

**1272 Effect of *Lactococcus lactis* ssp. *lactis* ml3 and c2 bacteriophage peptides and *Lactobacillus plantarum* yit0068 bacteriophage peptides on the growth of *L. lactis* ssp. *lactis* C2 and the inhibition of ml3 and c2 bacteriophage proliferation.** C. Hicks\*<sup>1</sup>, I. Surjawan<sup>1</sup>, N. Jose<sup>1</sup>, C. Jose<sup>2</sup>, and B. Barlow<sup>1</sup>, <sup>1</sup>University of Kentucky, Lexington, Kentucky, <sup>2</sup>University of Riau, Pekanbaru, Indonesia.

Phage-peptides were prepared from *L. lactis* ssp. *lactis* ml3 and *L. plantarum* yit0068 bacteriophage using a ficin hydrolysis. The ml3 and yit0068 peptides were compared to *L. lactis* ssp. *lactis* c2 bacteriophage peptides to determine growth inhibition of C2 host and inhibition of both ml3 and c2 bacteriophage. M17 with .011 mM CaCl<sub>2</sub> media with and without phage peptides (2 or 2.5%) were heat treated, cooled to 22°C, inoculated with C2 host, and infected with ml3 or c2 bacteriophage at 0, 10<sup>2</sup>, 10<sup>4</sup>, 10<sup>6</sup>, or 10<sup>8</sup> pfu/mL. Culture growth and lysis were monitored spectrophotometrically ( $\lambda_{600nm}$ ) for 6.5 h. Growth of C2 culture in ml3 or yit0068 peptide media was dramatically slower and cell density was lower at the stationary phase than when the culture was grown in a medium without peptides. Growth time to the stationary phase was essentially the same for media with and without peptides added. C2 culture grown c2 peptide medium was not significantly inhibited. When C2 culture was grown in medium containing ml3 or yit0068 peptide there was little inhibition of ml3 bacteriophage proliferation. When the medium contained c2 peptide the ml3 bacteriophage proliferation could be partially inhibited. When C2 host was grown in media containing ml3 peptide and infected with c2 bacteriophage only a slight inhibition of bacteriophage proliferation occurred. However, c2 bacteriophage proliferation was inhibited when C2 host was grown in media containing yit0068 or c2 peptide. Culture growth (C2) was extended by 20 and 50 min before the culture started to lyse when infection levels were 1 x 10<sup>3</sup> pfu/mL and 1 x 10<sup>4</sup> pfu/mL, respectively. Lysis did not occur when C2 culture was grown in media containing c2 or yit0068 peptide and infected with 1 x 10<sup>2</sup> pfu/mL of c2 bacteriophage. These data suggest that the best growth medium and phage inhibitory medium was the medium that contained the c2 bacteriophage peptide.

**Key Words:** *Lactococcus lactis* ssp, bacteriophage inhibition, ml3, yit0068, c2.

**1273 Effect of black pepper essential oils and orange peel terpenes on the inhibition of *Lactobacillus plantarum* and the inhibition of *L. plantarum* yit0068 bacteriophage proliferation.** C. Jose<sup>1</sup>, N. Jose<sup>2</sup>, C. Hicks\*<sup>2</sup>, and I. Surjawan<sup>2</sup>, <sup>1</sup>University of Riau, Pekanbaru, Indonesia, <sup>2</sup>University of Kentucky, Lexington, Kentucky.

Effect of essential oils of black pepper and orange peel terpenes on the growth of *L. plantarum* and proliferation of yit0068 bacteriophage was determined collaboratively at University of Kentucky and University of Riau. Orange peel terpene and Indonesian extracted black pepper essential oils were used to determine the concentration that would allow the growth of *L. plantarum* but inhibit the proliferation of yit0068 bacteriophage. Bacteriophage inhibition tests were done by growing *L. plantarum* (4%) in MRS medium (25 ml) containing .011 mM CaCl<sub>2</sub> with and without terpenes (0, 5, and 25 ppm) at 35°C. One half of the media were infected with *L. plantarum* yit0068 bacteriophage (0.01%; 1 X 10<sup>8</sup> pfu/ml) after 1 h of incubation or after 0.1 increase in absorbance ( $\lambda_{600nm}$ ). Dilutions were made by dissolving the essential oil stock in 1,2 -propanediol. Cell growth and lysis was spectrophotometrically observed ( $\lambda_{600nm}$ ) after inoculation for up to 6 h at 10 min intervals. Other studies (in Indonesia) of essential oils of black pepper fractionated using n-hexane or methanol were conducted in a similar manner, except that the concentration of the oil fraction in the media were 1.0, 1.5, 2.0, and 2.5 %. Results from both laboratories suggested that essential oil of black pepper, its fractions (n-hexane and methanol extracts) and orange peel terpene were effective inhibitors of culture growth at the concentrations tested. Both laboratories confirmed that there was no concentration of black pepper essential oil nor orange peel terpene where *L. plantarum* grew that yit0068 bacteriophage was inhibited. These negative results do not agree with a previous study suggesting that black pepper essential oils are inhibitory to *L. plantarum* bacteriophage proliferation during vegetable fermentations.

**Key Words:** *Lactobacillus plantarum*, yit0068 bacteriophage, essential oils, black pepper, terpenes, inhibition

**1274 Estimation of vitamin D<sub>3</sub> content in process cheese.** P. Upreti\*<sup>1</sup>, V. V. Mistry<sup>1</sup>, and J. J. Warthesen<sup>2</sup>, <sup>1</sup>MN-SD Dairy Foods Research Center, South Dakota State University, Brookings, <sup>2</sup>University of Minnesota, St. Paul.

The goal of this project is to develop a procedure for the fortification of process cheese with vitamin D<sub>3</sub>. To monitor the vitamin D<sub>3</sub> content of this process cheese a high-performance liquid chromatographic (HPLC) method was developed and is presented here. 4 to 5 g of shredded cheese sample was saponified in a low-actinic Erlenmeyer flask using 20-mL KOH (60%) and 20-mL ethanol (95%) at 70°C for 30 min in the presence of 50 mL of 6% ethanolic pyrogallol as an antioxidant. The unsaponifiable matter from cheese was extracted using a mixture of petroleum ether (90 parts) and diethyl ether (10 parts). The ether layer was washed with ice-cold water until neutralization. It was then separated and partially dried in a rotary evaporator, and then completely dried under nitrogen. The dried extract was reconstituted in 3-mL hexane and applied to a 1-mL silica cartridge for solid phase extraction. Vitamin D<sub>3</sub> was eluted with 5-mL of a mixture of hexane (21.5 parts) and chloroform (78.5 parts). The eluent was evaporated to dryness under nitrogen and reconstituted in 2-mL acetonitrile. Of this 100  $\mu$ L was injected into the HPLC. The HPLC system included a C<sub>18</sub> liquid chromatographic column (4.6-mm ID x 15 cm, 5- $\mu$ m particle size) with UV detection at 254 nm and a flow rate of 1 mL/min. A mixture of acetonitrile and methanol in the ratio of 30 to 70 was the mobile phase. This ratio was selected on the basis of satisfactory resolution of vitamin D<sub>3</sub> and separation of the vitamin from interfering shouldering peaks. Vitamin D<sub>3</sub> eluted at approximately 9 min. A standard curve of vitamin D<sub>3</sub> was prepared by adding known quantities of crystalline vitamin D<sub>3</sub> to the cheese at six levels (0, 4.46, 8.93, 17.86, 26.79, 35.71 IU per 5 g of cheese) and quantitated using the above procedure. This was replicated five times. The standard curve had a r<sup>2</sup> of 0.9722. The method can be used to assess the process cheeses fortified with vitamin D<sub>3</sub>. Process cheeses fortified with Vitamin D<sub>3</sub> will be manufactured and the above procedure will be used for quantification.

**Key Words:** vitamin D, process cheese, HPLC

**1275 Cheese treated by high pressure in an early stage of ripening. Changes in textural attributes.** J. Saldo<sup>1</sup>, E. Sendra\*<sup>2</sup>, and B. Guamis<sup>1</sup>, <sup>1</sup>Planta de Tecnologia d'Aliments, UAB. CeRTA. XiT. Bellaterra, Spain, <sup>2</sup>Divisin de Tecnologa de Alimentos. Universidad Miguel Hernandez. Orihuela, Spain.

High pressure (400 MPa, 5 min) was applied to cheese the day after salting in brine to promote accelerated ripening. The high pressure treatment (HPT) caused changes in cheese texture. The extent of these changes and its evolution along ripening will be studied. Two batches of Garrotxa cheese were manufactured. This is a mixed curd, uncooked, pressed, goat milk cheese. Uniaxial compression and stress relaxation tests were performed on cylindrical cheese samples (2.54 cm diameter and 2 cm height) and replicated 6 times for each cheese piece. All parameters measured from uniaxial compression test were different between treatments (p<0.05). Stress at breaking point ( $\sigma_f$ ) and at maximum strain ( $\sigma_{0.7}$ ) decreased in treated samples, while strain at breaking point ( $\epsilon_f$ ) was higher. The adherence of samples to the probe ( $\sigma_{ad}$ ) was higher for treated cheeses. HPT cheese became softer and yielding, with an increased adherence. Stress relaxation was modelised using a linear model which parameters are k<sub>1</sub> and k<sub>2</sub>, according Peleg & Normand (1983). Moisture in HPT samples were higher than in control ones and proteolysis was increased too. This would help those changes in texture. Multivariate analysis discriminate between young and old cheese along first principal component. Dramatic changes were observed after day 30 of ripening. Second principal component discriminate between treatments.  $\sigma_f$ ,  $\epsilon_f$  and  $\sigma_{0.7}$  were significant for PC1, while  $\sigma_{ad}$ , k<sub>1</sub> and k<sub>2</sub> were significant for PC2.

	$\sigma_f$	(N)	$\epsilon_f$	(-)	$\sigma_{0.7}$	(N)	$\sigma_{ad}$	(N)
	Ctrl	HPT	Ctrl	HPT	Ctrl	HPT	Ctrl	HPT
day 3	37.6 <sup>a</sup>	30.3 <sup>b</sup>	0.332 <sup>a</sup>	0.430 <sup>b</sup>	25.3 <sup>a</sup>	20.5 <sup>b</sup>	-0.187 <sup>a</sup>	-0.602 <sup>b</sup>
day 7	47.9 <sup>a</sup>	47.5 <sup>a</sup>	0.282 <sup>a</sup>	0.356 <sup>b</sup>	37.2 <sup>a</sup>	30.7 <sup>b</sup>	-0.189 <sup>a</sup>	-1.695 <sup>b</sup>
day 14	50.3 <sup>a</sup>	63.4 <sup>b</sup>	0.282 <sup>a</sup>	0.326 <sup>b</sup>	37.3 <sup>a</sup>	41.5 <sup>b</sup>	-1.017 <sup>a</sup>	-1.955 <sup>b</sup>
day 21	69.7 <sup>a</sup>	55.3 <sup>b</sup>	0.245 <sup>a</sup>	0.320 <sup>b</sup>	51.4 <sup>a</sup>	33.7 <sup>b</sup>	-0.949 <sup>a</sup>	-2.831 <sup>b</sup>
day 30	74.6 <sup>a</sup>	44.1 <sup>b</sup>	0.281 <sup>a</sup>	0.317 <sup>b</sup>	46.6 <sup>a</sup>	27.4 <sup>b</sup>	-0.817 <sup>a</sup>	-1.484 <sup>b</sup>
day 45	125.6 <sup>a</sup>	76.7 <sup>b</sup>	0.243 <sup>a</sup>	0.253 <sup>a</sup>	88.2 <sup>a</sup>	48.6 <sup>b</sup>	-3.440 <sup>a</sup>	-4.389 <sup>a</sup>
day 60	132.9 <sup>a</sup>	99.9 <sup>b</sup>	0.231 <sup>a</sup>	0.263 <sup>b</sup>	90.3 <sup>a</sup>	60.7 <sup>b</sup>	-3.960 <sup>a</sup>	-5.226 <sup>a</sup>

Uniaxial compression parameters along ripening time. Values with the same letter within the same row for each parameter do not differ significantly ( $p < 0.05$ ) by two way ANOVA analysis (batch and treatment).

**Key Words:** High Pressure, Goat Cheese, Texture

**1276 Cheeses of Spain: classification and description.** M. Almena Aliste<sup>\*1,2</sup>, A. Cepeda Sez<sup>1</sup>, and Y. Nol<sup>2</sup>, <sup>1</sup>*Hygiene and Food Inspection, Faculty of Veterinary Lugo-University of Santiago de Compostela, Spain*, <sup>2</sup>*INRA Dairy Technology and Analysis Research unit Poligny, France*.

In Spain there are more than 100 varieties of traditional cheeses. Eleven such cheeses already carry the quality label of origin "Protected Designation of Origin" (PDO), and other six denominations have recently been submitted. However, Manchego cheese is the only internationally known cheese from Spain. The diversity of the cheese varieties makes the classification of Spanish cheeses difficult. Furthermore, a one-dimensional classification based on a single criterion would be insufficient and confusing. This work showed the great variety of cheeses from Spain by using two kinds of classifications that combine different criteria: origin of milk, technology of elaboration and cheese characteristics (composition, texture and other key properties). The study was completed with a table that closely described the main properties of the current PDO-Spanish cheeses: *Tetilla*; *Cabrales*; *Picn Bejes-Tresviso*; *Quesucos de Libana*; *Cantabria cheese*; *Idizabal*; *Roncal*; *Zamorano*; *Mahn*; *Manchego*; *Majonero* and *La Serena* cheese. The approach used allows a general but precious overview of the cheeses manufactured in Spain and illustrates possible strategies for classifications that could be applied to other cheeses.

**Key Words:** Cheeses from Spain, Multidimensional Classification, Description

**1277 Stress relaxation test: an approach to study cheese openness.** C. Achilleos, M. Almena Aliste\*, and Y. Nol, *INRA Dairy Technology and Analysis Research unit Poligny, France*.

The quality of Comt cheese is determined not only by flavor and texture but also by openness: few eyes, slits, or none (blind cheese). In order to investigate mechanisms of eye development, we need instrumental methods to characterize the mechanical properties of cheese, which affect eye development. Stress relaxation tests at different initial deformation levels (from 5% to 60%) were evaluated with 3 Comt cheeses in comparison with uniaxial compression at constant displacement rate. Each cheese selected had a typical openness, either eyes, or slits or none. For stress relaxation, the initial compression that appeared interesting for the characterisation of the mechanical properties was 20%, which was large enough to avoid errors due to sampling while cracks did not develop in the samples. At this initial deformation level, stress relaxation allowed a quite good characterisation of the cheeses with the 3 typical openness (84.6% of the observations were well classified). Nevertheless, compression test allowed the best discrimination between the 3 types of Comt cheeses with 100% of the observations well classified. A set of 30 experimental Comt cheeses was analysed to evaluate the stress relaxation as a method to characterize the mechanical properties in relation with openness. The well classified cheeses represented 73.3% of the whole set. Stress relaxation provided promising results since the set of cheeses had complex openness associating slits and eyes.

**Key Words:** Relaxation and compression tests, Openness, Comt cheese

**1278 Evaluation of reduced fat Cheddar cheese made with attenuated and not attenuated adjunct culture of *Lactobacillus helveticus* I: Effect of make procedure and cell attenuation.** S.A. Madkor<sup>1</sup>, P.S. Tong<sup>\*1</sup>, and M. El-Soda<sup>2</sup>, <sup>1</sup>*California Polytechnic State University*, <sup>2</sup>*Alexandria University*.

Adjunct culture of *Lb. helveticus* I was selected for evaluation in stirred and milled curd reduced fat Cheddar cheese making trials. *Lb. helveticus* I was cultivated in MRS broth and harvested by centrifugation at 4°C (4000 g for 30 min). A portion of the culture suspension was attenuated by freeze shocking at -20°C. Untreated (not attenuated), freeze-shocked or untreated/freeze-shocked mixture of *Lb. helveticus* I cells were inoculated ( $10^8$  cfu/gm) in the starter-treated cheese milk prior to rennet coagulation. Inoculation of adjunct did not affect the manufacturing parameters in stirred or milled curd cheese. Cell counts of both

*lactococcus* on M17 agar and *lactobacilli* on Rogosa agar were determined after one day of ripening and monthly for 6 months thereafter. The viable count of *lactobacilli* in one day cheese was lower than the number expected to be retained in cheese possibly due the loss of culture cells in the whey during cheese processing. The number of *lactobacilli* increased up to the third month of ripening and slightly decline thereafter. The rate of *lactobacilli* reduction was greater in cheese made with freeze-shocked *lactobacilli*. Cheeses made with adjunct *Lb. helveticus* I showed higher levels of proteolysis as indicated by FAA levels with the progress of ripening when compared to control cheese without adjunct. A slightly higher level of free amino acids were observed in stirred curd cheese compared to milled curd cheeses. Taste panel results indicated addition of *Lb. helveticus* adjunct culture to milled or stirred curd reduced fat Cheddar cheese positively influenced the flavor/aroma score and prevented bitterness throughout the entire ripening period. This effect was most pronounced in cheese treated with a mixture of untreated and attenuated adjunct cultures. The general conclusion reached from this work indicate that we believe that *Lb. helveticus* I adjunct will lead to enhanced and improved flavor quality in stirred or milled curd reduced fat Cheddar cheese.

**Key Words:** Cheddar, cheese, adjunct

**1279 Genetic typing of Swiss cheese starter culture strains by pulsed field gel electrophoresis and arbitrarily primed-PCR.** J. K. Jenkins\*, W. J. Harper, and P. D. Courtney, *The Ohio State University Columbus, Ohio*.

The manufacture of Swiss cheese requires growth of three bacterial species, *Propionibacterium freundenreichii*, *Lactobacillus helveticus* and *Streptococcus thermophilus*. Two genetic typing methods were compared for their ability to discriminate between strains of each species. Isolated colonies from single and multiple strain cultures were obtained on appropriate selective media. For multiple-strain cultures, ten to twenty single colonies were typed by one or both methods to separate the strains. The arbitrarily primed-PCR (AP-PCR) was performed at three annealing temperatures, 38, 40 and 42C as described by Cusick and O'Sullivan (Appl. Environ. Microbiol. 66:2227-2231), followed by electrophoresis of products. Pulsed field gel electrophoresis (PFGE) was used to analyze *Apa*I and *Sma*I digestions of *Lactobacillus* and *Streptococcus* genomes and *Spe*I digestions of *Propionibacterium* genomes. Other enzymes or enzyme combinations tested for *Propionibacterium* genome digestion (*Apa*I, *Bam*HI, *Bgl*II, *Cla*I, *Eco*RV, *Hind*III, *Pst*I, *Sma*I and *Sph*I) were unable to yield the appropriate number of fragments for analysis. Of the nine *S. thermophilus* strains analyzed, AP-PCR distinguished six electrophoretic patterns. PFGE of both *Apa*I and *Sma*I digestions revealed eight distinct patterns with two strains having identical patterns. The results with all three species indicate that pulsed field gel electrophoresis, though more labor intensive, is more effective at discriminating between strains than AP-PCR.

**Key Words:** Lactic acid bacteria, Pulsed field gel electrophoresis, Arbitrarily primed-PCR

**1280 Salt tolerance of dairy propionibacteria.** O. Anggraeni, J. K. Jenkins\*, and P. D. Courtney, *The Ohio State University Columbus, Ohio*.

*Propionibacterium* species are required to produce the characteristic appearance and flavor of Swiss cheeses. Differences in salt tolerance among propionibacteria strains may influence strain performance depending on conditions in a specific cheese. Many bacteria can import compatible solutes, such as betaine and proline, to counteract the effects of high osmotic pressure in the environment. Six strains of *Propionibacterium freundenreichii* were grown in a minimal defined medium with various levels of NaCl for seven days at 28C, anaerobically. Growth was monitored by measuring culture turbidity (absorbance at 600 nm). All strains grew in the minimal medium without salt, though the lag phase varied among the strains from one to four days. 1% NaCl extended the lag phase of all strains by approximately one day and reduced the maximum cell density reached by four of the strains. Growth of three strains was completely inhibited by 2 and 3% NaCl. Two strains had markedly suppressed growth at these concentrations. One strain, P273, was able to reach the same cell density in 2% salt as in the absence of salt, though with an extended lag phase. In medium containing 3% salt, strain P273 grew at a significantly faster rate than the other five strains. No significant growth of any strain was observed at salt concentrations

of 4, 5, 6 and 7%. Betaine (1 mM) or proline (5.9 mM) was added to each growth medium to assess their effectiveness as osmoprotectants. Neither betaine nor proline improved growth of four of the strains at any salt concentration. Strains P273 and P812 were able to grow faster and to a higher cell density with betaine or proline in medium containing 3 or 4% salt. Salt tolerance varies among dairy propionibacteria and the

presence of proline or betaine in the medium can dramatically improve the salt tolerance of some strains. During cheese ripening, proteolytic breakdown of caseins, which have high proline contents, may contribute to osmoprotection of some *Propionibacterium* strains.

**Key Words:** *Propionibacterium*, salt tolerance, osmoprotectant

## ASAS/ADSA Milk Synthesis

### 1281 Feeding dairy cattle to increase the content of conjugated linoleic acid in milk. Ying Huang, Barry Bradford\*, Nicholas Heig, Jerry Young, and Donald Beitz, Iowa State University.

To evaluate effectiveness of conjugated linoleic acid (CLA) as the free acid and as the calcium salt to increase CLA in milk fat, 36 Holstein cows were fed six diets in a completely randomized block design with a 4-wk period for each replication. The control diet consisted of corn silage, alfalfa hay, and concentrates, and supplements were 1) 5% soy oil, 2) 1% CLA as free acid, 3) 1% CLA as the calcium salt, 4) 4% soy oil plus 1% CLA as the free acid, or 5) 4% soy oil plus 1% CLA as the calcium salt (Ca(CLA)2). No significant effects of dietary supplementation were found on daily milk yield, milk protein concentration and production, or milk lactose concentration and production. Supplementation of soy oil, CLA, or Ca(CLA)2 decreased milk fat concentration and production but had no effects on rumen VFA concentrations. Milk CLA was increased from 0.4% to 0.7% with 1% dietary CLA and to 1.3% with 1% dietary CLA plus soy oil. Dietary Ca(CLA)2 increased CLA milk fat to 0.9%; feeding Ca(CLA)2 with soy oil increased CLA in milk fat to 1.4%. Soy oil supplementation alone increased CLA content to 1.2%. In summary, dietary soy oil (5%) was as effective in increasing milk CLA as feeding dietary CLA (1%) or (Ca(CLA)2) (1%) with or without 4% soy oil. Dietary (Ca(CLA)2) resulted in greater concentrations of CLA in milk than did dietary CLA as free acids.

**Key Words:** Dairy Cows, Conjugated Linoleic Acid, Milk Fat

### 1282 Dietary fish oil plus vegetable oil maximizes trans-18:1 and ruminic acids in milk fat. D.L. Palmquist\*<sup>1</sup> and J.M. Grinari<sup>2</sup>, <sup>1</sup>OARDC/The Ohio State University, Wooster, Ohio, <sup>2</sup>University of Helsinki, Finland.

Four Holstein cows were fed 4 diets in a 4 x 4 latin square design with 3-week periods. Diets were 60% haylage/40% corn-soy base concentrate (DM) with 3% added oil, consisting of 0, 33, 67 or 100% fish oil, with the balance made up with sunflower oil. Cows were fed for ad libitum intake; rumen contents were taken at 2 and 6 hours postfeeding, and milk was sampled on the last day of each period. Dry matter intake, milk yield and milk fat percentage were not different ( $P > 0.05$ ) among treatments, nor were ruminal pH and concentrations and proportions of VFA at 2 and 6 hours postfeeding. However, there was a tendency ( $P = 0.09$ ) for fish oil to increase molar proportion of butyrate at 2 hours postfeeding. All saturated milk fatty acids (4-16), 16:1, 20:4 n-6, and 20:5 n-3 were lowest with no fish oil, whereas fish oil decreased stearic, oleic and linoleic acids; linear regressions of percentage fatty acid on proportion of fish oil were significant. Quadratic effects of fish oil were significant for trans 18:1 ( $P < 0.10$ ) and ruminic ( $P < 0.01$ ) acids; treatment means (+/- SD) for 0 to 3% fish oil, respectively, were for trans 18:1: 14.2 (7.5), 18.1 (3.6), 18.6 (6.1), 13.7 (4.4)%, and for ruminic acid: 4.0 (1.2), 6.1 (1.7), 5.8 (2.1), and 3.4 (1.2)% of total milk fatty acids. Fish oil fatty acids may influence ruminal metabolism to maximize conversion of linoleic acid provided by sunflower oil to vaccenic acid. This approach increases milk fat ruminic acid up to ten-fold.

**Key Words:** Milk Fat, Vaccenic Acid, CLA

### 1283 Effect of dietary conjugated linoleic acids on the yield and composition of cow's milk. K.N. Simard\*<sup>1</sup>, P. Lacasse<sup>2</sup>, L. Delbecchi<sup>2</sup>, and P.Y. Chouinard<sup>1</sup>, <sup>1</sup>Universite Laval, QC, Canada, <sup>2</sup>Agriculture and Agri-Food Canada.

Conjugated linoleic acid (CLA) is an octadecadienoic fatty acid (FA) which has several effects on lipid metabolism. Previous work (Chouinard et al., 1999. J. Nutr. 129:1579) showed that abomasal infusion of CLA (CLA-60; Natural Lipids LTD, Hovdebygda, Norway) resulted in a significant reduction of milk fat synthesis. The objective of the present

study was to evaluate the effects of different forms of inclusion of dietary CLA (protected vs. unprotected) and the site of infusion in the gastrointestinal tract (rumen vs. abomasum) on milk fat synthesis in lactating dairy cows. Four multiparous Holstein cows in mid-lactation were utilized in a 4 x 4 Latin square design. Treatments were C) control (no CLA-60); R) addition of unprotected CLA-60 in the rumen; P) addition of protected CLA-60 in the rumen; and A) addition of unprotected CLA-60 in the abomasum. The CLA-60 mixture (unprotected and protected) contained 61% of CLA and was added (150 g/d) in the rumen or the abomasum three times daily (50 g at 0800, 1300, and 1800 h). At each period, treatments were administered for 7 days followed by 7 days of washout. Rumen protection of lipids was obtained by microencapsulation. Data from days 6 and 7 of the treatment periods were used for statistical analysis. Treatments had no effect on DMI and milk protein content. Milk yield was increased by protected CLA and averaged 23.7<sup>bc</sup>, 24.7<sup>ab</sup>, 26.8<sup>a</sup>, and 21.7<sup>c</sup> kg/d for C, R, P, and A, respectively ( $P < 0.01$ ). Administration of CLA reduced milk fat content which averaged 3.94<sup>a</sup>, 3.38<sup>b</sup>, 2.94<sup>c</sup>, and 1.86<sup>d</sup> for C, R, P, and A, respectively ( $P < 0.01$ ). Addition of CLA-60 in the abomasum increased CLA content of milk fat from 8.6<sup>b</sup> (control) to 40.4<sup>a</sup> mg/g FA. Milk CLA contents were not affected by R (11.8<sup>b</sup> mg/g FA) or P (9.4<sup>b</sup> mg/g FA). Feeding CLA-60 in unprotected form decreased milk fat content, but this decrease was of lower magnitude than that obtained with protected or abomasally infused CLA-60. Supported by Agribrands Purina Canada Inc.

**Key Words:** Dairy cows, Milk fat, CLA

### 1284 The effect of trans-10,cis12 conjugated linoleic acid (CLA) infusion on milk fat synthesis and expression of lipogenic enzymes in the mammary gland of lactating cows. E. Matitashvili\*<sup>1</sup>, L.H. Baumgard<sup>1</sup>, and D.E. Bauman<sup>1</sup>, <sup>1</sup>Department of Animal Science, Cornell University.

Four cows in late lactation were used in a 2x2 crossover design, receiving either 0 or 14 g/d of trans-10,cis-12 CLA. Treatments were emulsified in skim milk and delivered by continuous abomasal infusion (5 d) with a 14-d interval between treatments. Mammary gland biopsies were taken on d 5 of treatment. One portion was used for metabolic flux measurements and the other for Northern blot analysis. cDNA probes included: ovine acetyl-CoA carboxylase (ACC) and delta-9 desaturase (SCD), (both from M.T. Travers and M.C. Barber, Hannah Res. Inst., UK), ovine fatty acid synthase (FAS) (C. Leroux, LGBC-INRA, France), fatty acid binding protein (FABP) (purchased from ATCC), and bovine ESTs with sequence homology to glycerol phosphate acyltransferase (GPAT) and lipoprotein lipase (LPL) (both from J.C. Byatt, Monsanto Co.). The CLA treatment decreased milk fat percent and yield by 45% and 47%, respectively. Mammary explant incubations were designed to measure metabolic capacity and results indicated that CLA treatment reduced rates of acetate incorporation into fatty acids and oxidation to CO<sub>2</sub> by 82% and 61%, respectively. RNA analysis demonstrated that CLA treatment reduced abundance of all specific mRNA measured by 39 to 54%. Thus, treatment with trans-10,cis12 CLA altered processes associated with *de novo* synthesis (ACC and FAS), uptake of preformed fatty acids (LPL), fatty acid transport and esterification (FABP, GPAT), and plasticity of milk fat (SCD). Furthermore, the magnitude of the reduction in message for these enzymes observed with CLA treatment was similar to that observed with *in vitro* rates of acetate utilization for fatty acid synthesis and oxidation to CO<sub>2</sub> and *in vivo* measurements of milk fat yield.

**Key Words:** Conjugated linoleic acid, Mammary, Lipogenesis