

cows milk were characterized by growth on selective media, Gram stain, coagulase gene polymorphism, thermonuclease gene fragment amplification and activity on toluidine blue-o agar. SE production was evaluated using PCR and immunoassay. Growth and SE production in UHT milk were quantified at temperatures ranging from 21 to 45°C over 72 h. Two strains did not exhibit the expected coagulase gene polymorphism profile and were discarded from the study. Two of 15 strains were positive for toxin production. One expressed gene fragments for SEC and TSST-1 and one for SEA and SEC. SEC and SEA production by these two strains was confirmed by immunoassay. Two control strains and two SE-producing isolates were evaluated for growth and SE production. Strains grew at temperatures ranging from 21 to 45°C with an optimum at 40°C. The highest concentration of SE was produced at 31°C after 72 h incubation and the shortest time to SE production was observed at 40°C. Enterotoxin production data was used to estimate the amount of SE that might be produced in PHE as a function of processing time, temperature, eddy size and batch size. Preliminary calculations revealed production of SE during extended runs would not lead to human illness.

**Key Words:** Staphylococcus aureus, Staphylococcal enterotoxin, Extended run

**72 Development of a novel immunoassay system for immunobiotics that modulate intestinal immunity through Toll-like receptor 2.** M. Tohno\*, T. Shimosato, Y. Kawai, T. Saito, and H. Kitazawa, *Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.*

Studies on the biological functions of immunobiotic lactic acid bacteria (LAB) have contributed to their worldwide application as functional

foods and supplements. The beneficial effects of activating intestinal immunity with LAB are very important, but the cellular and molecular mechanisms by which immunobiotics regulate intestinal immune homeostasis have not been elucidated. Recently, Toll-like receptor (TLR) 2 was identified as a specific receptor for bacterial cell wall components, although some of the biochemical and immunological mechanisms by which TLR2 recognize and respond to immunobiotic bacteria remain unclear. In the present study, we investigated the role of TLR2 in the modulation of intestinal immunity by immunobiotic LAB. First, we isolated a cDNA encoding TLR2 from swine Peyer's patches, which are considered to be a good model of the human intestinal immune system. The complete open reading frame of swine TLR2 contained 2358 bp, corresponding to a 785-amino acid polypeptide with a calculated molecular mass of 89.6 kDa. We then transfected mammalian cells with the swine TLR2 cDNA to develop an immunoassay for immunobiotic LAB. The swine TLR2-expressing transfectant was able to recognize not only yeast cell wall zymosan but also intact LAB, which resulted in the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). Furthermore, high levels of TLR2 were detected in the follicle-associated epithelium of swine gut-associated lymphoid tissues, including membranous (M) cells and antigen-presenting cells such as dendritic cells. These findings indicate that TLR2-expressing cells in the gut-associated lymphoid tissues allow the host defense to respond to a variety of immunobiotic LAB. This finding may help clarify how LAB modulate intestinal immunity through TLR2, information that can aid in the development of immunobiotic foods.

**Key Words:** Immunobiotics, Lactic acid bacteria, Toll-like receptor 2

## Graduate Student Paper Competition: National ADSA Production Division

**73 Evaluation of feeding dried distillers grains plus solubles (DDGS) with corn silage or alfalfa hay as the primary forage source.** D. H. Kleinschmit\*, D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur, *South Dakota State University, Brookings.*

Nine multiparous ( $250 \pm 6$  DIM) and three primiparous ( $204 \pm 6$  DIM) Holstein cows were utilized in a  $3 \times 3$  Latin-square design to evaluate the lactation performance of dairy cows fed a diet containing DDGS with either corn silage or alfalfa hay as forage. All cows were fed a total mixed diet containing corn silage (CS), 50% corn silage and 50% alfalfa hay (CSAH), or alfalfa hay (AH) as the forage source. All diets had a 50:50 forage to concentrate ratio and contained 15% DDGS in the concentrate mix. Diets were formulated to provide similar amounts of metabolizable protein but concentrations of CP increased when alfalfa was added. Dry matter intake (22.5, 23.5, and 20.5 kg/d for CS, CSAH, and AH, respectively) had a quadratic relationship ( $P < 0.01$ ) with the addition of alfalfa. Yields of milk, 4% FCM, and energy-corrected milk (29.0, 30.7, and 31.0 kg/d) were similar for all diets. Feed efficiency (1.33, 1.39, and 1.54 kg ECM/kg DM intake) improved linearly ( $P < 0.01$ ) with increased concentrations of alfalfa in the diet. Milk fat concentration (3.86, 3.72, and 3.58%) decreased linearly ( $P < 0.01$ ) with addition of alfalfa, but this result was more drastic in primiparous cows than in multiparous cows. Differences in milk fat yield were not observed among diets. Milk protein concentration (3.32, 3.29, and 3.29%) and yield (0.90, 0.96, and 0.98 kg/d) were not affected by diet. Increasing the alfalfa content in the diets increased ( $P < 0.01$ ) the concentration of milk urea nitrogen

linearly due to greater concentrations of dietary CP. Ruminal molar proportions of acetate (63.4%), propionate (21.4%), and butyrate (10.1%) were similar across diets. Concentrations of ruminal ammonia were also similar (5.75 mg/dL). In conclusion, with the exception of a depression in milk fat content, replacing corn silage with alfalfa hay in diets containing 15% DDGS did not affect yields of milk and milk components, milk composition, and ruminal VFA and ammonia. The addition of alfalfa decreased DMI while maintaining milk production thus improving feed efficiency.

**Key Words:** Dried distillers grains plus solubles, Dairy cattle, Forage source

**74 The effect of supplemental dietary forage on the concentration of phosphorus and nitrogen in feces of lactating cows.** E. M. O'Rourke\*<sup>1</sup>, J. J. Michal<sup>1</sup>, R. L. Kincaid<sup>1</sup>, J. H. Harrison<sup>2</sup>, and C. T. Gaskins<sup>1</sup>, <sup>1</sup>Washington State University, Pullman, <sup>2</sup>Washington State University, Puyallup.

Sixteen multiparous Holstein cows were assigned to a study to determine if added dietary forage affected diurnal variation in the concentration of phosphorus (P) and nitrogen (N) in feces. At the start of the experiment, the cows averaged 262 DIM, 757 kg BW, and 37 kg daily milk yield. Dietary treatments were a control TMR consisting of 25% alfalfa haylage, 23% alfalfa hay, 10.3% whole cottonseeds, 7.3% wheat millrun, and 34.4% concentrate, and a treatment diet where cows were fed 2.27 kg alfalfa hay as a top-dress supplement to the

control ration. The TMR contained 19% CP, 36 % NDF, 27% ADF, and 0.42 % P. The treatment diet contained 19.2 % CP, 36.3 % NDF, 27.3 % ADF, and 0.42 % P. All cows were fed the TMR at 1400 h daily and the alfalfa hay offered as a top-dress at 0700 h. Cows had ad libitum access to feed except between 0800 to 1000 h, and 1100 to 1400 h. On d 21, fecal grab samples (200 g) were collected approximately every 4 h for 48 h in such a manner as to represent every 2 h in a diurnal period. The dry matter intake (DMI) averaged 26 kg/d and was not affected by treatment. Similarly, average milk yield was 38 kg/d and not affected by treatment. The concentration of P in the feces (0.70 and 0.71% for control and supplemental cows, respectively) was not affected ( $P > 0.1$ ) by either supplemental forage or time of sampling. Although the concentration of N in the feces was affected ( $P < 0.05$ ) by both the supplemental forage and time of sampling, the ratio of P to N (mean = 0.285) was not significantly changed by either supplemental forage or time of sampling. In conclusion, for dairy cows fed a TMR containing 0.42 % P there was no diurnal change in the % P in feces nor did a forage top-dress affect the % P in feces. However, both time of sampling and the forage top-dress affected the % N in the fecal samples. Fecal grab sampling appears to be a reasonable method by which P excretion may be determined.

**Key Words:** Phosphorus, Feces, Nitrogen

**75 Suppressor of cytokine signaling-2 mRNA increases after calving in dairy cows and is associated with elevated estradiol-17 $\beta$  concentrations before calving.** L. A. Winkelman<sup>\*1</sup>, M. C. Lucy<sup>2</sup>, and C. K. Reynolds<sup>1</sup>, <sup>1</sup>The Ohio State University, Columbus, <sup>2</sup>University of Missouri, Columbia.

Suppressors of cytokine signaling (SOCS) inhibit intracellular growth hormone (GH) signaling. After calving, the somatotrophic axis of the dairy cow is uncoupled, partially due to reduced liver specific GH receptor (GHR 1A) on the liver cell surface. Estradiol-17 $\beta$  (E2) concentrations increase at calving, and E2 upregulates SOCS-2 mRNA. The presence of SOCS in the liver of transition dairy cows has yet to be examined. We hypothesize SOCS-2 mRNA is upregulated after calving. Multiparous Holstein cows (n = 18) were dried off 45 d before expected calving and fed diets at ad libitum (AL) or restricted (R) DMI during the dry period. The R diet was formulated to meet nutrient requirements at a DMI of 9.4 kg/d, while AL cows consumed 13.7 kg DM/d. All cows were fed the same diet ad libitum for 4 wk after calving. Coccygeal or jugular vein blood samples were collected weekly and more frequently near calving. Liver biopsies obtained at -21, -7, +2, and +28 d relative to calving were assessed for SOCS-2 and GHR 1A mRNA by real time RT-qPCR and were normalized to controls. The relative amount of SOCS-2 mRNA increased after calving ( $P < 0.01$ ) and was greater on +2 d for R vs. AL cows (0.81 vs 0.45,  $P < 0.05$ ). Plasma E2 was greater in R vs. AL (304 vs. 215 pM,  $P < 0.05$ ) and increased before calving ( $P < 0.01$ ). Amount of GHR 1A mRNA did not differ between diets, but tended to decrease on +2 d ( $P < 0.20$ ). In addition to reduced GHR 1A, increased SOCS-2 mRNA after calving, perhaps due to increased E2, may further uncouple GH signaling in the liver of the transition dairy cow.

**Table 1. Relative amounts of SOCS-2 and GHR 1A mRNA**

Day relative to calving		-21	-7	+2	+28
Relative amount of SOCS-2 mRNA <sup>1</sup>					
	Restricted	0.24	0.36	0.81	0.47
	Ad Libitum	0.26	0.45	0.52	0.62
	SEM	0.045	0.074	0.070	0.095
Relative amount of GHR 1A mRNA <sup>2</sup>					
	Restricted	370.5	226.1	41.5	174.5
	Ad Libitum	292.0	289.7	103.7	309.8
	SEM	133.3	442.9	79.0	301.3

<sup>1</sup>Relative steady state concentration of mRNA, normalized to  $\beta$ -actin. <sup>2</sup>Values are expressed as the fold difference in arbitrary units (AU) relative to the amount in a medium control.

**Key Words:** SOCS-2, Liver, GHR 1A

**76 Effects of dietary allocation of barley grains differing in expected starch digestion on rumen fermentation and productivity of lactating dairy cows.** C. Silveira<sup>\*1</sup>, M. Oba<sup>1</sup>, W. Z. Yang<sup>2</sup>, and K. A. Beauchemin<sup>2</sup>, <sup>1</sup>University of Alberta, Edmonton, AB, Canada, <sup>2</sup>Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.

The objective was to evaluate effects of dietary allocation of barley grains differing in expected starch digestion on dry matter intake, rumen fermentation, and milk production of dairy cows. Four primiparous ruminally cannulated (123  $\pm$  69 d in milk; mean  $\pm$  SD) and 4 multiparous ruminally and duodenally cannulated (46  $\pm$  14 d in milk) cows were used in a 4  $\times$  4 Latin Square design with a 2  $\times$  2 factorial arrangement of treatments with 16 d per period. Treatments were two levels of dietary grain allocation (39.4 vs. 26.7% of dietary DM) and two steam-rolled barley grains differing in expected ruminal starch digestion (Xena vs. Dillon). Xena used in this study had higher starch concentration (58.7 vs. 50.0%) and greater in vitro 6-h starch digestibility (78.0 vs. 73.5%) compared with Dillon. All experimental diets were formulated for 17.5% CP and 20.0% forage NDF. Dry matter intake and milk yield were not affected by treatment. Milk fat concentration (3.55 vs. 3.29%;  $P < 0.01$ ) was greater for cows fed Dillon compared with Xena, but not affected by dietary grain allocation. Ruminal acetate concentration was lower, and propionate concentration was greater for cows fed Xena and high grain diets compared with cows fed Dillon and low grain diets, respectively. High grain diets tended to decrease mean ruminal pH (6.10 vs. 6.17;  $P < 0.10$ ) and to increase time below pH 5.8 (6.4 vs. 4.2 h/d;  $P < 0.06$ ), and increased the area below pH 5.8 (5715 vs. 3375 pH  $\times$  sec;  $P < 0.05$ ) compared with low grain diets. Cows fed Xena had longer time that ruminal pH was below 5.8 (6.6 vs. 4.0 h/d;  $P < 0.03$ ) and tended to have lower minimum pH (5.45 vs. 5.59;  $P < 0.06$ ) compared with cows fed Dillon. These results indicate that selection of barley grain can affect milk production and rumen fermentation to an extent at least as great as changes in dietary grain allocation.

**Key Words:** Barley grain, Starch digestibility, Rumen fermentation

**77 Characterization of cytokine gene expression in periparturient dairy cows naturally infected with *Mycobacterium avium* subsp. paratuberculosis.** E. L. Williams\* and J. R. Stabel, *USDA-ARS-National Animal Disease Center, Ames, IA.*

Johne's disease (JD), caused by *Mycobacterium avium* subsp. paratuberculosis (MAP), is estimated to infect more than 22% of US dairy herds. Periods of immunosuppression, typically seen at parturition, may contribute to the transition from subclinical, or asymptomatic, to clinical stage of infection. Understanding the effects of stressors disease may provide information that will help manage JD. The objective of this study was to characterize cytokine gene expression in periparturient dairy cows naturally infected with MAP. Twenty-two multiparous Holstein cows were placed into 3 groups consisting of 5 noninfected healthy cows, 12 subclinical cows, and 5 clinical cows. Blood was collected from the jugular vein 3 wks pre- and 4 wks post-calving. Peripheral blood mononuclear cells (PBMC) were isolated from the buffy coat fractions of blood and cultured for 24 h with and without concanavalin A. At 24 h, RNA was extracted from all cells and converted to first-strand cDNA. Real-time PCR was performed on each sample to evaluate the expression of the following genes: IFN- $\gamma$ , TNF- $\alpha$ , IL-12, IL-10, TGF- $\beta$ , IL-4 and  $\beta$ -actin gene. All reactions were performed in triplicate and RT-PCR data was analyzed using delta-Ct values and Proc Mix procedure of SAS. Across the periparturient period, expression of IFN- $\gamma$ , TNF- $\alpha$ , and TGF- $\beta$  mRNA did not differ between nil- and stimulated PBMCs isolated from infected and control cows. ConA-stimulated PBMCs showed greater IL-12 mRNA expression for both subclinical ( $P < 0.03$ ) and clinical ( $P < 0.04$ ) cows compared to controls. Expression of IL-4 mRNA from nil- ( $P < 0.03$ ) and stimulated ( $P < 0.01$ ) PBMCs was greater in infected compared to healthy control cows. Expression of IL-10 mRNA from nil- ( $P < 0.08$ ) and stimulated ( $P < 0.05$ ) PBMCs was greater for infected cows compared to control cows. The data indicate an ability of parturition to regulate IL-12 and IL-4 gene expression, but not IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ , or IL-10. Expression for the Th1 cytokine IL-12 and the Th2 cytokines IL-4 and IL-10 from infected cows were significantly elevated compared to healthy controls.

**Key Words:** Periparturient, Cytokines, Johne's disease

**78 Response in diurnal variation of circulating blood metabolites to nocturnal vs. diurnal provision of fresh feed in lactating cows.** A. Nikkiah\*, J. C. Plaizier, C. Furedi, and A. D. Kennedy, *University of Manitoba, Winnipeg, MB, Canada.*

Our objective was to evaluate the impact of providing fresh TMR at 9 am or at 9 pm on daily average and 24-h variation of blood metabolites in lactating cows. Four multiparous ( $645 \pm 75$  kg body weight, BW;  $90 \pm 33$  days in milk) and 4 primiparous ( $576 \pm 46$  kg BW;  $77 \pm 25$  days in milk) lactating Holsteins were used in a  $2 \times 2$  cross-over design with two 6-week periods. Each period consisted of a 4-week adaptation period followed by 2 sampling weeks. A TMR containing 50% concentrate on a dry mater basis was fed ad libitum allowing for between 5 and 10% orts. Blood was sampled via jugular catheter every 2 h for 24-h at week-5 of each period. Data were analyzed using Proc Mixed of SAS (v. 9.1) with appropriate covariance structure for repeated measures. Fresh feed delivery at 9 pm instead of 9 am did not affect daily averages of glucose, lactate, and urea in blood plasma (Table 1). All metabolites showed diurnal variation (H,  $P < 0.05$ ) which for glucose and urea was altered markedly by time of feeding ( $T \times H$ ,  $P < 0.0001$ , Table 1). Blood glucose in cows fed at 9 pm showed a dramatic postprandial decline lasting until 2 h after feeding, and a

remarkable postprandial rise shortly thereafter. Although less dramatic than in evening fed cows, blood glucose in morning fed cows started to drop at 4 h before feeding and rose gradually at 2 h after feeding. Both groups exhibited a postprandial increase in blood lactate, which was higher and more long-lasting in cows fed at 9 pm than in cows fed at 9 am. Blood urea increased shortly after feeding in morning fed cows but not in evening fed cows. After peak at 2 h post-feeding, blood urea declined progressively until 10 h post-feeding in all cows. Results showed that provision of fresh TMR at different times of a 24-h period alters the diurnal variation of blood metabolites and, thereby, may affect the peripheral nutrient utilization by lactating cows.

**Table 1. Effect of time of feeding (T), parity (Par), hour of sampling (H), and the interactions on blood metabolites**

Item	Time of feeding			Fixed effect, P					
	9 am	9 pm	SEM T	Par	T $\times$ Par	H	T $\times$ H	Par $\times$ H	
Glucose, mg/dL	75.7	74.9	1.1	0.43	0.60	0.33	0.04	<0.0001	0.01
Lactate, mM	0.67	0.72	0.03	0.23	0.97	0.99	<0.0001	0.19	0.64
Urea, mM	5.06	5.35	0.46	0.32	0.47	0.90	<0.0001	<0.0001	0.66

**Key Words:** Time of feeding, Diurnal variation, Blood metabolites

**79 Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems.**

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The Rumen Simulation Technique (RUSITEC) and a dual flow (DF) continuous culture system were used to investigate the effects of cinnamon leaf oil on rumen microbial fermentation. Incubations were conducted concurrently in the two systems, with the oil added at 0 (control, CON), and 500  $\mu$ g/mL (CIN). Eight RUSITEC (920 mL;  $n=4$ ) and six DF (1300 mL;  $n=3$ ) fermenters were randomly assigned to treatment. Inoculum was prepared from four ruminally cannulated, lactating Holstein cattle fed a 50:50 forage to concentrate TMR diet (16.7% CP, 34.4% NDF). Data were analyzed using the PROC MIXED procedure of SAS with a repeated measures analysis of covariance. The model tested main and interactive effects of system type (RUSITEC vs. DF), treatment (CON vs. CIN), and sampling day. In both systems, mean pH was similar ( $P > 0.05$ ) between treatments. Concentration of NH<sub>3</sub> was decreased ( $P < 0.01$ ) by CIN in the RUSITEC (15.4 vs. 20.7 mM) but not in the DF system (average 18.2 mM). In both systems total VFA ( $PP < 0.05$ ) and propionate concentrations ( $P < 0.01$ ) were reduced by CIN, but acetate, butyrate, and branched chain VFA concentrations were unaffected ( $P > 0.05$ ). Protozoa were less numerous ( $P < 0.01$ ) with CIN than in the controls (both systems). Headspace CH<sub>4</sub> concentration was unaffected ( $P > 0.05$ ) by treatment in the RUSITEC (average 1.95%), but was increased ( $P < 0.01$ ) by CIN in the DF system (3.0 vs. 5.6%). Digestibility of DM was similar between CIN and CON in DF (61.8%) but was decreased by CIN (68 vs. 82%;  $P < 0.01$ ) in the RUSITEC for both silage and concentrate feeds. This study suggests that CIN reduces fermentative activity in continuous cultures, possibly as result of its antiprotozoal activity.

**Key Words:** Essential oils, Rumen microbial fermentation, Continuous culture system

**80 Feed peas can successfully replace soybean meal and corn grain in dairy cow diets.** M. Vander Pol\* and A. N. Hristov, *University of Idaho, Moscow.*

The objective of this experiment was to investigate the effect of partial substitution of soybean meal and corn grain with feed peas in dairy cow diets on DM intake and milk yield and composition. Twenty-four lactating Holstein cows were blocked into 12 groups based on DIM and milk yield at the end of a 2-week covariate period (average DIM, 104±8.0 and milk yield, 39±1.2 kg/d). Cows within group were randomly assigned to two treatments: Control diet (% on DM basis): alfalfa hay, 28.0; corn silage, 17.7; whole cottonseed, 7.0; dry distillers grains, 6.0; rolled barley grain, 12.0; solvent-extracted soybean meal, 7.4; steam-rolled corn, 19.9; and mineral/vitamin supplement, 2.0) and Pea diet (alfalfa hay, 28.1; corn silage, 17.7; whole cottonseed, 7.0; dry distillers grains, 6.0; rolled barley grain, 11.8; solvent-extracted soybean meal, 1.6; steam-rolled corn grain, 10.8; feed peas, 15; and mineral/vitamin supplement, 2.0). Diets were isonitrogenous (18.4% CP) and isoenergetic (1.58 Mcal/kg NE<sub>L</sub>). The peas used in the trial (U.S. No. 1 feed peas), contained 25% CP and estimated 1.98 Mcal/kg NE<sub>L</sub>. The experiment continued for 70 days. Dry matter intake (25.9 and 26.3 kg/d; SE = 0.67; Control and Pea diet, respectively), milk yield (35.8 and 35.6 kg/d; SE = 0.75), 4% FCM yield (32.6 and 34.3 kg/d; SE = 1.37), milk fat (3.54 and 3.76%; SE = 0.124) and protein (3.00 and 2.99%; SE = 0.047) content and yields were not affected by diet ( $P = 0.148$  to  $0.851$ ). Concentration of milk urea nitrogen was also not affected ( $P = 0.239$ ) by treatment (14.4 and 15.0 mg/dl, SE = 0.35, respectively). Organoleptic characteristics of milk were evaluated by a consumer panel. Regardless of whether the treatment or the control sample was presented as the reference, panelists guessed right or wrong about 50% of the time ( $P = 0.712$ ). This experiment demonstrated that inclusion of 15% (DM basis) feed peas in dairy cow diets, replacing solvent-extracted soybean meal and corn grain had no effect on DM intake and milk yield, composition, and quality.

**Key Words:** Feed pea, Dairy cow, Milk production

**81 17 $\beta$ -estradiol concentration in raw and pasteurized/homogenized whole milk.** D. A. Pape-Zambito\*, R. F. Roberts, N. M. Kauffman, and R. S. Kensinger, *Pennsylvania State University, University Park.*

Many fear that estrogens in dairy products may lead to growth of estrogen sensitive cancers in humans. The presence of estradiol (E<sub>2</sub>) in raw whole cow's milk has been previously confirmed. The objective of this study was to determine if pasteurization/homogenization affects E<sub>2</sub> concentration in milk. One hundred kg of fresh raw milk was collected from the PSU dairy herd bulk tank on 4 consecutive days. On each day 1 liter of raw milk was sub-sampled and subjected to no further treatment (C). The remaining milk was pasteurized at 79.4C for 16-18 s and either homogenized at 6.89 MPa (1<sup>st</sup> stage) and 3.45 MPa (2<sup>nd</sup> stage) (A), or 17.23 MPa (1<sup>st</sup> stage) and 3.45 MPa (2<sup>nd</sup> stage) (B). Milk fat and solids were analyzed with a Smart Trac analyzer. Fat globule size was determined using a Horiba laser scattering particle size analyzer. E<sub>2</sub> was quantified by radioimmunoassay after ethyl

acetate extraction, triglyceride precipitation, and Sephadex LH-20 column chromatography. Tritiated E<sub>2</sub> was used as an internal standard to determine recovery. Statistical analyses were completed using SAS. Milk fat and solids averaged 3.56 ± 0.01 % and 12.44 ± 0.02 %, respectively, and were not affected by treatment. Pasteurization/homogenization reduced fat globule size with mean diameters (D<sub>3,2</sub>) of 4.58, 0.59, and 0.39  $\mu$ m for treatments C, A, and B, respectively. E<sub>2</sub> was correlated with milk fat percent ( $R = 0.58$ ,  $P < 0.05$ ). Concentrations of E<sub>2</sub> were not affected by treatment with means of 0.70, 0.58, and 0.64 pg/ml for C, A, and B, respectively ( $P > 0.3$ ). Based upon these findings, this method can be used to quantify E<sub>2</sub> in fluid milk products. Concentrations of E<sub>2</sub> in raw and pasteurized/homogenized whole milk are low relative to endogenous E<sub>2</sub> in humans, thus are unlikely to cause health problems.

**Key Words:** 17 $\beta$ -estradiol, Milk, Pasteurization/homogenization

**82 Effects of varying CLA doses on production and bioenergetic variables during the transition period.** L. J. Odens\*<sup>1</sup>, R. Burgos<sup>1</sup>, B. C. Pollard<sup>1</sup>, M. L. Innocenti<sup>1,2</sup>, S. H. Baker<sup>1</sup>, S. R. Sanders<sup>1</sup>, J. K. Kay<sup>1</sup>, M. L. Rhoads<sup>1</sup>, C. E. Moore<sup>1</sup>, M. J. VanBaale<sup>1</sup>, and L. H. Baumgard<sup>1</sup>, <sup>1</sup>*The University of Arizona, Tucson,* <sup>2</sup>*University of Milan, Milan, Italy.*

Supplementing a high dose (600 g/d) of rumen-inert CLA inhibits milk fat synthesis in dairy cows immediately postpartum. During negative EBAL it appears moderate CLA-induced milk fat depression (MFD) causes a positive response in milk yield (MY); however, as MFD becomes more severe (>35%), the MY response diminishes. Multiparous Holstein cows (n=31) were randomly assigned to 1 of 3 trts beginning ~10 d prior to expected calving and ceased at 40 DIM: 1) 578 g/d of a RI palm fatty acid (FA) distillate (control), 2) 600 g/d of CLA for the entire trial period (CLA-1), and 3) 600 g/d of CLA until 10 DIM followed by 200 g/d (CLA-2) for the remainder of the trial. Each dose provided equal amounts of FA by replacing and balancing trt with a RI palm FA distillate. Doses provided a total of 522 g of FA/d and either 0, 58 or 174 g of CLA (mixed isomers)/d. To improve palatability, doses were mixed with 600 g/d of dried molasses; one-half of the supplement was fed at 0800 h, and the remaining at 1900 h. Individual MY, DMI and BW were recorded daily and milk composition determined every other d. There was no overall CLA effect on either the content or yield of milk protein or lactose. Both CLA trts decreased overall milk fat content (26.0 & 18.3%) and yield (22.5 & 17.3%) with CLA-induced MFD becoming significant by d 8. The CLA-induced MFD increased in severity with progressing DIM until plateauing on d 18 for CLA-1 (43%) and d 20 for CLA-2 (24%); although neither milk fat t10, c12 CLA content (1.8 mg/g) nor its transfer efficiency (7.3%) changed over time. Trts had no effect on overall DMI or MY, but there was a trt x time interaction ( $P < 0.05$ ) for MY, as cows fed either CLA trt tended to have an increased MY at wk 3, 4 & 6. In addition, cows fed either CLA trt had a significant improvement in overall EBAL (-5.1 vs. -1.8 Mcal/d), a decrease in overall NEFA levels (12%) and an overall increase in glucose levels (11%).

**Key Words:** CLA, Transition, Milk fat