SDS were effective at different levels to solubilize aggregates indicating that hydrogen bonds and hydrophobic interactions are involved in the gel network. The decrease in turbidity of enzyme-induced gels when the dielectric constant of the solvent was decreased also showed the importance of hydrophobic interactions. Reducing reagents such as β-mercaptoethanol and DTT did not break aggregates and suggests that disulfide bonds do not play a major role in the aggregation process. Analysis by native-PAGE and SDS-PAGE also demonstrated that most of the aggregates could be disrupted. HPSEC results have determined that 80% of the peptides present were $<$ 2000 Da. Therefore, physical aggregation via hydrogen bonds and hydrophobic interactions, with ionic bonding playing a minor role are the most probable type of interactions involved the gelation process.

Key Words: Enzymatic hydrolysis, Gelation, Interactions

73 Process analysis of skim milk microfiltration for selective concentration of casein. M Singh*, G Solakni, and S.S.H. Rizvi, *Institute of Food Science, Cornell University, Ithaca NY 14853*.

Cheese making from concentrated cheese milk has been of interest to the food industry for well over the past two decades. As more and more cheese plants incorporate membrane processing in cheese manufacture to standardize cheese milk and increase total solids, the need to analyze this process becomes more critical. In this study we report the effects of concentration factor (CF), cross flow velocity (CFV), and uniform trans-membrane pressure (UTMP) on energy requirements for selectively concentrating skim milk upto 8 times using cross flow microfiltration (CFM). CFM (0.2μm) of pasteurized skim milk was carried out at 50°C at three CFVs and three UTMPs. Volumetric CF of 8x was achieved at each combination of CFV and TMP, permeate flux and longitudinal pressure drop were recorded at each CF, and power consumption was calculated. Power consumption increased upto 1398% with CF (23.6 to 353.5 kJ/kg at 8x). A transition from turbulent to laminar flow was observed at 6x and retentate behavior became shear thinning. Increase in CFV from 5.3 to 6.3 m/s at 8x increased flux and reduced power consumption by 25% (353.1 to 263.1 kJ/kg). Although, increasing the UTMP (68.9 to 137.9 kPa) enhanced the starting flux (51.1 to 62.2 $kgm^{-2}h^{-1}$) and lowered the corresponding power consumption by 18% (28.8 to 23.6 kJ/kg), above 6x, higher UTMP caused excessive fouling, lowered the flux from 14.0 to 8.0 $kgm^{-2}h^{-1}$ and the power consumption increased from 179.9 to 353.1 kJ/kg. Overall power consumption is always lower due to shorter CFM process when skim milk is microfiltered to 8x at higher CFV (6.3m/s) as compared to 5.3m/s. Overall power consumption increased only marginally when UTMP was increased (68.9 to 137.9kPa). However at higher UTMP and above 6x, the permeate flux dropped precipitously below the minimally acceptable rate, which limited the performance of the CFM system.

Key Words: Microfiltration, Skim milk, Process Analysis

Graduate Paper Competition
ADSA Production Division and ADSA Southern Branch

74 Effects high wheat bran rations and different sources of protein on the milk constituents and production. Moslem Bashtani*, Abbasali Naserian, and Reza Valizadeh, *Ferdowsi University Of Mashhad, Mashhad, khorasan, Iran*.

The effect of including an increased amount of wheat bran on performance of Holstein dairy cows was investigated using a change over design with four treatment periods. Eight multiparous Holstein cow weighting 56319 kg and average days in milk of 48.5 26 and mean milk production of 30 1.94 kg/day were adapted to the experimental rations for 14 days and then entered into a collection period of 7 days. The proportion of concentrate and roughages in the total mixed ration were 60% and 40%. The treatments were 1) 25% wheat bran supplemented with cottonseed meal 2,3,4) 40% wheat bran supplemented with cottonseed meal, fish meal and urea respectively. The animals were individually kept indoors and had free access to fresh water and salt blocks. The daily feed intake was recorded and milk yield, blood, feces, and rumen liquor were sampled on a regular basis for analysis. There were no treatment effects on average daily dry matter and nutrient intakes. Dry matter, organic matter and crude protein digestibility were significantly increased with 40% level of wheat bran while NDF and ADF digestibility were similar in the different treatments. The digestibility values, which were calculated by AIA marker, were similar to the in vivo results. Feces, urine and ruminal pH were not affected by the different treatments. Changing the level of wheat bran did not significantly increase rumen ammonia-N, but urine utilization led to significant increases in rumen ammonia-N contents. A significant increase in blood glucose was observed in the supplemented fish meal diet. Daily milk production, percentage and daily yield of protein, lactose, casein, NPN and SNF milk were not significantly affected by different diets. It appeared that the level of wheat bran in dairy diets can be increased up to 40% with any adverse effects.

Key Words: Dairy cows, Wheat bran, Milk production and composition

75 Effect of lauric acid on ruminal fermentation, nutrient digestibility and milk yield of dairy cows. K. L. Grandeen*, A. N. Hristov, and J. K. Ropp, *Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844-2330*.

Medium-chain saturated fatty acids have been shown to inhibit ruminal protozoa and ammonia production in the rumen in vitro. A cross-over design trial with four ruminally and duodenally cannulated lactating dairy cows (268±30.5 DIM; 768±36.9 kg BW) was conducted to study the effect of lauric acid-Na (LA) on ruminal fermentation, nutrient digestibility, and milk yield and composition. Cows were fed (DM basis) a 46% concentrate (barley, corn, cottonseed, soybean meal):52% forage (alfalfa hay, triticale silage) diet, twice a day (0600 and 1800). The daily dose of LA (0, Control or 240 g/cow, LA) was divided into two equal portions and introduced directly into the rumen through the cannula before the two feedings. Cows were treated with LA for 14 days before sampling and rumens were inoculated with ruminal contents (20% on weight basis) from donor cows fed on the same diet on day 1 of each study period. Ruminal samples (28 in 5 days) were analyzed for fermentation variables and protozoal counts. Digestibility was determined using acid insoluble ash as a marker. LA had no effect ($P > 0.05$) on ruminal pH (6.0 and 6.1), ammonia (12.0 and 11.8 mM), and VFA concentration (128.0 and 121.4 mM) and composition (Control and LA, respectively). Compared to Control, protozoal counts were reduced ($P < 0.05$) by LA (11.14 *vs* 0.98 $\times$ 10^5/ml, respectively). Carboxymethylcellulase and xylanase activities of ruminal fluid were lowered (by 40 and 36%, respectively; $P < 0.05$) and amylase activity was not affected ($P > 0.05$) by LA compared to Control. DM intake and DM, OM, CP, NDF, and ADF digestibility were not different ($P > 0.05$) between the two treatments. Milk yield (28.8 and 29.6 kg/d), FCM yield, milk fat (3.43 and 3.38%) and protein (2.92 and 2.79%) concentrations and yields and milk urea N content (24.6 and 21.1 mg/dl; Control and LA, respectively) were not affected ($P > 0.05$) by treatment. In conclusion, compared to untreated Control, lauric acid introduced into the rumen daily at approximately 0.3% of the rumen weight reduced protozoal numbers and fibrolytic activities of ruminal fluid but had no other effects on ruminal fermentation, total tract digestion of nutrients, or milk yield and composition.

Key Words: Lauric acid, Protozoa, Dairy cows

76 Production and metabolic responses to dietary conjugated linoleic acid (CLA) and trans-octadecenoic acid isomers in periparturient Holstein cows. KT Selberg*, CR Staples, and L Badinga, *University of Florida, Gainesville, FL*.

Thirty-nine multiparous Holstein cows were utilized in a completely randomized design to examine the effects of feeding ruminally protected CLA and trans-octadecenoic acid isomers on animal productivity and metabolism during the transition to lactation. Dietary treatments were initiated approximately 28 days (D) prior to expected calving date and continued through D 49 postpartum (PP). Treatments consisted of 1) a basal TMR diet (CON), 2) basal diet + 150 g/d CLA mix (CLAM), and 3) basal diet + 150 g/d trans-octadecenoic acid mix (TRANS). The amounts of CLA and trans-octadecenoic acid mixes fed were adjusted to 225 g/d during the seven-week (wk) PP treatment period. Liver biopsies

were obtained at D 2, 14 and 28 PP to evaluate treatment effects on hepatic lipid accumulation. Dietary treatments had no detectable effects on pre- or PP dry matter intakes, body weights and body condition scores. Treatment x D interactions were detected for yield of milk ($P < 0.001$) and fat ($P < 0.001$). Milk yield increased and peaked earlier (by wk 3) in the TRANS group, compared to CON and CLAM groups. In contrast, dietary CLA stimulated milk production only after wk 4 of lactation. Milk fat percentage decreased sharply between wk 1 and 3, and did not change thereafter in the TRANS group. Dietary CLA caused a slower, but more drastic decrease in milk fat by wk 6 of lactation. Average milk fat yield (CON, 1.4 kg/d; CLAM, 1.2 kg/d; TRANS, 1.4 kg/d) and 3.5% fat-corrected milk production (CON, 40.0 kg/d; CLAM, 37.4 kg/d; TRANS, 40.0 kg/d) did not differ among diets. Feeding CLA or trans-octadecenoic acids had no detectable effect on milk protein percentage or somatic cell count. Liver fat and triacylglycerol (TAG) concentrations increased between D 2 and 14 PP, and then decreased by D 28 in CON and CLA groups. Total lipid and TAG concentrations in liver biopsies collected from cows in the TRANS group were similar across D. Differential kinetics of CLA and trans-octadecenoic acid-mediated effects on production and metabolic responses would indicate potential converging as well as distinct signaling mechanisms for these fatty acid isomers in the dairy cow.

Key Words: Conjugated linoleic acid, Trans-octadecenoic acids, Transition period

77 Intramammary infusion of IGF-I increases BrdU-labeling in mammary epithelial cells of prepubertal heifers. L.F.P. Silva*, M.J. VandeHaar, and M.S. Weber Nielsen, *Michigan State University, East Lansing MI.*

In vitro studies with bovine mammary tissue strongly suggest that insulin-like growth factor-I (IGF-I) stimulates mammogenesis in cattle before puberty. However, this effect has never been demonstrated *in vivo*. Our objective was to determine if intramammary infusions of IGF-I would stimulate mammogenesis in prepubertal heifers. Ten μg of rhIGF-I diluted in 10 ml of sterile saline (1mg/ml albumin) was infused once per day, via the streak canal, into two quarters, one front and one rear, of six prepubertal dairy heifers (222 $\pm$10 kg BW). Contralateral quarters received saline with albumin. This dose of IGF-I was calculated to increase the concentration of IGF-I in the parenchyma by 50 ng/ml. After seven days of treatment, bromodeoxyuridine (BrdU) was infused intravenously at 5 mg/kg BW, and heifers were slaughtered 3 h later. Samples from three regions of the mammary parenchyma (proximal, intermediate, and distal to the teat) were collected, fixed, sliced, and incubated with BrdU monoclonal antibody to identify cells in the S-phase of the cell cycle. Total number of epithelial cells and BrdU-labeled cells were quantified in three microscopic fields from each slide section. An average of 3,200 cells were counted in each quarter. Intramammary infusion of IGF-I increased ($P<0.001$) the percentage of epithelial cells in the S-phase by 60% (6.4 vs. 4.0%, $\pm$0.6%). Proliferation was similar ($P>0.35$) in all three parenchymal regions, and the response to IGF-I was similar in each region. This similar response indicates that IGF-I translocated homogeneously throughout the parenchyma. The effects of IGF-I in front quarters was the same as in rear quarters. Also, infusion of IGF-I in diagonal quarters gave a response identical to that of infusion in quarters of the same side, suggesting that each quarter could serve as a separate experimental unit. Statistical power calculations showed that five animals would be required to detect a 30% difference in cell proliferation with 95% confidence. We conclude that local IGF-I increases proliferation of mammary parenchymal epithelial cells in prepubertal heifers. Moreover, combining intramammary infusion with the BrdU-labeling technique is a sensitive method for measuring effects of metabolic compounds on mammogenesis.

Key Words: Bovine, Proliferation, Mammary gland

78 Use of insulin-like growth factor-1 in culture and administration of GnRH to recipients to improve pregnancy rates following timed embryo transfer of in vitro-produced embryos to lactating dairy cows. J. Block*[1], M. Drost[1], R.L. Monson[2], J.J. Rutledge[2], R.M. Rivera[1], F.F. Paula-Lopes[1], O.M. Ocon[1], and P.J. Hansen[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Wisconsin, Madison, WI.*

Studies have reported the positive effect of insulin-like growth factor-1 (IGF-1) on embryonic development in vitro. In addition, there are reports that administration of GnRH on d 11 improves pregnancy rates following artificial insemination. Objectives of this study were to determine if pregnancy rate following timed embryo transfer would be improved by 1) culturing embryos in the presence of IGF-1 and 2) treating recipients with GnRH on d 11 after putative estrus. The experiment was conducted between June and September, 2001. Embryos were produced from Holstein oocytes collected from ovaries from a Wisconsin abbatoir. Oocytes were shipped overnight to Gainesville where fertilization took place. Following fertilization, oocytes were cultured in the presence or absence of 100 ng/ml IGF-1. A total of 210 primiparous and multiparous lactating Holstein cows were synchronized using the OvSynch protocol and used as recipients in 13 replicates (6 to 24 recipients/replicate). Fair to excellent quality blastocysts and morulae were collected at d 8 after fertilization and randomly transferred to day 7 (d 0 = the day following the 2nd GnRH injection) recipients. For the first 3 replicates (n = 46), recipients received no additional treatment. For the remaining 10 replicates (n = 164), recipients randomly received either GnRH (Cystorelin®, 100 ug) or placebo on d 11. Pregnancy was diagnosed 45 d after embryo transfer. Recipients which received IGF-1 treated embryos had higher pregnancy rates than controls ($p<0.05$, 29/124 = 23.4% vs. 10/86 = 11.6%). Among cows receiving GnRH or placebo at d 11, pregnancy rate was higher ($p<0.05$) for those receiving GnRH (22/93 = 23.7%) than for those receiving placebo (8/71 = 11.3%). Results indicate that addition of IGF-1 to embryo culture and administration of GnRH on day 11 can improve pregnancy rates to timed embryo transfer in lactating dairy cows. Support: USDA IFAFS 2001-52101-11318 and USDA TSTAR 2001-34135-11150, Florida Milk Checkoff Program, and the Babcock Institute for International Dairy Research and Development, UW-Madison.

Key Words: Embryo Transfer, IGF-1, GnRH

79 Expression of fibronectin, laminin and type IV collagen in mammary tissue from ovariectomized and intact prepubertal heifers. S. D. Berry*[1], R. D. Howard[2], and R. M. Akers[1], [1]*Virginia Tech,* [2]*Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061.*

The objective of this experiment was to investigate the potential role of the extracellular matrix proteins fibronectin, laminin and collagen in regulating prepubertal heifer mammary development. Mammary parenchyma and fat pad tissue was collected from fourteen six-month old heifers, eight of which were ovariectomized between one and three months of age, and six which served as intact controls. Distribution of total collagen was assessed by sirius red staining of tissue sections and fibronectin, laminin and type IV collagen were assessed by immunohistochemistry. Abundance of fibronectin and laminin was also analyzed by western blotting. Total mammary mass was dramatically reduced in ovariectomized animals (130 21 g vs. 304 25g, $p < 0.001$). Histological structure also differed: while parenchyma from intact animals contained abundant branching epithelial structures (TDU), TDU from ovariectomized animals essentially consisted of major ductal structures with little or no branching. Collagen fibers were abundant and densely packed throughout inter-lobular stroma and were less abundant and more diffuse within intra-lobular stroma. Type IV collagen was present primarily in the basal lamina of capillaries, whereas fibronectin and laminin staining was present throughout parenchymal stroma, in both intact and ovariectomized animals. Western blotting showed that fibronectin was more abundant within parenchyma than the mammary fat pad (182 vs. 21 densitometric units/mg tissue; $p < 0.0001$). Laminin was more abundant in parenchyma from intact than ovariectomized animals (30 vs. 17 densitometric units/mg tissue; $p < 0.05$), but laminin abundance did not differ between parenchyma and fat pad tissues. These results provide initial evidence that fibronectin, laminin and collagen participate in regulation of prepubertal mammary development in heifers.

Key Words: Mammary, Bovine, Extracellular matrix

80 Comparison of high-molecular weight glycoproteins, MUC1 and MUCX, in porcine and bovine milks. C. Liu*, A.K. Erickson, D.R. Henning, and D.H. Francis, *South Dakota State University, Brookings, SD.*

Using periodic acid Schiff's (PAS) reagents, a modified silver staining method, and wheat germ agglutinin (WGA) blot assay, two polymorphic high-molecular weight glycoproteins in porcine milk samples were resolved and detected on SDS-PAGE. Both proteins showed polymorphism in band number (one or two) and band mobility. Based on this observation and the data obtained from other species, these two glycoproteins were expected to be porcine homologues of milk MUC1 and MUCX. Porcine MUC1 was resolved in 6% running gel and had an estimated molecular weight varying from 300,000 to 400,000. Porcine MUC1 was detected in both milk fat globule membrane (MFGM) and skim milk (whey protein portion) preparations, while porcine MUCX, resolved in 3% stacking gel, was only found in the skim milk phase. With similar methods, polymorphic MUC1 was found in both MFGM and skim milk phases of bovine milk but exhibited a much lower molecular mass (usually <250,000), which agrees with earlier studies on bovine MUC1. However, in contrast to porcine MUCX, bovine MUCX was prsent only in skim milk, appearing as a nonpolymorphic single band on 3% stacking gel and has a relatively higher molecular weight than porcine MUCX. Regardless of species, PAS staining of MUC1 bands was much stronger than that of MUCX bands within each individual sample, suggesting either much less glycosylation or much lower concentration of MUCX in the skim milk.

Key Words: Porcine milk mucins, Bovine milk musins, Milk glycoproteins

81 Short day photoperiod enhances lymphocyte proliferation in dairy cattle. T.L. Auchtung*, J.L. Salak-Johnson, and G.E. Dahl, *University of Illinois, Urbana, IL.*

The periparturient period is a time of increased immunosuppression and risk of mastitis in cows. Cows on short day photoperiod (SDPP) during the dry period have higher milk production in the subsequent lactation than cows on long day photoperiod (LDPP) when dry. Of interest, rodents treated with SDPP have increased immune cell function relative to LDPP animals. The objective of this study was to determine whether immune cell function could be improved in dairy cattle treated with SDPP as compared to LDPP. Holstein steers (n = 12) were used as the model. Treatments were LDPP (16 h light:8 h darkness) and SDPP (8 h light:16 h darkness). After 9 wk on treatment, animals were switched to the opposite photoperiod treatment. Blood (20 mL) was collected on heparin via jugular venipuncture at Weeks 4 and 13. The buffy coat was mixed with RPMI-1640 cell growth media and was centrifuged through Histopaque-1077 density gradient. Lymphocytes were then washed with RPMI and brought to a final concentration of 5 x 10^6 cells/ml in RPMI supplemented with 10% FBS and gentamicin. Lymphocytes were stimulated by each of three mitogens: Concanavalin A (20 μg/mL), phytohemaglutinin (10 μg/mL), and pokeweed mitogen (10 μg/mL). After incubation for 48 h at 37°C and 5% CO2, thiazolyl blue (MTT) was added to quantify lymphocyte proliferation. At Week 4, mitogen-stimulated lymphocyte proliferation was greater ($P < 0.05$) in animals treated with SDPP than those treated with LDPP for all three mitogens. Results were similar at Week 13, with animals treated with LDPP through Week 9 but then switched to SDPP having greater ($P < 0.05$) lymphocyte proliferation for all three mitogens as compared with the animals that were switched from SDPP to LDPP. In conclusion, SDPP significantly improves immune function, as measured by lymphocyte proliferation, compared with LDPP in dairy cattle.

Key Words: Cattle, Photoperiod, Lymphocytes

82 Prevention of fatty liver in transition dairy cows by glucagon. R. A. Nafikov*[1], B. N. Ametaj[2], G. Bobe[1], J. W. Young[1], and D. C. Beitz[1], [1]*Iowa State University, Ames, IA,* [2]*Purdue University, West Lafayette, IN.*

The objective of this study was to determine whether administration of glucagon at day two postpartum would prevent the development of fatty liver (hepatic lipidosis) in dairy cows. Twenty-four multiparous Holstein cows were used. During the dry period, cows were fed cracked corn in addition to their normal diet for the last 30 days before calving to induce pathological fatty liver. Then, they were assigned randomly to one of three different treatment groups of eight cows in each and injected subcutaneously with either saline, 7.5 mg/day, or 15 mg/day of glucagon for 14 days starting at day two postpartum. Liver samples were obtained on -4, 2, 6, 9, 16, 20, 27, 34, and 41 days postpartum by puncture biopsy. Blood samples were taken from the coccygeal vein every day starting 4 days prepartum until the day after the last injection of glucagon and then every time before liver biopsies. Liver samples were analyzed for lipid composition. Blood samples were analyzed for concentrations of glucose, nonesterified fatty acids, β-hydroxybutyrate, and other blood constituents. We found that glucagon administered at day two postpartum prevented the accumulation of total lipids in cows' liver during the first two weeks after calving. Glucagon at both dosages increased blood glucose concentration but did not alter nonesterified fatty acid concentration in blood. This experiment demonstrates that glucagon given during the early postpartal period will prevent fatty liver in dairy cows. (supported in part by USDA grant 99-35204-8576)

Key Words: Fatty liver, Glucagon, NEFA

83 Oxytetracycline resistant gram-negative bacteria in dairy cattle: risk factors and implications on food safety. A. A. Sawant, N. V. Hegde, B. C. Love, and B. M. Jayarao, *The Pennsylvania State University, University Park, PA, USA.*

Over the last decade, scientists, food animal producers, medical and veterinary clinicians and the general public have become increasingly concerned about the development of antibiotic resistant microorganisms and its subsequent transmission from the animal to human populations. This issue is perceived as a significant public health concern, particularly in terms of food safety. A study conducted in our laboratory showed that 104 of 344 (30%) lactating cows on 23 of 33 (69%) dairy farms were positive for oxytetracycline-resistant gram-negative bacteria (OXY-GNB) in feces. The OXY-GNB accounted for 0.2 to 99% of the total fecal flora in the feces. *Escherichia coli* accounted for 96% of the oxytetracycline-resistant isolates. Isolates resistant to oxytetracycline were also observed to be resistant to clindamycin (100%), florfenicol (99%), penicillin (100%), tiamulin (100%), tilmicosin (97%), tylosin (100%), and sulpha drugs (88-95%). The isolates were able to grow at high concentration (32ug/ml) of oxytetracycline than the cutoff level for MIC_{90} (16ug/ml). Resistance to oxytetracycline persisted even on removal of "selection pressure" (culture on antibiotic free medium); suggestive of resistance being regulated by genetic element(s). It was observed that dairy producers who fed milk replacers with oxytetracycline to calves were 12-fold more likely to have lactating cows shedding OXY-GNB as compared to dairy producers who did not feed milk replacers containing oxytetracycline. Further, bulk tank milk (BTM) from 3 of the 33 dairy herds had *Escherichia coli* that were resistant to oxytetracycline. The findings are compelling and strongly suggest that oxytetracycline-resistant gram-negative bacteria could be an important food safety issue.

Key Words: antimicrobial resistance, gram negative bacteria, milk replacer

84 Antimicrobial resistance patterns of bacteria cultured from milk samples in Wisconsin from 1994 - 2001. J. A. Makovec* and P. L. Ruegg, *University of Wisconsin, Madison.*

Concern about antimicrobial resistance exists throughout the world. The objective of this study was to determine antimicrobial resistance of bacteria cultured from milk samples submitted to the WI Veterinary Diagnostic Laboratory from Jan 1994 - June 2001. Clinical case records from milk samples were retrieved for analysis. Antimicrobial susceptibility tests were performed on 8,905 of 83,650 samples. Bacteria were tested for susceptibility using Kirby-Bauer disk diffusion and classified as sensitive, intermediate or resistant based on NCCLS standards. For analysis, intermediate results were classified as resistant. Antimicrobial resistance was examined for *Staph aureus*, *Staph sp.*, *Strep sp.* and *E.coli*. Year was significantly associated with proportion of *Staph aureus* resistant to ampicillin, erythromycin, lincomycin and penicillin (P<0.0001) as well as pirlimycin (P=0.0078) and sulfa (P=0.0076). There was no significant relationship between year and proportion of *Staph aureus* resistant to tetracycline (P=0.58). Year was significantly associated with proportion of *Staph sp.* resistant to erythromycin (P=0.03), lincomycin, pirlimycin, sulfa (P<0.0001) and SXT (P=0.0004). There was no significant association between year and proportion of *Staph sp.* resistant to cloxacillin (P=0.12), penicillin (P=0.74) and tetracycline (P=0.23). Year was significantly associated with proportion of *Strep sp.* resistant to cloxacillin

(P=0.0007) and tetracycline (P=0.0441). There was no significant association between year and proportion of *Strep sp.* resistant to ampicillin (P=0.22), cephalothin (P=0.337), erythromycin (P=0.07), lincomycin (P=0.95), penicillin (P=0.21), pirlimycin (P=0.13), sulfa (P=0.37) and SXT (P=0.78). Year was significantly associated with proportion of *E.coli* resistant to cephalothin and sulfa (P=0.0006). There was no significant association between year and proportion of *E.coli* resistant to ampicillin (P=0.10), SXT (P=0.49) and tetracycline (P=0.10). Some variation was noted in the antimicrobial resistance of these pathogens.

Key Words: Antibiotic, Antimicrobial resistance, Mastitis

85 Effects of storage time and thawing methods on the recovery of Mycoplasma in milk samples from cows with intramammary infections. M. Biddle*, L. Fox, M. Evans, and C. Gaskins, *Washington State University*.

This study was executed to determine the effects of storage and thawing on the viability of *Mycoplasma species* in cow#s milk. The trial was designed using a control sample and seven treatments subjected to two methods. Treatments 1, 2, 3 and 4 were the same sample repeatedly frozen and thawed for 4 weeks starting on Week 1 after collection. Thawing, plating, and refreezing of this sample were repeated on Weeks 2, 3, and 4, after original collection. Treatments 5, 6, and 7 were three individual samples stored for varying lengths of time. Treatment 5 samples were stored for two weeks and a portion plated on Week 2. Treatment 6 samples were stored for three weeks and plated on Week 3. Treatment 7 samples were stored for four weeks and plated on Week 4. There was a significant treatment effect (p< 0.0001) on the recovery of colony forming units (CFU) in milk samples when comparing the control sample to Treatments 1 through 7. There was a linear decline in mean number of CFU in samples that were repeatedly frozen and thawed. Control sample CFU was 6.29, Treatment 1 CFU 4.64, Treatment 2 CFU 3.69, Treatment 3 CFU 3.01, and Treatment 4 CFU 1.86. A linear decline in mean number of CFU was also present for milk samples that were stored for varying lengths of time. Treatment 5 CFU was 4.41, Treatment 6 CFU 4.13, and Treatment 7 CFU 3.18 which are 2-3 fold log reductions in comparison to the control sample. To determine the best thawing method, Treatment 1 through 7 samples, previously split, were thawed using two methods. In Method 1, samples were thawed at ambient temperature for one-half hour. In Method 2, samples were thawed at 37°C in a water bath. More mycoplasma were recovered from milk samples thawed at ambient temperature than milk samples thawed in a 37°C water bath (p< 0.0001). In comparing mean numbers of CFU, Method 1 CFU was 4.04 and Method 2 CFU was 3.76. A final comparison was made between individual treatments. All treatments were significant (p< 0.0001), with the exception of the Treatment 5 to Treatment 6 pairwise comparison. The results of this study indicate that storage and thawing of milk samples is harmful to mycoplasma organisms.

86 Performance of lactating dairy cows fed gamagrass as hay or silage. J-S. Eun*[1], V. Fellner[1], J. C. Burns[2], and M. L. Gumpertz[1], [1]*North Carolina State University, Raleigh, NC, USA,* [2]*USDA-ARS, Raleigh, NC, USA*.

Twenty lactating Holstein cows were used to determine feeding value of gamagrass as hay or silage and effect of supplemental corn on gamagrass silage utilization. Cows were grouped by DIM, milk yield, and parity into 5 groups. Each group was assigned to one of 5 dietary treatments: 1) gamagrass hay (GH), 2) gamagrass silage (GS), 3) GS + low corn (GSLC), 4) GS + medium corn (GSMC), and 5) GS + high corn (GSHC). A protein supplement mix was offered to all cows to keep crude protein levels similar across treatments. All silage diets were offered for 6 weeks and hay was offered for 3 weeks. Data were analyzed according to a completely randomized design using the proc GLM procedure of SAS. Feeding gamagrass as hay or silage did not change milk yield. Compared to gamagrass silage, feeding supplemental corn increased milk yield but only at the medium and high levels of corn inclusion ($P < 0.05$). Milk fat, protein, and lactose contents were similar across all treatments; there was a tendency for milk protein to be higher with GSHC diet ($P < 0.07$). Yields of milk fat, protein, and lactose tended to be higher with GS compared with GH and corn supplementation supported higher yields when compared to gamagrass silage. Gamagrass fed as silage resulted in a higher feed conversion efficiency compared to gamagrass fed as hay ($P < 0.01$). Including corn with the silage resulted in a lower feed efficiency with GSHC being the lowest. Conversion of feed N to milk N was greater with gamagrass fed as silage compared to hay ($P < 0.01$) and supplementation of GS with corn failed to improve N efficiency. Milk urea nitrogen (MUN) was significantly higher ($P < 0.01$) for cows fed GH compared to all other treatments. Feeding GS significantly lowered MUN, and corn supplementation at the medium and high levels further reduced MUN ($P < 0.05$). Milk lipid profile was similar between GH and GS. Supplementing corn at the high level increased $C_{18:0}$, *trans*-$C_{18:1}$, and $C_{18:2}$ contents. Gamagrass silage supported similar milk yield compared to gamagrass hay. Increased energy from supplemental corn increased milk yield and tended to increase conversion of feed N into milk protein. Gamagrass fed as silage without or with corn improved the N status of the cows as indicated by lower MUN concentrations.

Key Words: Gamagrass, Corn, Dairy cows

87 Dietary cation-anion difference and K:Na ratio effect on performance of lactating dairy cows during hot weather. C.D. Wildman*, J.W. West, and J.K. Bernard, *The University of Georgia, Tifton, GA*.

Forty-two lactating Holstein cows averaging 187.6 DIM (±58.5d) were used in an 8 wk trial to determine the response to dietary K:Na ratio and dietary cation-anion difference (DCAD) levels fed during hot weather. The study duration was June 6 through July 31. Mean maximum and minimum temperature, relative humidity, and temperature-humidity index (THI) were 31.2 and 22.6°C; 95.8 and 58.8%; and 85.9 and 75.3. Treatments were arranged as a 2x3 factorial within a randomized block design to provide 30 or 45 meq/100g DM (Na + K - Cl - S) and 2:1, 3.5:1, and 5:1 K:Na ratios using sodium bicarbonate and potassium carbonate to modify diets. Intake of DM, energy-corrected milk yield, fat percentage, and protein percentage for low and high DCAD and low, moderate, and high K:Na ratios were 22.8, 22.8, 22.7, 22.7, 22.9 kg/d; 27.7, 28.2, 28.2, 27.6, 28.0 kg/d; 3.9, 3.7, 3.8, 3.9, 3.8%. No significant main effects or interactions were detected (P>.10). A significant effect for K:Na ratio was observed in protein percentage (P<.08), with the highest ratio yielding the lowest protein percentage (2.9%) compared to the low and moderate ratios, which yielded 3.0 and 3.1%. Blood urea nitrogen (BUN), blood bicarbonate, urinary bicarbonate, blood Na and K, and urinary Na and K for low and high DCAD and low, moderate, and high K:Na ratios were 17.3, 14.8, 15.8, 15.2, 17.1 mg/dl; 22.1, 22.5, 22.1, 21.9, 22.8 mmol/L; 94.7, 106.1, 102.6, 100.6, 97.9 mmol/L; 147.8, 147.5, 147.5, 147.6, 147.8 mmol/L; 4.9, 4.8, 4.7, 4.8, 4.9 mmol/L; 99.5, 110.6, 141.9, 105.4, 67.9 mmol/L; 128.8, 153.7, 116.2, 148.2, 159.4 mmol/L. A significant effect of DCAD was seen for BUN (P<.01). A significant ratio effect was seen for urinary K and Na (P<.10). There was a tendency toward a DCAD effect for urinary bicarbonate (P<.11). No other main treatment effects were seen. Results suggest that sufficient blood buffering existed with the lower DCAD diet, with additional cation and bicarbonate being excreted in the urine.

Key Words: Dietary Cation-Anion Difference, Electrolytes, Heat stress

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88 Potential mechanisms for increased milk yield due to increased milking frequency during early lactation. S. A. Hale*[1], A. V. Capuco[2], and R. A. Erdman[1], [1]*University of Maryland, College Park,* [2]*USDA-ARS, Beltsville, MD*.

Increased milking frequency (IMF) at the beginning of lactation has been shown to increase milk yield not only during IMF but also after its cessation. This experiment evaluated the immediate effects of IMF initiated during early lactation, on mammary growth and long-term effects on milk yield. Thirty-one cows were divided into three treatment groups: 1) controls: cows milked twice daily (2X) beginning at parturition (d 1), 2) IMF1: cows milked four times daily (4X) from d 1 to 21 postpartum (pp) and 3) IMF4: cows milked 2X d 1 to 3 and 4X d 4 to 21 pp. The 4X cows were milked immediately before 2X cows and again 3 h later, at the end of the normal milking routine. All cows were