71 Retention of vitamin D fortified emulsions in bench-top cheese. M. Tippetts*[1,2], S. Martini[1,2], C. Brothersen[2,1], and D. McMahon[1,2], [1]*Utah State University*, *Logan*, [2]*Western Dairy Centrer*, *Logan, UT*.

The objective of this research was to evaluate the retention of vitamin D in bench-top cheese by using different delivery matrices. Vitamin D was incorporated in the cheese using a 5% oil-in-water emulsion. Emulsions were formulated with different emulsifiers (non-fat dairy powder, whey protein concentrate, calcium and sodium caseinates) in triplicate to determine the efficiency in vitamin D delivery. The oil phase was composed of 50:50 wt% of vitamin D and soybean oil; the water phase consisted of 2 wt% protein in a 0.01M Na2HPO4-7H2O buffer (pH 7). The amount of each emulsifier added varied according to protein content. The oil and water phases were homogenized using a high shear force for 1 min at 18,000 rpm and then passed through a microfluidizer (2,500psi). Vitamin D dissolved in medium chain triacylglycerides (Continental Custom Ingredients; 40,000 IU/mL) was used as a control. Bench-top cheese was made in triplicate with pasteurized (72 °C 15 s) whole (non-homogenized) milk. For each sample, 200 µl of vitamin D emulsion or 125 µl pre-emulsified vitamin D (control) was added to 200 ml of milk (250 IU per gram of curd). Each fortified milk solution was prepared in 250 ml polycarbonate Nalgene centrifuge bottles. Glucono-delta-lactone (2% w/v) and double strength rennet diluted to 1.8 rennet units per ml with cold water (0.2% v/v) were added to each. Mixtures were incubated 30 min at 35°C for coagulum formation, then coagulum was cut and centrifuged at 1000 x g at 25 °C for 30 min. The curd and whey were measured and 100 g of whey and 5 g of curd were retained and frozen for vitamin D analysis (O'Neil Laboratories). Results from this research show that the retention of vitamin D in the curd was 98.4 ± 0.2% for the samples formulated with non-fat dairy powder, 97.1 ± 0.5% for samples with calcium caseinate, 98.1 ± 0.1% for samples with sodium caseinate and 97.1 ± 0.2% for samples with whey protein. These values were significantly higher than the ones obtained for the control delivery matrix (93.3 ± 0.7%). These results suggest that using an oil-in-water emulsion is a better delivery matrix than using the vitamin D dissolved in medium chain triacylglycerides.

Key Words: vitamin D, cheese, retention

72 Low fat Mozzarella cheese with improved baking and melting properties. R. Wadhwani* and D. J. McMahon, *Utah State University*, *Logan*.

Low fat cheeses dehydrate too quickly when baked using a forced air convection that prevents proper melting on a pizza. Low fat Mozzarella cheeses were made using direct acidification with citric acid to pH 5.9, containing 1.5, 3.0, 4.5, or 6.0% fat. The cheese curd was salted and placed in a bowl chopper and 4.5, 3.0, 1.5, or 0.0% respectively, of melted butter was added along with glucono-δlactone. The curd mixture was comminuted, hooped, and pressed into a block using 100 kPa pressure. Cheese was analyzed for melting, texture, free oil, and performance when baked on a pizza at 250°C for 6 min in an Impinger oven after 15, 30, 60, and 120 days of storage at 5°C. All cheeses contained 5.5-6.0% fat and 52-53% moisture. Compared to the control cheese in which all 6% fat was from the curd, the treated cheese had higher stretchability tested using a fork test after baking the pizza. Melting also increased which was particularly evident after baking on a pizza. The control cheese remained in the form of shreds and did not melt, while the cheeses made with added butter melted and flowed together. The cheeses with added butter had higher free oil and less hardness, gumminess, and chewiness than the control. Low fat Mozzarella cheese with free oil availability during baking is very important for retaining moisture. This study helped in availing free oil in cheese using melted butter.

Key Words: Mozzarella, melting, baking

73 Effects of starch addition on a low-fat cheese model system. K. M. Larsen*[1,2], D. J. McMahon[1,2], and W. R. McManus[1,2], [1]*Western Dairy Center*, *Logan, UT*, [2]*Utah State University*, *Logan*.

Addition of starch to low-fat cheese has the potential to create discontinuities in the protein matrix and alleviate formation of a rubbery texture. The purpose of this research was to 1) determine if various starches are retained with casein when a rennet curd is formed, and to quantify the percentage of starch lost into the whey during syneresis, and 2) determine starch impact on microstructure of rennet-induced milk gels using laser scanning confocal microscopy (LSCM). Model low-fat cheeses were made by renneting and acidifying skim milk containing one of five starches at 0.5% (wt./vol). Starches used included modified waxy cornstarch (WC), waxy rice starch (RS), instant tapioca starch (IT), dextrin (D), and modified food starch (MF). Milks were heated prior to renneting to gelatinize starch. After curd formation, curd was cut and centrifuged at 25°C at 500 x g then 1,000 x g for 15 min each. Starch content in whey was determined by alcohol precipitation. Curd samples for LSCM imaging were fixed in osmium tetroxide. Acriflavin HCl at 2% in water was used to stain carbohydrate, and 0.01% Rhodamine B in water was used for protein. Curd yields were 18.9, 21.7, 14.0, 25.1, 21.2, and 13.4% for WC, RS, D, MF, IT and control cheeses, respectively. Apparent starch losses in whey were 0, 0.7, 15.4, 18.6, and 0%, from centrifuged curd containing WC, RS, D, MF, and IT, respectively. The proportion of carbohydrate observed in the curd structure with LCSM was related to the level of starch retention. There were differences in the distribution of starch between the protein matrix and the serum pockets depending on the starch used. The MF apparently interacts most strongly with the matrix, D interacts less, and WC, RS and IT are intermediate.

Key Words: starch, cheese, low-fat

Graduate Student Paper Competition: National ADSA Production Division Oral MS Competition

74 Effects of conjugated linoleic acid isomers on mammary gland development in BALB/cJ mice. J. M. Gloviczki*[1], J. Kraft[2], A. L. Lock[2], J. F. Trott[1], and R. C. Hovey[1], [1]*University of California*, *Davis*, [2]*University of Vermont*, *Burlington*.

Conjugated linoleic acids (CLA) are isomers of octadecadienoic acid that are present in ruminant-derived products. *Cis*-9, *trans*-11 (9,11) CLA is the most abundant, while the less abundant *trans*-10, *cis*-12 (10,12) isomer is a major constituent of mixed isomer supplements. Both isomers suppress mammary gland (MG) tumorigenesis *in vitro* and *in vivo*, whereas 10,12 CLA has been implicated as affecting the incidence of obesity and insulin resistance. Intriguingly, recent data indicate that a diet supplemented with 10,12 CLA stimulates MG tumorigenesis in transgenic mice overexpressing the ErbB2 oncogene. These conflicting data emphasize the need to further investigate MG development

in response to the intake of specific CLA isomers. The objective of these studies was to determine the effects of these CLA isomers on MG development in female BALB/cJ mice and the responsiveness of their MG to ovarian hormone stimulation. In the first study, mice were weaned onto a control diet (ctrl) (AIN-93G) or the same diet with 1% of the fat from soybean oil replaced with either 9,11 CLA or 10,12 CLA. The MG were collected at 35d and 55d of age for tissue analysis. At 35d mice fed 10,12 CLA had increased ductal elongation in the MG ($P<0.05$) in comparison to mice fed ctrl. The females fed 10,12 CLA had an increase in supramammary lymph node size at 55d ($P<0.01$). At both ages the MG from females fed 10,12 CLA weighed less than those fed ctrl ($P<0.01$). MG weight from females fed 9,11 CLA was reduced at 55d ($P<0.05$). In the second study, pubertal mice were fed either the control diet or one of the treatment diets for 28d. Mice were then ovariectomized, and 7d later commenced receiving 17β-estradiol (1ug) and/or progesterone (1mg), or vehicle for 4d. The MG from females fed 9,11 CLA or 10,12 CLA weighed less than those from females fed ctrl ($P<0.05$ and $P<0.001$). Mice fed 10,12 CLA also had larger ($P<0.001$) supramammary lymph nodes ($P<0.001$). There was no interactive response between hormone treatment and diet on MG weight($P>0.05$). Taken together, these data support previous suggestions that 10,12 CLA stimulates ductal elongation in the developing mouse MG, potentially via its effects on the adipose stroma.

75 The effects of TGF-β1 on mammary stroma during the dry period of dairy cows. L. De Vries*, J. Liesman, K. Weiss, H. Dover, T. Casey, M. VandeHaar, and K. Plaut, *Michigan State University*, *East Lansing*.

During the dry period in dairy cows, the mammary gland undergoes remodeling in preparation for the next lactation. This remodeling occurs in part through the coordination and activation of various cell types, and the production of structural proteins, growth factors and proteases. Transforming growth factor-β1 (TGF-β1) can activate human mammary fibroblasts, and TGF-β mRNAs are present in the bovine mammary gland. Therefore, we hypothesized that TGF-β1 plays a role in bovine mammary remodeling during involution and mammogenesis. Our objective was to determine if TGF-β1 affects the expression of stromelysin-1 and fibronectin, and the activation of stromal cells during the dry period. Tissue was biopsied from 7 multigravid Holstein cows at 4 time points: late lactation, 7 d after dry-off, 21 d before expected calving and 7 d before expected calving. Explants of biopsied tissue were incubated for 2 h in Waymouth's media containing insulin, hydrocortisone, and 0 or 5 ng/ml TGF-β1. Immunohistochemistries of 5-μm sections of formalin-fixed, paraffin-embedded explant tissue were analyzed with Image Pro Plus software, and a mixed model analysis was performed with Statistical Analysis Software. TGF-β1 increased the total number of stromal cells expressing smooth muscle α-actin (SMA, a marker of stromal cell activation) from 640 to 760 cells per mm^2 *($P=0.03$)* across all 4 mammary biopsies. TGF-β1 also tended to increase the percent of stromal cells expressing SMA from 22% to 26% *($P=0.06$)*. TGF-β1 had no effect on the percent of the intralobular stroma area expressing fibronectin or stromelysin-1. The effect of TGF-β1 was similar for all biopsies for all variables measured (no significant treatment × biopsy interaction). These findings support our hypothesis that TGF-β1 plays a role in mammary remodeling of dry cows through the activation of stromal cells. *This project was supported by National Research Initiative Competitive Grant no. 2006-35206-16719 from the USDA Cooperative State Research, Education, and Extension Service.*

Key Words: transforming growth factor β1, remodeling, dry period

76 Comparison of real-time PCR and culture for detection and speciation of *Mycoplasma* species in bulk tank milk samples. A. Justice-Allen*[1], G. Goodell[2], J. Trujillo[1], and D. Wilson[1], [1]*Utah State University*, *Logan*, [2]*Dairy Authority*, *Greeley, CO*.

Intra-mammary infections caused by mycoplasma species have been recognized for more than fifty years. In addition to causing mastitis, these organisms also cause pneumonia, joint infections, and otitis. The impact of a herd level outbreak of disease can be a marked decrease in milk production, and result in the culling of a significant percentage of animals. The standard detection method for mycoplasma mastitis at the herd level has been repeated bulk-tank sampling and culture using specialized media. The goal of our research was to develop a real-time PCR method which could detect and differentiate mycoplasma species in bulk tank milk at rates comparable to standard culture techniques. 180 previously cultured bulk tank samples were extracted with a Qiagen DNEasy DNA isolation kit. The PCR reaction was carried out using a PerfeCTa Sybr master mix and two primers keyed to the 16S-23S ribosome RNA spacer region of *M. bovis*. This protocol had been previously tested with several mycoplasma type species from American Type Culture Collection (ATCC). The results from the bulk tank samples were compared to these standards. For 70% of the samples, the culture and PCR methods of detection agreed. PCR detected mycoplasma organisms in 24 (13%) samples that were culture negative. In 20 samples, the mycoplasma species identified was not *M. bovis*. Twelve samples had *M. bovis* and a second species. Four dairies had two different species of mycoplasma in bulk tank milk. Three dairies had only non-*M. bovis* mycoplasma. PCR did not detect *M. bovis* in 10 (5.5%) culture positive samples. Samples that were not in agreement or that identified a species of mycoplasma other than *M. bovis* were confirmed by sequencing the amplicon. The failure of the PCR to detect organisms in 10 samples is under investigation. This could be attributed to the loss of DNA during storage and shipment or the presence of inhibiting substances in the milk. Since the impact of mycoplasma mastitis varies with the species involved, a diagnostic method which can rapidly and accurately identify the species will greatly assist in the management of the disease.

Key Words: mycoplasma, mastitis, real-time PCR

77 Intermediates of linoleic acid biohydrogenation in ruminal batch cultures dosed with uniformly ^{13}C labeled linoleic acid. C. M. Klein* and T. C. Jenkins, *Clemson University*, *Clemson, SC*.

Most depictions of linoleic acid biohydrogenation do not explain all of the fatty acid isomers found in the rumen. Linoleic acid is generally accepted to be converted to *cis*-9 *trans*-11 conjugated linoleic acid (CLA), *trans*-C18:1 and then stearic acid. In this study ^{13}13C labeled linoleic acid was used as a metabolic tracer to determine other intermediates of linoleic acid biohydrogenation. Ruminal microorganisms were maintained in 10 ml batch cultures for 3, 6, 12 and 24 h. A 0 h sample was taken by acidifying media prior to addition of mixed ruminal microorganisms. Duplicate cultures were methylated and then separated on a 100-m CP-Sil 88 column and abundances of the quasimolecular (M) and M+18 ions were determined by mass spectroscopy in electron impact ionization mode. Enrichment was calculated as [M+18/M]*100 in labeled minus unlabeled cultures and tested for their difference from zero by t-test ($P<0.05$). Enrichments for linoleic acid were not different for 0 through 12 h (12.5, 13.6, 14.0, 14.2%), with an increase ($P<0.05$) to 17.9% at 24 h. By 24 h incubation significant tracer was found in 5 isomers of C18:2, 4 isomers of C18:1 and stearic acid ($P<0.01$). No enrichment of stearic acid was seen from 0 to 6 h, which then increased ($P<0.05$) to 0.43 and 3.05% at 12 and 24 h respectively, *trans*-9 C18:1

was not enriched before 6h and increased (P<0.05) to 6.4 and 9.4% from 12 to 24 h. There was no linolenic acid enrichment at any time point. The appearance of tracer in 4 isomers of C18:2 indicate that linoleic acid is acted on by isomerase enzymes and converted into a multitude of CLA isomers. *Cis*-9 *trans*-11 CLA enrichment increased from 0 at 0 and 3h to 5.4, 8.4 and 7.9% at 6, 12 and 24 h. *Trans*-10 *cis*-12 CLA enrichment averaged 0.0, 3.6, 5.3, 7.5 and 7.4% at 0, 3, 6, 12 and 24 h, respectively. Two currently unidentified C18:2 isomers in the CLA range were also found to be labeled (P<0.01). These results indicate that linoleic acid is converted into several CLA and *trans* monoene isomers prior to complete saturation to stearic acid.

Key Words: linoleic acid, biohydrogenation, conjugated linoleic acid

78 Effect of an exogenous fibrolytic enzyme or ammonia on fiber concentration, feed intake, digestibility, and ruminal pH of steers fed bermudagrass hay harvested at two maturity stages. J. J. Romero*, A. T. Adesogan, M. A. Zarate, O. C. M. Queiroz, J. Han, K. G. Arriola, C. M. Huisden, C. R. Staples, and M. Garcia, *University of Florida*, *Gainesville*.

The objectives were to compare the effect of treatment without (control) or with either a fibrolytic enzyme (ENZ) (Biocellulase A) or anhydrous ammonia (NH3; 4% DM) on the quality of bermudagrass (Cynodon dactylon) hay harvested as 5- and 10-wk regrowths and the performance of steers. Six ruminally cannulated Brangus steers (average BW 216.4 ± 5.9kg) were used in an experiment with a 6 × 6 Latin Square design with a 3 (treatments) × 2 (maturities) factorial arrangement. Each period consisted of 14 days of adaptation, followed by 7 days of digestibility (total collection) measurements and 1 day of ruminal pH measurement. Steers were housed in individual pens and fed 110% of their previous days hay consumption twice daily (1000 and 1600 h) with 2.66 kg DM/hd/d of a supplement consisting of sugar cane molasses, distillers grain and a mineral and vitamin supplement. Urea (0.03 kg/hd/d) was provided to steers fed Control and enzyme-treated hays to ensure diets were isonitrogenous. No treatment × maturity interactions existed (P>0.05) for performance measures except CP and NDF%. Ammoniation reduced NDF% (P=0.01) and increased CP% (P<0.001) to a lesser extent (P<0.01) at 5 wk (74.7 vs. 78.6 and 78.1; 17.8 vs 12.9 and 12.1) than at 10 wk (68.0 vs vs 74.4 and 71.1;16.3 vs 9.1 and 9.2). The ADF % of hays were lower at 10 vs 5 wk (39.0 vs 40.6). Hay (4.8 kg/d) and total (7.51 kg/d) DMI and ruminal pH (6.50) were unaffected (P>0.05) by treatment or maturity. Dry matter digestibility decreased with maturity (64.3 vs. 63.1; P<0.05) and was increased by NH3 (65.9 vs. 62.4%; P<0.001) but not ENZ (62.9%; P>0.09).

Key Words: enzyme, ammonia, beef cattle

79 Supplemental starch in postpartum dairy cow diets: 2. Effects on reproduction. B. L. Dyck*[1], M. G. Colazo[2], D. J. Ambrose[1,2], M. K. Dyck[1], and L. Doepel[1], [1]*University of Alberta*, *Edmonton, AB, Canada*, [2]*Alberta Agriculture and Rural Development*, *Edmonton, AB, Canada*.

Negative energy balance in early lactation cows has been associated with reduced reproductive performance. This study was conducted to determine if increasing the level of dietary starch would improve energy balance and positively impact reproduction. One of 3 diets was fed in a randomized block design to Holstein cows from calving until 70 DIM. All diets contained 45% barley-based concentrate and 10% alfalfa hay. In addition, the low (LSD, n=14) medium (MSD, n=13) and high starch diets (HSD, n=13) contained 45% barley silage, 45% alfalfa silage, and 41% barley silage + 4% corn starch, respectively. Resulting starch levels were 23.5%, 25.3% and 26.9% for LSD, MSD, and HSD, respectively. Transrectal ultrasonography was performed biweekly from 7 d after calving until 1st ovulation or 62 DIM, and a complete estrous cycle was monitored for ovarian dynamics in 8 LSD, 7 MSD and 9 HSD cows. Blood samples were collected every 2nd d during the cycle for determination of progesterone and estradiol concentrations; LH pulsatility was determined (5 cows/trt) approximately 15 d post calving. Cows fed HSD had a shorter interval from calving to 1st ovulation than cows fed MSD (30.6 vs. 43.2 d; *P*<0.01) and those fed LSD (38.1 d; *P*=0.07). The incidence of double 1st ovulation was higher (*P*=0.03) in cows fed HSD (39%) and MSD (27%) compared to those fed LSD (0%). There were no treatment effects on LH pulse frequency or amplitude, ovarian dynamics, or progesterone and estradiol concentrations during the observed estrous cycle. Energy balance did not differ between the 3 treatments and averaged -2.6±1.4 Mcal/d. Number of cows confirmed pregnant 30 d after 1st AI did not differ between treatments and averaged 36%. Increasing dietary starch decreased the interval from calving to 1st ovulation without improving energy balance but had no impact on pregnancy to 1st AI.

Key Words: starch, energy balance, ovulation

80 Accuracy of an on-farm blood test for pregnancy in dairy and beef cattle. J. C. Green*[1], D. H. Volkmann[1], S. E. Poock[1], M. F. McGrath[2], M. Ehrhardt[2], A. E. Moseley[2], and M. C. Lucy[1], [1]*University of Missouri*, *Columbia*, [2]*Monsanto Co.*, *St. Louis, MO*.

The ruminant placenta produces pregnancy-associated glycoproteins (PAG) that can be detected in the blood. The objective was to determine the accuracy of an on-farm PAG test for the purpose of pregnancy detection. Blood was sampled from dairy cattle (539 Holstein cows, 173 Holstein heifers, 73 Guernsey cows, 22 Guernsey heifers, and 12 Jersey heifers) and crossbred beef cattle (145 cows and 46 heifers) that were greater than or equal to 25 d after insemination (range = 25 to 45 d for dairy and 29 to 56 d for beef). Cattle were ultrasounded for detection of pregnancy within 2 d of blood collection. Blood was collected in EDTA-coated tubes and assayed either as whole blood or plasma. Whole blood or plasma was incubated in a polystyrene tube coated with a monoclonal PAG antibody for 15 min. The tubes were then washed with a phosphate-buffered saline-tween (PBST) solution and subjected to sequential incubations with a biotinylated polyclonal PAG antibody (15 min, followed by PBST wash), a horseradish peroxidase-streptavidin solution (15 min, followed by PBST wash) and a peroxidase substrate. Tubes were visually assessed for color (i.e., PAG/pregnancy status) after 15 min (clear solution = PAG negative, not pregnant; blue solution = PAG positive, pregnant). Total assay time was approximately 90 min. The ultrasound exam was used as the gold standard for pregnancy diagnosis. The sensitivity (99.8 ± 0.2%), specificity (91.7 ± 1.4%), and negative predictive value (99.7 ± 0.3%) for the PAG test used in dairy cattle were similar for different breeds and for cows and heifers (P>0.10). The positive predictive value for the test was greater in dairy heifers when compared with dairy cows (96.5 ± 1.4% versus 90.5 ± 1.7%, respectively; P<0.05). In beef cattle, the sensitivity (100%), specificity (92.3 ± 3.0%), positive predictive value (95.0 ± 2.0%), and negative predictive value (100%) for the PAG test were similar for cows and heifers (P>0.10). The accuracy of the test was similar for dairy and beef cattle (P>0.10). The conclusion was that the on-farm pregnancy test

based on PAG is highly sensitive and specific for pregnancy detection in dairy and beef cattle.

Key Words: pregnancy test, PAG, cattle

81 Financial analysis of direct comparison of natural service sires and timed artificial insemination in a dairy herd. F. Lima*, A. deVries, and C. Risco, *University of Florida*, *Gainesville*.

Objective was to compare the cost and profitability of natural service (NS) vs. timed artificial insemination (TAI) as the sole reproductive program on a commercial dairy farm in random allocated field study. Both programs were directly compared in 2007 and managed by the researchers. The NS group had a bull/cow ratio of 1/28 including open and pregnant cows. Bulls rested for 2 wk after 2 wks of exposure with cows. Both groups were presynchronized with 2 injections of PGF 14 days a part and the TAI group received Ovsynch and Ovsynch+CIDR for resynchronization of non-pregnant cows with 35 d intervals between AI up to 5 services. Pregnancy rate (PR) for NS (70 to 230 d) was 25.1% and for TAI (80 to 230 d) was 24.2%. Direct net cost of the NS program during the trial was $92.87/cow/yr. Direct net cost of the TAI program during the trial was $52.76/cow/yr. Costs per eligible day were estimated at $1.32 for the NS program and $0.76 for the TAI program. A herd budget was developed to account for the effects of differences in voluntary waiting periods and pregnancy rates on cost and profitability. The advantage of the TAI program after inclusion of these costs was $31.44/cow/yr (default). The advantage of the TAI program was $16.72 if genetic progress was not considered. A decrease in milk price from $20 to $14/cwt reduced the advantage of the TAI program to $26.44/cow/yr. Considering opportunity cost, if each bull was replaced by an additional cow, the advantage of the TAI program was $98.95/slot/yr. When milk price was reduced from $20 to $14/cwt, the advantage of the TAI program was $58.30/slot/yr. Changing PR for each program for 16.5% resulted in an advantage of $50.52/cow/yr for the TAI program. Considering opportunity cost, the advantage rose to $138.68/slot/yr, mainly because more bulls were needed. In conclusion, PR of the NS and TAI programs were similar and if well managed could both be much better than typical on US dairy farms. The TAI program was cheaper and its advantage increased substantially when opportunity costs of additional cows were considered.

Key Words: economics, bulls, estrus synchronization

82 Fecal and urinary estrogens in dairy heifers during the estrous cycle. H. A. Tucker*[1], K. F. Knowlton[1], and N. G. Love[2], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg*, [2]*University of Michigan*, *Ann Arbor*.

The US EPA has identified estrogens from animal feeding operations as a major environmental concern, but little data is available quantifying estrogen excretion by dairy cattle. The objectives of this study were to quantify estrogenic activity in feces and urine during the estrous cycle in dairy heifers, and evaluate relationships with plasma 17-β estradiol (E2). Seven Holstein heifers were fed a common diet for the 28 d study. Plasma was obtained via jugular venipuncture and grab samples of feces and urine were collected daily. Plasma E2 was quantified with RIA and used to confirm day of estrous. Feces and urine samples from days -12, -6, -2, -1, 0, 1, 2, 6, 12 of the estrous cycle were analyzed with Yeast Estrogen Screen (YES) bioassay. The YES assay utilizes a recombinant yeast strain (*Saccharomyces cerevisiae*) containing the human estrogen receptor gene and a chromogenic reporter system to indicate total estrogenic activity. Proc Mixed (SAS) was used to evaluate the effect of day on concentration of estrogens. The effect of day was significant, and patterns of estrogenic activity in feces and urine mirrored plasma E2 concentrations during the estrous cycle. Feces and urine estrogenic activity peaked a day after plasma E2 peaked. Identifying sources of variation in estrogen excretion by livestock will aid in the development of practices to reduce environmental estrogen accumulation.

Table 1. Average concentrations of plasma 17-β estradiol and fecal and urinary estrogenic activity by day of estrous

	Day of Estrous								
	-12	-6	-2	-1	0	1	2	6	12
Plasma[1], pg/mL	1.66	0.88	0.92	1.57	22.86	2.48	1.54	1.12	0.89
Feces[2], µg/mL	11.74	18.94	53.61	73.31	87.00	106.79	82.21	20.10	15.85
Urine[3], µg/mL	10.87	20.42	36.00	79.09	90.18	112.58	89.65	14.27	9.47

[1] SEM=2.39 [2]SEM=12.15 [3]SEM= 13.67

Key Words: estrogen, estrous, feces

83 Low progesterone concentration during the development of the first follicular wave impairs fertility of lactating dairy cows. A. C. Denicol*[1], G. Lopes Jr.[1], L. G. D. Mendonça[1], F. A. Rivera[1], F. Guagnini[1], R. V. Perez[1], J. R. Lima[1], R. G. S. Bruno[1], J. E. P. Santos[2], and R. C. Chebel[1], [1]*University of California*, *Tulare*, [2]*University of Florida*, *Gainesville*.

Objectives were to determine the influence of progesterone (P4) concentration on fertility of lactating dairy cows induced to ovulate follicles of the first follicular wave. Lactating dairy cows (n = 988) received 2 injections of PGF2α 14 d apart (Presynch). Cows were then randomly assigned to first follicular wave (FW), first follicular wave with CIDR (FWC), or second follicular wave (SW) treatments. FW and FWC cows started the timed AI (TAI) protocol 3 d after the last PGF2α of the Presynch protocol, whereas SW cows received GnRH 3 d after the last PGF2α of the Presynch and started the TAI protocol 7 d later. The TAI protocol consisted of GnRH on d 0, PGF2α on d 7, and GnRH + TAI on d 10. FWC cows received 2 CIDR inserts from d 2 to 7. A subgroup of cows (n = 719) had their ovaries scanned by ultrasound on d 0, 7, and 17. Pregnancy was diagnosed at 38 d after AI. Although similar (P = 0.49) proportion of cows ovulated in response to the first GnRH of the TAI protocol (66.9%), FW cows had larger (P < 0.06) follicles on d 7 than FWC and SW cows (FW = 17.8 ± 0.4, FWC = 16.8 ± 0.4, SW = 16.0 ± 0.4 mm). Pregnancy per AI (P/AI) at 38 d tended (P = 0.06) to be and was smaller (P = 0.02) for FW (28.7%) cows than FWC (35.9%) and SW (37.2%) cows, respectively. Among cows scanned by ultrasound, the interaction between treatment and ovulation in response to the first GnRH injection tended (P = 0.10) to affect P/AI, because among FW (21.1 vs. 34.9%) and SW (26.0 vs. 40.2%) cows those that did not ovulate had smaller P/AI, but among FWC cows ovulation to the first GnRH did not affect P/AI (35.4 vs. 34.5%). FW cows had more (P = 0.04) corpora lutea 7 d after AI than FWC and SW cows (FW = 1.26 ± 0.04, FWC = 1.13 ± 0.04, SW = 1.12 ± 0.04) and were more likely (P<0.01) to experience double ovulation (FW = 32.2, FWC = 18.7, SW = 20.6%). Exposure to low concentrations of P4 during follicle development resulted in larger follicles before ovulation, increased multiple ovulations, and reduced fertility in lactating dairy cows induced to ovulate follicles of the first follicular wave.

Key Words: progesterone, first follicular wave, lactating cow