and additional management requirements may limit the acceptability of this approach.

Key Words: Tall Fescue, Orchardgrass, Cow-Calf Performance

59 Using orchardgrass and endophyte-free fescue versus endophyte-infected fescue overseeded on bermudagrass for cow herds: four-year summary of forage characteristics. W. K. Coblentz*, K. P. Coffey, D. A. Scarbrough, T. F. Smith, K. F. Harrison, D. S. Hubbell, III, B. C. McGinley, J. E. Turner, and J. B. Humphry, *University of Arkansas, Fayetteville*.

A four-year trial was initiated in January 2000 to evaluate forage production, basal cover, and persistence of endophyte-infected tall fescue (IF; *Festuca arundinacea* Schreb.), endophyte-free tall fescue (FF), or orchardgrass (OG; *Dactylis glomerata* L.) overseeded into dormant common bermudagrass [*Cynodon dactylon* (L.) Pers.] for spring-calving cows. The FF and OG pastures were managed with either a twice weekly (HM) or twice monthly (LM) rotation schedule, while the IF pastures were managed with a LM rotation schedule only. Forage-related response variables were evaluated with a split-plot design, where forage system (IF-LM, FF-HM, FF-LM, OG-HM, and OG-LM) was the whole-plot term and sampling/evaluation date was analyzed as a repeated measure. Forage availability (four-year mean = 3809 kg/ha) was not affected ($P = 0.601$) by forage management system, but was affected by sampling date ($P < 0.0001$) and the forage system x sampling date interaction ($P = 0.010$). Total basal cover varied ($P < 0.0001$) with sampling date, ranging from 36.3 to 51.5%, but was not affected by forage system ($P = 0.679$) or the interaction of main effects ($P = 0.354$). The interaction of forage system and evaluation date affected ($P = 0.038$) the percentage of the desired cool-season species in each pasture. Generally, FF and IF remained relatively stable over the entire study, ranging from 49.2 to 72.5% for FF-HM, 54.0 to 73.5% for FF-LM, and 46.9 to 65.8% for IF. However, the percentage of OG declined markedly ($P < 0.1$) since June 2002 in both the HM and LM grazing systems, ending at 36.2 and 14.7%, respectively, on the final (November 2003) evaluation date. After four years, FF has persisted well, but OG stands have thinned markedly, particularly under the LM rotation schedule. Therefore, FF may be a better choice than OG as an alternative to IF for spring-calving cow herds in the Upper South.

Key Words: Tall Fescue, Orchardgrass, Bermudagrass

National ADSA Foods Only (Graduate): ADSA Dairy Foods Graduate Paper Contest

60 Influence of pre-exposure of mycobacterium avium sub sp paratuberculosis to different environments on invasion of bovine epithelial cells in in vitro cell culture system. D. A. Patel*[1], L. Meunier-Goddik[1], and L. Bermudez[2], [1]*Food Science and Technology, Oregon State University, Corvallis,* [2]*Department of Biomedical Sciences, Oregon State University, Corvallis*.

Mycobacterium avium sub sp paratuberculosis (MAP) is a causative agent of Johnes disease (JD) in cattle having significant economic impact. It is known that after crossing the mucosal barrier, MAP can disseminate and infection can spread to various sites including mammary gland. MAP can be exposed to high osmolarity and the intracellular environment of mammary gland epithelial cells. To study the influence of the environment on MAP invasion of bovine epithelial cells; MAP strain ATCC 19698 was used in the study. Immortalized MDBK (Madin Darby Bovine Kidney) cells were used as a model of bovine epithelial mucosa. MAC-T cells (mammary epithelial cells) were used for intracellular passage of MAP. MAP culture was inoculated in to raw milk, water and Middlebrook 7H9 broth (7H9 broth) containing Mycobactin J and OADC (10 % V/V) at 37 °C in the presence of antibiotics. MAP was also used to infect MAC-T cells for 1d and 4d at 37°C. MAC-T were then lysed and bacteria separated from host cell by differential centrifugation at 4 °C. MAP exposed to several environments was then added to polarized MDBK cells for 2hr. The percentage MAP invasion was calculated as percent of MAP inoculum that internalized in to MDBK cells. Statistical significance was determined by students t test and ANOVA. Our results indicated that MAP invasion is not significantly different at 1d among milk, broth and water preexposed samples. However, MAP passage in MAC-T cells significantly increased invasion percent (15 fold at 1d, p-value < 0.001 and 10 fold at 4d, p-value < 0.001) compared to control. Employment of RNA subtraction hybridization will help to define which MAP genes are associated with the invasive phenotype. These results indicate that exposure of intracellular MAP significantly increases its invasion efficiency for bovine epithelial cells. This characteristic may be important in establishing successful infection in susceptible host.

Key Words: Mycobacterium avium sub sp paratuberculosis, Invasion Assay, Mammary Epithelial Cells

61 Elucidation of the role of chymosin-mediated proteolysis in texture development during Cheddar cheese ripening. J. A. O'Mahony*[1,2], J. A. Lucey[2], and P. L. H. McSweeney[1], [1]*Department of Food and Nutritional Sciences, University College, Cork, Ireland,* [2]*Department of Food Science, University of Wisconsin, Madison*.

More than 20 years ago, it was hypothesised that proteolysis of α_{s1}-casein by residual chymosin, early in ripening, is responsible for the initial softening observed in Cheddar cheese. To investigate this hypothesis, full-fat, Cheddar cheeses (2 kg) were manufactured with 0.1, 1.0 or 10.0 μmol pepstatin (a potent inhibitor of chymosin) added per litre of curd/whey mixture at the start of cooking to obtain residual chymosin levels of 89, 55 and 16% of the activity in the control cheese, respectively. Texture profile analysis of cheeses was performed by compression (2 cycles) to 25% of original height. Levels of intact α_{s1}- and β-caseins were measured by densitometric analysis of urea-polyacrylamide gel electrophoretograms of pH 4.6-insoluble fractions from the cheeses. During the first 21 d of ripening, the rate of development of pH 4.6-soluble nitrogen (expressed as % of total nitrogen) was 0.29, 0.14, 0.06 and 0.04 %/d for control cheese and cheeses made with 0.1, 1.0 or 10.0 μmol L^{-1} pepstatin, respectively. Concurrently, the level of intact α_{s1}-casein decreased by 49, 25, 7 and 3% in these cheeses. At 21 d of ripening, there was a significant ($P<0.001$) reduction in hardness (maximum force on first compression cycle) in each of the 4 cheeses with initial (1 d) hardness values of 189, 204, 207 and 233 N decreasing to 115, 139, 163 and 187 N, in cheeses made with 0.0 (control), 0.1, 1.0 or 10.0 μmol L^{-1} pepstatin, respectively. It was concluded that, irrespective of the extent of α_{s1}-casein hydrolysis, there was a significant softening of Cheddar cheese texture during the first 21 d of ripening and it appears that the hydrolysis of α_{s1}-casein by chymosin is not a prerequisite for Cheddar cheese softening. We believe that some physicochemical change(s), such as a reduction in the amount of calcium associated with the *para*-casein matrix of the curd may be responsible for this textural change.

Key Words: Proteolysis, Texture, Cheddar Cheese

62 Compositional factors associated with calcium lactate crystal formation in naturally smoked Cheddar cheese. P. Rajbhandari* and P. S. Kindstedt, *University of Vermont, Burlington*.

We have observed a high incidence of surface crystals among retail samples of naturally smoked Cheddar cheese. We have also occasionally observed that some samples displayed crystals whereas others originating from the same vat of cheese did not. This study compared the compositions of naturally smoked Cheddar cheese samples that contained surface crystals (Cry+) with those of samples originating from the same vat that were crystal-free (Cry-). Six pairs of retail samples (Cry+ and Cry-) produced at the same cheese plant on different days were obtained from a commercial source. Each pair was 5-6 mo old upon receipt and was stored for up to 18 mo at 4°C to insure that the Cry- sample remained crystal-free. Then, the crystalline material was scraped off the surfaces of Cry+ samples and analyzed for lactic acid, Ca, P, moisture and CP. Cry+ and Cry- samples were then sectioned into 3 concentric subsamples (0-5mm, 6-10mm and > 10mm depth from the surface) and analyzed for pH, TA, TS, NaCl, total and water-soluble Ca and P, and CP. The data were analyzed by ANOVA according to a repeated measures design with 2 within-subjects variables. The crystalline material contained 52.9% lactate, 8.1% Ca, 0.1% P, 28.5% water and 8.9% CP on average. Both Cry+ and Cry- cheese samples contained significant gradients of decreasing moisture from center to surface. Compared to Cry- samples, Cry+ samples possessed significantly higher moisture,

TA, water-soluble Ca and P, and significantly lower salt, pH and total Ca. The data suggest that within-vat variation in salting efficacy may have influenced calcium lactate crystal formation. Lower salt uptake by Cry+ cheese curd during salting may have resulted in higher moisture (and thus lactose) retention, which caused more lactic acid to be produced in the cheese. Higher lactic acid resulted in lower pH, which shifted calcium to the soluble state. Lactate and soluble Ca in Cry+ cheese became further elevated at the cheese surface as a result of dehydration during natural smoking, possibly triggering the formation of calcium lactate crystals.

Key Words: Cheddar, Calcium Lactate, Crystals

63 Influence of calcium, phosphorus, residual lactose, and salt-to-moisture ratio on cheese quality: pH changes during ripening. P. Upreti*, P. S. Lehtola, and L. E. Metzger, *Department of Food Science and Nutrition, MN-SD Dairy Food Research Center, University of Minnesota, St. Paul.*

The pH of cheese is an important attribute that influences its quality. Large changes in cheese pH are often observed during ripening. Changes in cheese pH during early ripening are associated with calcium (Ca), phosphorus (P), residual lactose, and salt-to-moisture ratio (S/M) of the cheese. Ca and P have the ability to act as a buffer that stabilizes cheese pH, whereas lactose is converted to lactic acid that causes a decrease in cheese pH. The S/M can influence bacterial growth that can affect the rate of conversion of lactose to lactic acid. The net balance between the buffering capacity and the formation of lactic acid should, therefore, determine cheese pH. In order to assess this hypothesis, 4 treatments of Cheddar cheese with 2 levels (high and low) of calcium (Ca) and phosphorus (P), and 2 levels (high and low) of residual lactose were manufactured. Each treatment was subsequently split in half and salted at 2 levels (high and low) for a total of 8 treatments. The detailed experimental design and manufacture of these cheeses is a subject of another abstract submitted for this conference. All the cheeses were salted at a pH of 5.4, pressed for 5h, and then ripened at 6-8°C. The pH of the salted curds before pressing, cheese at day 1, and weeks 1, 2, 3, and 4 of ripening was measured. The cheeses with low levels of Ca and P, high lactose, and low S/M showed a considerable drop in pH (mean=0.20 units) from salting to d1 of ripening, whereas, cheeses with high levels of Ca and P, low lactose, and high S/M showed an average drop in pH of 0.07 units. The comparison of pH of cheeses at 4wk indicated that cheeses with higher levels of Ca and P had higher pH ($p<0.05$) as compared to lower Ca and P cheeses. Also, cheeses with higher S/M were higher in pH ($p<0.05$) as compared to lower S/M cheeses. Residual lactose content of cheeses had a significant effect ($p<0.05$) on cheeses at low salt content. However, at high salt content the effect of residual lactose was not significant. This study determined that changes in cheese pH during early ripening were influenced by Ca and P, lactose, and S/M.

64 Effect of starter inoculation rates and incubation temperatures on physical properties of yogurt. W. J. Lee* and J. A. Lucey, *Department of Food Science, University of Wisconsin, Madison.*

Textural attributes are considered as important criteria for the quality of yogurt. The objectives of this research were to investigate the effect of different starter culture inoculation rates and incubation temperatures on physical properties and microstructure of yogurt gels. A two factor (5 × 2) experimental design was used for data analysis. Yogurt gels were made with 0.5, 1, 2, 3, or 4% (w/w) inoculation rates and incubated at 40 or 46°C. Dynamic low amplitude oscillatory rheology was performed to monitor rheological properties of yogurt gels. Gel permeability and amount of surface whey were determined. Confocal scanning laser microscopy was used to examine gel structure. Storage modulus values (stiffness) increased with increased inoculation rate and decreased incubation temperature. Gels made at higher inoculation rate and incubation temperature exhibited higher yield stress and had higher loss tangent values (ratio of viscous to elastic moduli) during gelation, respectively. Higher permeability and whey separation values were observed in yogurt gels made at lower inoculation rate and higher incubation temperature, which indicated an increased susceptibility of the network to rearrange. These rearrangements resulted in the formation of large pores in gel network. An increase in inoculation rate resulted in a decrease in the pH where the maximum in loss tangent occurred, presumably reflecting less efficient solubilization of colloidal calcium phosphate (which is a slow process) and the need to attain a lower pH to complete the solubilization. Whey separation was positively correlated with the value for maximum in loss tangent ($r = 0.94$) and permeability ($r = 0.89$), respectively. A negative correlation was observed between whey separation and storage modulus ($r = -0.48$). It was concluded that rearrangements of casein particles in gel network and pH at which the solubilization of colloidal calcium phosphate occurred were important driving forces involved in whey separation and a weak network.

Key Words: Yogurt, Rheology, Starter Culture

National ADSA Production Only (Graduate)

65 Cloning the genomic sequence and proximal promoter of bovine pyruvate carboxylase. S. M. Rodriguez*, C. A. Bidwell, and S. S. Donkin, *Purdue University, West Lafayette, IN.*

Pyruvate carboxylase (PC) catalyzes a pivotal reaction in gluconeogenesis and lipid metabolism in liver. We previously identified six unique alternative splice variants in the 5 untranslated region (UTR) of PC mRNA. These splice variants may have a role in translational regulation of PC protein abundance. The objectives of this experiment were to clone and sequence the bovine PC gene, to determine the intron/exon organization of the 5UTR and to identify PC promoter elements. The RPCI-42 Bovine Bacterial Artificial Chromosome (BAC) library was screened with oligonucleotide sequences corresponding to specific elements of the 5 UTR sequence of bovine PC and to a region of the coding sequence. Two BACs that hybridized to all probes were selected for further analysis. A partial restriction map of the BACs was made with oligonucleotides corresponding to the coding region and the 89 and 110 bp elements of bovine PC 5 UTR. The BAC fragments that hybridized to the oligonucleotide probes were isolated and sequenced. The sizes of the cloned genomic PC 5 UTR fragments were verified by PCR, using genomic DNA from four cows. Sequencing data confirms the existence of a 178 bp exon that contains the 68 and 110 bp sequence elements of the 5 UTR for PC mRNA. The 178 bp exon appears to be the first transcribed exon in PC and the 68 and 110 bp 5 UTR sequences are most likely generated by alternative transcription start sites. Genomic sequence data also confirms that the 3 end of the 89 bp element is a discrete 41 bp exon. Regions within the genomic sequence adjacent to the 178 and 41 bp exons of the PC 5 UTR contain binding sites for TBP, Sp1, Ap1 and/or CEBP transcription factors. These data provide information about the arrangement of exons in the 5 UTR of PC and about putative promoter regions.

Key Words: Pyruvate Carboxylase Gene, Liver, Promoter

66 Relationship between antibiotic susceptibility of mastitis pathogens and treatment outcomes. F. Hoe* and P. Ruegg, *University of Wisconsin, Madison.*

Antimicrobial susceptibility testing is commonly used to guide mastitis treatments. Broth microdilution is used to obtain quantative results that are recorded as minimum inhibitory concentrations (MIC). The objective of this study was to determine the relationship between MIC values of mastitis pathogens and clinical outcomes. Duplicate quarter milk samples were obtained from cows observed with mild to moderate mastitis in a single quarter. Cows were ineligible if they had secondary clinical signs or had received antibiotics within the previous 30 days. Cows were treated with intrammary pirlimycin and could not receive ancillary treatments. Milk samples were collected before treatment and 14 and 21 days after treatment. Microbiological procedures were as described by the NMC. MIC values were determined using a commercial microdilution method (Sensititre, Westlake, OH). Of eligible milk samples ($n = 217$), 58 samples were no growth, 17 produced different growth on the duplicates and 6 were contaminated. MICs were obtained for: Strep spp. (34.6%); E.coli (25.7%); CNS (19.1%); Klebsiella spp. (9.6%) and other minor pathogens (14.0%). No significant difference was observed for days of treatment (2.8 and 2.9) and days until clinical cure (3.5 and 3.8) for gram positive and gram negative isolates, respectively