshooting performance problems and developing practical solutions to those problems, as will be discussed.

Key Words: Silage, Fermentation, Performance

41 Improving protein utilization in silages to increase animal performance and reduce environmental burden. Ed Charmley*, *AAFC Crops and Livestock Research Centre, Nappan, NS, Canada.*

Silage normally contains between 12 and 22 % crude protein (CP). While such levels should be adequate for most productive livestock, in silages they are not. This is because of poor utilization of N in silage. Most silage-based production rations require supplemental protein to meet requirements for growth or lactation in ruminants. Silage protein is highly degradable in the rumen and as such is a poor supply of dietary protein and peptides to the small intestine. The situation is exacerbated by a scarcity of available energy in the rumen which limits microbial protein synthesis. Associated with poor utilization of silage N by ruminants are concomitant high losses of silage N to the environment. Poor utilization of silage N by ruminants is attributed to proteolytic activity by crop enzymes shortly after harvest and further microbial breakdown of protein during ensilage. The production of low molecular weight nitrogenous compounds, including ammonia, amines and amides, has a negative effect on voluntary intake of silages. This appears to relate to an ammonia burden in the blood stream as well as specific appetite suppressing characteristics of certain compounds. The poor utilization of silage N in combination with lower voluntary intake of silages can seriously limit ruminant production. Availability of protein supplements is likely to be reduced in future, making it more important than ever to maximize the use of the protein in forages and particularly silage. Methods to improve silage protein utilization exist, including improved crop drying rates, use of microbial and chemical additives and use of, or selection for, crops having characteristics that reduce protein solubility. In future, the emphasis will move away from additives and towards plant breeding and management practices. The ultimate goal will be to use ensiling technology to improve upon the protein quality characteristics of the original crop, thus tailoring the protein characteristics of the silage with the requirements of the animal.

Key Words: Silage, Protein utilization, Animal performance

42 Reflections and concluding remarks. J.W. Thomas*, *Michigan State University.*

Titles of abstracts and symposia about forages in 2002 are somewhat similar to those of the l950s and l960s. This similarity disappears when one examines the complexity and significance of the contents, the results and the discussions of current presentations. Silage microbiology has progressed from attempting to identify the bacteria to using strains of desirable bacteria as innoculants to improve silage quality and decrease losses. Chemical changes during ensiling are being directed to make a more nutritious product and to improve animal performance instead of merely identifying and characterizing these chemicals. When optimum end products are identified, modified organisms could be used as innoculants. Procedures that can be economically viable in large animal facilities have been developed in place of those used in smaller units. For instance, we evaluate silage in bunker silos rather than small cement stave silos and large hay bales not 40 lb. bales. We evaluate forages for large groups of cows using total mixed raions not a forage for individual cows. All this has been accomplished by the innnovative and cooperative efforts in the research by academic staff, graduate students and industry personnel. My investigations involved over 23 faculty and 59 students in the areas of forage harvesting, storing and feeding practices.

Key Words: Silage innoculants, Silage chemistry, Forages

Physiology
Improving Reproductive Efficiency with Hormone Treatments

43 Optimization of timed insemination programs and integration with bST to increase pregnancy rates in lactating dairy cows. W. W. Thatcher*[1], L. Badinga[1], S. M. Pancarci[1], F. Moreira[1], R. Pershing[1], A. Guzeloglu[1], T. R. Bilby[1], S. Kamimura[1], and J. Santos[2], [1]*University of Florida, Gainesville, FL, USA,* [2]*University of California, Davis, CA, USA.*

Ovsynch permitted timed insemination with normal fertility. Pregnancy rate of cyclic cows was increased ∼20% with a pre-synchronization program of two $PGF_{2\alpha}$ injections 14 days apart and initiation of the Ovsynch program 12 days after the 2nd $PGF_{2\alpha}$. This enhancement in fertility is associated with placement of cows in early diestrus (i.e., d5-10) at initiation of Ovsynch to avoid early metestrus and late diestrus. Following pre-synchronization with $PGF_{2\alpha}$, ECP has provided alternative systems for timed insemination (Heat Synch: GnRH[d0]-$PGF_{2\alpha}$ [d7]-ECP[d8;1mg]; Double ECP: ECP[d0; 2mg]-$PGF_{2\alpha}$ [d10]-ECP[d11;1mg]) with pregnancy rates comparable to Ovsynch. Strategies to enhance embryonic development and survival include injection of bST (Posilac, 500 mg) at the time of insemination within a Pre-synchronization/Ovsynch program that increased pregnancy rates