

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, cis-12 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 unfusion (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, cis-12 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

**1285 In vitro lipid synthesis using bovine mammary homogenate.** T. C. Wright\*, J. P. Cant, and B. W. McBride, *University of Guelph*.

An in vitro system for lipid synthesis using bovine mammary homogenate was validated. Mammary tissue from Holstein cows producing  $26.4 \pm 3.5$  kg/d (mean and standard error) of milk was isolated immediately after slaughter. Tissue samples were ground under liquid N to a fine powder and stored at  $-70^\circ\text{C}$  until analysis. Tissue was homogenized in two volumes of isotonic sucrose in a Potter-Elvehjem tissue grinder and centrifuged at  $15\,000 \times g$ . Incubations were done at  $37^\circ\text{C}$  for 1h at pH 7.0 in a shaking water bath. The incubation solution (3.0ml) contained: 80 mM Tris-HCl, 0.80 mM  $\text{MnCl}_2$ , 20 mM  $\text{NaHCO}_3$ , 0.05 mM Coenzyme A, 1.7 mM ATP, 10 mM sodium citrate, 0.5 mM glucose 6-phosphate, 1.7 mM sodium acetate, 4.2 mM glutathione, 0.05 NADP, 20 mg/ml fatty acid free bovine serum albumin and 1 to 5 mg mammary protein. Incubations also contained approximately  $1 \mu\text{Ci } 1\text{-}^{14}\text{C}$  sodium acetate. Exogenous fatty acids or synthetic detergent was bound to albumin before introduction to the incubation medium. Final exogenous concentrations ranged from 70 to  $350 \mu\text{M}$ . Lipid synthesis was linear with respect to protein content and time. Lipid products were extracted and an aliquot was counted in a scintillation counter. Supernatant protein concentration was estimated using the bicinchoninic acid method with bovine serum albumin as the standard. Results ( $n=3$ ) indicated that when caprylic acid was incubated at a  $350\text{-}\mu\text{M}$  concentration there was a 2.4% ( $\pm 5.0\%$ ) increase in lipid synthesis compared to control incubations. At all concentrations from 70 to  $350 \mu\text{M}$  exogenous palmitic acid decreased lipid synthesis (12 to 25% respectively). Albumin bound detergent did not decrease lipid synthesis. Results indicated that palmitic acid inhibits lipid synthesis in vitro and results are not a non-specific detergent effect.

**Key Words:** Lipid, In vitro, Mammary

**1286 Kinetics of glucose transport by isolated bovine mammary epithelial cells.** Changting Xiao\*, John P. Cant, Michael I. Lindinger, and Brian W. McBride, *University of Guelph, Guelph, Ontario, Canada*.

Glucose is the sole precursor for lactose synthesis in lactating-cow mammary gland. The purpose of this research was to identify an appropriate description of glucose transport kinetics from extracellular fluid into lactating-cow mammary epithelial cells. Viable epithelial cells were isolated from lactating-cow mammary tissue by a collagenase dissociation technique. Cells were incubated with 3-O-methyl-D-[1- $^3\text{H}$ ]glucose (3OMG) at  $37^\circ\text{C}$ , and initial rates of 3OMG entry were measured under four different experimental conditions: zero-*trans*, where extracellular [3OMG] were varied while intracellular [3OMG] was kept at zero; equilibrium-exchange, where intracellular and extracellular [3OMG] were the same and varied simultaneously; infinite-*cis*, where extracellular [3OMG] was saturating and intracellular [3OMG] varied; and infinite-*trans*, where extracellular [3OMG] varied and intracellular [3OMG] was saturating. Uptake of 3OMG by isolated mammary epithelial cells exhibited saturable kinetics and was inhibited by phloretin and  $\text{HgCl}_2$ . The  $K_m$  and  $V_{max}$  for zero-*trans* entry and equilibrium-exchange measured in 3 cows were  $6.95 \pm 1.01$  mM and  $24.92 \pm 3.41$  nmol per min per mg cell protein, and  $17.78 \pm 1.74$  mM and  $39.77 \pm 0.91$  nmol per min per mg cell protein, respectively. The results demonstrated that both extracellular and intracellular concentrations of glucose need to be considered when simulating glucose transport by bovine mammary epithelial cells.

**Key Words:** cow, mammary epithelial cells, glucose transport

**1287 Factors affecting lactose production of lactating rat mammary acini.** K. H. Myung\*<sup>1</sup> and S. R. Davis<sup>2</sup>, <sup>1</sup>Chonnam National University, Kwangju, Korea, <sup>2</sup>AgResearch, Rukura Research Centre, Hamilton, New Zealand.

In vitro performance of mammary acini from lactating rats was assessed by the measurement of lactose secreted into media during 24 h of culture using a highly sensitive bioluminescence assay. Lactose production was significantly higher in cells from fed relative to starved (18h) animals ( $p < 0.01$ ) and increased linearly with increasing glucose concentrations in the media ( $p < 0.0001$ ) over 0-6h culture period. Lactose production of cells prepared from fed rats maintained in media containing 30 mM

glucose was 8.9 fmol/cell/h over 0-6h of culture, but declined thereafter. Over 6-24h of culture period the highest lactose production was 3.6 fmol/cell/h of fed cells in 40 mM glucose concentration media and there was no significant difference in the productivity of cells prepared from rats starved for 18h. Prolactin, hydrocortisone or a combination of prolactin, hydrocortisone and insulin significantly ( $p < 0.0002$ ) decreased lactose production over both 0-6h and 6-24h culture periods. Insulin alone had no effect. No effects of shaking, Matrigel coating or cell density at seeding on lactose secretion were found. Aeration during tissue digestion significantly ( $p < 0.05$ ) increased lactose production over 0-6h culture period. In conclusion, lactose production by mammary acini in vitro approached in vivo rates for 6h of culture and was sensitive to nutritional state of donor animals and aeration during tissue digestion. Hormonal additions had a negative effect on cell performance.

**Key Words:** Lactose, Lactation, Cell culture

**1288 The expression polymorphism of kappa-casein gene affects cheese yield.** G Robitaille\*<sup>1</sup>, D Petitclerc<sup>1</sup>, J Morisset<sup>2</sup>, and M Britten<sup>3</sup>, <sup>1</sup>DSRDC, Agriculture and Agri-Food Canada, Lennoxville, Canada, <sup>2</sup>Sherbrooke University, Sherbrooke, Canada, <sup>3</sup>FRDC, Agriculture and Agri-Food Canada, St-Hyacinthe, Canada.

A differential allele-specific expression of kappa( $\kappa$ )-casein gene was recently detected in Holstein cows genotyped  $\kappa$ -casein AB. Two different populations were defined: a group of cows presenting a similar level of expression for alleles A and B- specific  $\kappa$ -casein gene (cows HH) and a group of cows over-expressing the allele B-specific  $\kappa$ -casein gene compared to allele-A (group HL). The objective of this study was to evaluate the effect of this expression polymorphism on cheese yield to verify that an optimal expression of  $\kappa$ -casein gene effectively affects milk composition so that the cheese yield can be improved. Laboratory-scale cheddar-type cheeses were made from 144 ml of blended-milk samples at 7 test-days. At each test-day, milk samples from individual cows were pooled based allele-specific expression of  $\kappa$ -casein ( $n=5$  for each group of cows), skimmed, dialyzed overnight at  $4^\circ\text{C}$  against bulk milk adjusted at pH 6.2 with lactic acid, and standardized for protein content. Cream was added so that protein to fat ratios were identical and close to 0.9 before cheese production. Data were analyzed by analysis of variance. The 37% (w/w) moisture-adjusted cheese yield from milk of cows HH was significantly higher ( $P < 0.005$ ) than the one obtained from milk of cows HL,  $9.95 \pm 0.03$  % and  $9.82 \pm 0.03$  % for cows HH and cows HL, respectively. The decrease in cheese yield observed for milk of cows HL was mainly due to protein lost in the whey. In conclusion, the 37% (w/w) moisture-adjusted cheese yield was higher when the  $\kappa$ -casein gene expression was improved. Funded by FCAR-NOVALAIT.

**Key Words:** kappa casein, gene expression, cheese yield

**1289 Distribution of delta-9 desaturase mRNA in bovine tissues: effect of physiological state and diet.** E. Matitashvili\*<sup>1</sup>, D.G. Peterson<sup>1</sup>, D.H. Beermann<sup>1</sup>, and D.E. Bauman<sup>1</sup>, <sup>1</sup>Dept. of Animal Science, Cornell University.

Delta-9 desaturase introduces a cis-9 double bond in fatty acids and plays an important role in determining the plasticity of synthesized lipids and the endogenous synthesis of CLA. Tissue distribution of delta-9 desaturase (stearoyl-CoA desaturase, or SCD) mRNA was analyzed by Northern blot in Holstein cows ( $n=9$ ) in different physiological states and in crossbred beef steers ( $n=10$ ) fed different diets. Tissues included liver, mammary gland, subcutaneous and intermuscular adipose tissue, semitendinosus and longissimus dorsi muscle, heart, kidney, lung and brain. We have identified SCD mRNA as a 5 kb transcript using ovine SCD cDNA (M.T. Travers and M.C. Barber, Hannah Res. Inst., UK). Comparisons of cows in early and late lactation, and non-lactating, non-pregnant cows indicated highest expression of SCD mRNA in lactating mammary gland, and in adipose tissue independent of physiological state. Hepatic SCD expression in cows was low in all physiological states. Expression of SCD mRNA was about 30 times greater in lactating mammary gland than in the non-lactating state. Expression of SCD mRNA in liver and adipose tissue was greatest in non-lactating cows. Highest expression of SCD mRNA in steers was found in subcutaneous and intermuscular adipose tissue. Lower levels of SCD mRNA were found in kidney, lung and brain, but expression in liver was negligible. Steers ( $n=5$ ) fed full-fat extruded soybeans at 25.6% of total dry matter intake for 111 days prior to slaughter exhibited markedly

elevated (2-3 times) levels of SCD mRNA in subcutaneous and intermuscular adipose tissue when compared to control steers (n=5) fed a corn-based diet. Overall, the greatest expression of SCD mRNA was observed in adipose tissue in steers and cows and in mammary gland in lactating cows and expression was regulated by physiological state and diet.

**Key Words:** Delta-9 desaturase, Expression, Tissue

**1290 Milk fat globule size is not affected by diet restriction or soy oil supplementation.** A.D. Beaulieu<sup>\*1</sup>, J.K. Drackley<sup>1</sup>, J.M. Lynch<sup>2</sup>, and D.M. Barbano<sup>2</sup>, <sup>1</sup>University of Illinois, Urbana, <sup>2</sup>Cornell University, Ithaca, NY.

Milk fat globule size and distribution influence the processing characteristics of milk fat. Abomasal infusion of high-oleic sunflower free fatty acids increased ( $P < 0.001$ ) milk fat globule volume mean diameter (VMD) and decreased ( $P < 0.001$ ) DMI (Overton et al., 1998, J. Dairy Sci. 81 (Suppl. 1): 352). An experiment was conducted to determine whether the increase in globule size was a result of the infusion or the DMI depression. Lactating Holstein cows (n=6) consumed a TMR ad libitum for 3 wk. During wk 4, 5, and 6, one-half of the group was restricted to 64%, 50%, and 37%, respectively, of ad libitum DMI. Milk production was decreased ( $P < 0.05$ ) and milk fat percent was increased ( $P < 0.05$ ) by 50% and 63% restriction. The proportions of C18:1<sub>cis-9</sub>, C18:1<sub>trans</sub>, and C18:2 in milk fat increased ( $P < 0.05$ ) and proportions of C12:0, C14:0, and C16:0 decreased ( $P < 0.05$ ) when cows consumed 50% or 37% of ad libitum intake. Neither the VMD nor the diameter below which 90% (d 0.9) or 50% (d 0.5) of the volume of milk fat is contained were affected ( $P > 0.05$ ) by diet restriction. Milk fat globule size was positively correlated ( $P < 0.05$ ) with C6:0 to C12:0 and C16:0 in milk fat and negatively correlated ( $P < 0.05$ ) with C18 fatty acids. A second experiment examined the effect of soy oil supplementation on milk fat globule size. Six lactating Holstein cows were adapted to a basal TMR. Three cows then were fed the basal diet supplemented with soy oil (4% of DM) for 4 wk. Soy oil did not affect milk production or fat content ( $P > 0.05$ ) but increased ( $P < 0.05$ ) milk protein content. Soy oil did not affect ( $P > 0.05$ ) milk fat particle VMD, d(0.9), or d(0.5) but decreased ( $P < 0.05$ ) the contents of C4:0 to C16:0 and increased ( $P < 0.05$ ) C18:1<sub>cis-9</sub>, C18:1<sub>trans</sub>, C18:2<sub>cis-9</sub>, C18:2<sub>cis-12</sub>, and C18:2<sub>cis-9, trans-11</sub>, in milk fat. Although DMI restriction and soy oil supplementation affected milk fatty acid composition, milk fat globule size was unchanged.

**Key Words:** Milk fat globule size, milk fatty acids

**1291 Supplementary infusion of amino acids and bovine somatotropin in atropine treated cows.** P.H. Luimes<sup>\*1</sup>, J.P. Cant<sup>2</sup>, X. Zhao<sup>1</sup>, and D. Petitclerc<sup>3</sup>, <sup>1</sup>McGill University, St.Anne-de-Bellevue, Quebec, <sup>2</sup>University of Guelph, Guelph, Ontario, <sup>3</sup>Agriculture and Agri-Food Canada, Lennoxville, Quebec.

In cattle and sheep, muscarinic cholinergic antagonists, such as atropine, will decrease milk and milk protein yield. In cattle, blood concentrations of amino acids (AA) have been shown to decrease due to atropine, whereas, in sheep, muscarinic cholinergic antagonists have been shown to decrease serum somatotropin (ST) concentrations. Thus, atropine may provide an interesting model to determine the relative importance of endocrine and nutrient factors involved in milk synthesis. An experiment was conducted in which AA, bovine (b) ST or both were infused over 8h into cattle treated with atropine to determine whether one or both of these factors were responsible for the decline in milk protein production. Five mature Holstein cows were used in a 5 x 5 Latin square design, the treatments being: saline (0.9%), atropine (120  $\mu\text{g}\cdot\text{kg}(\text{MBW})^{-1}\cdot\text{h}^{-1}$ ), atropine + bST (850  $\mu\text{g}\cdot\text{h}^{-1}$ ), atropine + AA (25  $\text{g}\cdot\text{h}^{-1}$ ) and atropine + AA + bST. The AA profile of the infusate was designed to emulate the profile of milk protein. Atropine was unable to decrease plasma bST concentrations in lactating cows, though it did decrease plasma  $\alpha$ -amino nitrogen ( $\alpha$ -AN). Neither bST nor AA infusion were able to restore milk protein percent or milk protein yield. Perhaps other nutrient or endocrine factors, such as glucose, somatostatin, insulin and/or IGF-1 are involved in these effects of atropine infusion.

	Atr +		Atr +		Atr +		SEM
	Saline	Atr	bST	AA	bST + AA	SEM	
Milk							
yield (kg/8h)	9.8	7.4	8.0	7.6	8.5	0.7	
protein (%)	3.34a	3.13b	3.05bc	2.96c	2.98bc	0.18	
protein (g/8h)	341a	232b	243ab	227b	252ab	30	
Plasma							
bST (ng/ml)	2.40a	2.39a	6.71b	2.20a	6.14b	0.42	
$\alpha$ -AN (mM)	2.71a	1.90b	1.98b	2.81a	2.55a	0.13	

<sup>a,b,c</sup>LSMeans are different within each row ( $P < 0.05$ ).

**Key Words:** Atropine, Bovine Somatotropin, Amino Acid

**1292 Correlations between specific binding of bST to desaturated hepatic membranes and various serum endocrine and nutrient components.** M. Lonard<sup>1</sup>, P.H. Luimes<sup>1</sup>, E. Block<sup>1</sup>, and D. Petitclerc<sup>\*2</sup>, <sup>1</sup>McGill University, St.Anne-de-Bellevue, Quebec, <sup>2</sup>Agriculture and Agri-Food Canada, Lennoxville, Quebec.

Six mature Holstein cows were injected with 30.9  $\text{mg}\cdot\text{d}^{-1}$  of recombinant bovine somatotropin (rbST) from d 15 to 41 of lactation. Cows were assigned to one of two 3 x 3 Latin squares in which periods were of 6 d duration. Rests of 3 d were allocated to cows between periods. The experimental treatments, which began on day 18 of lactation, were saline (0.9%), glucose (50  $\text{g}\cdot\text{h}^{-1}$ ) and insulin + glucose (12.5  $\text{IU}\cdot\text{h}^{-1}$  + 50  $\text{g}\cdot\text{h}^{-1}$ , respectively). Data concerning serum concentrations of endocrine and nutrient components were previously published (Léonard and Block, 1997; J.Dairy Sci. 80:127-143). Data regarding percent specific binding (<sup>125</sup>I-bST.200  $\text{mg}^{-1}$ ) of hepatic membrane protein to bST were published by Léonard et al. (1992; J.Dairy Sci. 75(Suppl.1):182). Pearson correlation coefficients (r) were determined between specific binding of bST to desaturated hepatic membranes and serum insulin concentrations ( $r = 0.82$ ,  $P < 0.01$ ), serum IGF-1 concentrations ( $r = 0.48$ ,  $P < 0.05$ ), energy balance ( $r = 0.47$ ,  $P < 0.05$ ) and serum concentrations of NEFA ( $r = -0.59$ ,  $P < 0.01$ ). Serum glucose was not correlated with specific binding of bST to desaturated hepatic membranes. The mechanism by which insulin, which is usually at its nadir during early lactation, increases serum IGF-1 concentrations appears to be via an increase in specific binding of bST to its hepatic receptors.

**Key Words:** Specific Binding, Growth Hormone, Insulin

**1293 Effect of 17-estradiol on milk production and mammary gland involution in Holstein cows in mid-late lactation.** L. Delbecchi<sup>\*</sup>, D. Petitclerc, and P. Lacasse, AAFC-Dairy and Swine R&D Centre, Lennoxville, Quebec, Canada.

This study was conducted to assess the effects of estrogens on milk production and persistency of lactation in mid-late lactating cows. The hypothesis was that the increasing amounts of estrogens in the blood of pregnant lactating cows is one of the factors inducing or accelerating the progressive involution of the mammary gland observed during the declining phase of lactation. Eight non-pregnant Holstein cows, ranging from 177 to 338 d of lactation, received subcutaneous injections of either 17-estradiol (15  $\text{mg}/\text{cow}/\text{day}$ ; treated group, n=4) or excipient (95% ethanol; control group, n=4) from d 0 to d 8. Treated and control cows were paired according to the number of parities and milk yield. Milk production was measured from d -10 to d 20. Milk composition was evaluated on samples harvested before, during and after estradiol injection. One treated cow presented signs of acute mastitis on d 4 and was removed from the experiment. Milk production was reduced ( $P < 0.01$ ) in treated versus control cows by 14.8% on d 3, 37.2% on d 6, 76.5% on d 8, and 81.6% on d 11. Two treated cows dried-off spontaneously by d 8 and d 9, respectively. Changes in milk composition characteristic of a mammary gland in involution were observed in treated cows. Between d 0 and d 7, milk fat content and lactose concentration decreased ( $P < 0.05$ ) by 37.6% and 15.9%, respectively. During the same interval, milk protein concentration increased by 61.9% ( $P < 0.05$ ). Control cows showed no significant variation in these parameters during the same period. Effects of estradiol at the molecular level are currently investigated. These results support the hypothesis that estrogens produced by the foeto-placental unit induce a gradual decline in milk production in pregnant lactating cows. This work was supported by Dairy Farmers of Ontario and Agriculture and Agri-food Canada.

**Key Words:** Involution, Mammary gland, Estrogens

**1294 Transgenic Sows Overexpressing Alpha-lactalbumin: Piglet Growth and Milk Component Intake Early in Lactation.** M.S. Noble\*, M.B. Wheeler, and W.L. Hurley, *University of Illinois, Urbana, IL.*

Piglets reared by transgenic sows overexpressing  $\alpha$ -lactalbumin ( $\alpha$ -LA) grow at a significantly faster rate compared to piglets reared by control sows. Differences in piglet weight gain occur ( $P < 0.05$ ) by d 6 of lactation and continue throughout the lactation period. By weaning (d 21 of lactation), transgenic reared piglets are 13% heavier than piglets reared by control sows. The objective of this study is to determine if increased preweaning growth by transgenic reared piglets is associated with altered milk component composition and component intake. Milk samples were collected from first parity  $\alpha$ -LA transgenic sows ( $n = 11$ ) and their non-transgenic littermates ( $n = 13$ ) on d 3, 6, 9, and 12 of lactation. Milk samples were analyzed for concentrations of bovine  $\alpha$ -LA, lactose, total protein, and total solids. Lactose, total protein, and total solids intake by transgenic reared and control reared piglets were determined from milk production data previously reported. Bovine  $\alpha$ -LA was expressed throughout the lactation period (approximate mean concentration = 400 g/ml). Lactose concentrations were greater ( $P < 0.05$ ) on d 3 and 6 of lactation, but not different on d 9 or 12 of lactation in milk of transgenic sows when compared with milk from control sows. Total protein and total solids concentrations in the milk of transgenic sows and control sows were not significantly different on d 3, 6, or 12 of lactation. Lactose and total solids intake were greater ( $P < 0.05$ ) on d 3 and 6 of lactation for transgenic reared piglets compared to control reared piglets. Total protein intake was greater on d 3, 6, and 9 of lactation for transgenic reared piglets compared to control reared piglets. These data suggest that the elevated levels of lactose, total protein, and total solids intake by transgenic reared piglets early in lactation may account for the significant increase in their rate of preweaning growth.

**Key Words:** alpha-lactalbumin, transgenic sow, piglet growth

**1295 A redefinition of the effects of mammary cell numbers and enzyme activities on predictions of milk yield and composition by a lactating dairy cow model.** M. D. Hanigan\*, F. E. Standaert, and D. C. Weakley, *Purina Mills, Inc., St. Louis, MO.*

The model of Baldwin (1995) provides a method to predict milk yield and composition over time. This allows accommodation of time-dependent effects such as previous plane of nutrition. However, in evaluating the model, it was found to be inadequate with respect to predictions of milk fat and protein. It was determined that an updated representation of mammary cell numbers and cell enzyme activities was required to resolve the problem. The model of Dijkstra et al. (1997) was adopted for representation of each.

$$Mass_{Mammary} = \alpha BW_{DIM0} e^{(-\beta + \chi Trt) \times DIM}$$

$$Synthesis_i = Enz_{i,DIM} e^{(\alpha_i (1 - e^{\beta_i \times DIM}))}$$

$$Degradation_i = Enz_i e^{(-\delta \times DIM)}$$

where cell numbers were calculated from mass of protein and total enzyme activity for each component ( $i =$  lactose, protein, or fat) was calculated as the product of cell numbers and enzyme per cell. The mammary mass equation was parameterized from the data of Gibb et al. (1992) (see table). After adoption of the protein equation as a predictor of cell numbers, mammary enzyme equations were parameterized using a small production data set assembled from past studies conducted at the Purina Mills, Inc. research unit. After fitting to the data, predictions of lactation curves for milk fat and protein were significantly improved with root mean square prediction errors for milk and milk component yields declining by approximately 2 fold suggesting that this alternative representation was justified.

	$\alpha$	$\beta$	$\chi$	$P \leq .05 Trt$
Udder Mass	.044(.0016) <sup>a</sup>	.0019(.0004)	.0006(.0002)	*
Udder Protein	.005(.0002)	.0015(.0004)	.0006(.0002)	
Udder Fat	.006(.0004)	.001(.0007)	.0009(.0004)	*

Parameter estimates (SE) for Eq. (1) when fitted to the udder composition data of Gibb et al. (1992). Treatments (Trt) were 3 dietary energy intakes. <sup>a</sup>All  $\alpha$  and  $\beta$  coefficients were significantly different from 0 excepting  $\beta$  for udder fat.

**Key Words:** Lactating Cow, Model, Mammary

**1296 Amino peptidase gene expression in caprine mammary gland; A possible role in peptide-bound amino acid uptake.** S.J. Mabweesh\*<sup>1</sup>, M. Cohen<sup>1</sup>, O. Gal-Garber<sup>1</sup>, A. Shamay<sup>2</sup>, and Z. Uni<sup>1</sup>, <sup>1</sup>The Hebrew University of Jerusalem, <sup>2</sup>Agricultural Research Organization, The Volcani Center.

This experiment was conducted to isolate gene that express the enzyme aminopeptidase N (APN) in mammary gland of lactating goats. APN are enzymes anchored to the cell membrane via an N-terminal hydrophobic sequence of 20 amino acids that span the membrane only once. APN are highly expressed at the brush border membrane of the small intestine and are responsible for peptides hydrolysis and protein digestion. The mammary gland of ruminants do not express gene for peptide transporter (Pept1), however indirect evidence for peptide uptake by the gland is apparent. We hypothesized that APN enzyme would be expressed in the mammary gland of lactating ruminants and it may play an important role in peptide-bound amino acids uptake in the mammary gland. The first step in investigation this hypothesis was to isolate the gene from the mammary tissue. Caprine mammary gland tissue was collected for RNA isolation and basal-membrane vesicles (BMV) preparation. Total RNA was isolated from the mammary tissue and the APN gene was detected by RT-PCR using specific primers chosen from conserved regions of APN genes which identified as 531 bp cDNA. This cDNA fragment showed 83% homology to human, rabbit and rat intestinal APN. This cDNA fragment was used as probe in northern blot analysis which revealed a transcript of approximately 4.0 kb. Western blot analysis detected a protein of 155 KDa in BMV prepared from the mammary tissue. APN kinetics measurements in BMV revealed a high affinity enzyme with  $k_m = 57 \mu M$  and  $V_{max} = 270 \text{ pmol. mg prot}^{-1} \cdot \text{min}^{-1}$ .

**Key Words:** Mammary gland, Amino peptidase, Peptide uptake

**1297 Analysis of the sources of variation in CLA production in dairy cows.** J.A. Kelsey\*, D.G. Peterson, and D.E. Bauman, *Cornell University, Ithaca, NY.*

Conjugated linoleic acid (CLA) has a wide variety of health benefits based on animal models. Ruminant derived foods are the major dietary source and this relates to incomplete biohydrogenation of fatty acids. Some CLA escapes complete biohydrogenation in the rumen, but the major source is endogenous synthesis via  $\Delta^9$ -desaturase from *trans*-11 C<sub>18:1</sub>, another biohydrogenation intermediate. Thus, milk fat CLA can vary because of rumen outflow of CLA and *trans*-11 C<sub>18:1</sub> or activity of  $\Delta^9$ -desaturase. Our objective was to examine individual consistency and sources of variation in milk fat CLA. Holstein cows were divided into 3 groups ( $n=10/\text{treatment}$ ) for the 12 wk study. One group (low) was fed a traditional corn-based TMR, a second group (high) was fed extruded, full-fat soybeans to achieve higher milk fat CLA, and the third group (switch) alternated treatments at 3 wk intervals between low (periods 1 and 3) and high (periods 2 and 4) diets. Average CLA concentration in milk fat was relatively constant among individuals and groups over the 12 wk for low and high treatments. Milk fat CLA was 3-fold greater in the high treatment and there was a 2- to 3-fold range among individuals for both diets. The switch treatment was also relatively constant within dietary treatment, with CLA concentration varying as expected, according to diet. In milk fat synthesis,  $\Delta^9$ -desaturase has four primary substrates; C<sub>14:0</sub>, C<sub>16:0</sub>, C<sub>18:0</sub>, and *trans*-11 C<sub>18:1</sub>. The ratio of these substrates and their corresponding products serves as a proxy for  $\Delta^9$ -desaturase and all 4 ratio comparisons allowed for similar conclusions. We focused on the C<sub>14:1</sub>/C<sub>14:0</sub> ratio because the predominant source of these fatty acids is mammary synthesis. Regardless of diet, ratios of C<sub>14:1</sub>/C<sub>14:0</sub> were similar for treatment groups with a 2- to 3-fold range among individuals. Furthermore, individual cows displayed remarkable consistency in the hierarchy across periods for all treatments, including the switch treatment where the diet and supply of substrate for desaturation varied in successive periods.

**Key Words:** CLA, milk fat, desaturase

**1298 Effect of postpartum changes in BCS on milk components.** DilipKumar Garikipati\*<sup>1</sup>, Sarjan Rao Kapa<sup>1</sup>, and Kailash M.M.<sup>2</sup>, <sup>1</sup>College of Vety Science, Tirupati, <sup>2</sup>College of Vety Science, Bangalore.

Body condition scoring system with 1 to 5 scale (Edmonson et al,1989) was used in 137 crossbred HF cows in early to mid lactation to evaluate the post partum losses in body fat reserves and its influence on milk components. The effect on dry matter intake (DMI) was linear and a reduction of DMI (kg/day) with the increase in BCS ranged from 10.78 to 13 Kg/day for BCS 4.5 to 2.5 DMI decreased by 1.3 Kg/day for an additional increase in one unit of condition score. This showed that the DMI of fatty cows was less than the thin cows. As the live weight (kg) of the animals increased the BCS also increased indicated increase, in body fat reserves. Mean daily and peak milk yield over the first 6 months of lactation in relation to BCS were linear and there was a increase of 4.1 Kg and 7.8 Kg of daily milk yield and mean peak milk yield, respectively for every increase of one unit of condition score. Milk fat yield ranged from 3.1 to 3.9 percent and there was a positive response of 5 g/kg with an increase of one unit of condition score over the range 3 to 4 BCS. A positive milk protein output response was also observed with milk protein yield ranged from 32 to 34 g/kg with a meagre increase of 1g/kg over the range of 3 to 3.5 BCS and a decrease of 2g/kg for every one unit increase of BCS over the range of 3.5 to 4.5. The pattern of prediction equations for change in BCS to 120 DIM and 305 days FCM yield showed the BCS loss of 0.75 to 1.0 unit which was associated with more milk production. Higher rates of loss in BCS in second and third lactation numbers that have been associated with diminished milk production compared with its potential production. This suggested that increased feeding levels are warranted as the number of lactations advanced. Post partum decrease in BCS was observed upto three months and this was due to loss of body reserves through milk production which gradually recouped from fourth month onwards. This suggested that the increase in the post partum feeding levels will prevent the loss in BCS.

**Key Words:** BCS=Body Condition Score, fat, milk

**1299 Evaluation of the antibacterial activities of lactoferrin derived peptides.** P.-W. Chen, C.-L. Shyu, and F. C. Mao\*, National Chung Hsing University, Taichung, Taiwan.

The hydrophobic and basic regions of N-terminal of lactoferrin, which contained 10 amino acids, originated from bovine (bLF20-29), caprine (cLF20-29), porcine(pLF20-29), human (hLF21-30) and murine (mLF20-29) were chemically synthesized. The minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) against Gram-positive bacteria (*S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212) and Gram-negative bacteria (*E. coli* ATCC 25922 and five wild strains of *E. coli* that resisted to broad spectra of antibiotics) were determined. The MIC and MBC of bovine lactoferrin and pepsin-digested bovine lactoferrin hydrolysates against selected bacteria were also determined and compared with that of synthesized peptides. The bLF20-29 had potent antibacterial activity that it inhibited and killed all the selected bacteria. The MIC for bLF20-29 was 30 µg/ml and the MBC was 60 µg/ml in selected *E. coli*. However, the peptides of cLF20-29, pLF20-29, hLF21-30 and mLF20-29 had less antibacterial activity. The MIC for these peptides were more than 500 µg/ml among the selected bacteria. According to the hydrophobic and basic pattern, two peptides modified from the bLF20-29 and cLF20-29 origin were synthesized, named LFM1 (Arg Arg Trp Trp Trp Arg Trp Arg Arg Trp) and LFM2 (Arg Arg Trp Trp Arg Arg Trp Arg Arg Trp). Both the LFM1 and LFM2 had excellent antibacterial activities. The MIC and MBC for these two peptides were similar in selected bacteria. The MIC and MBC were 2.8 µg/ml and 3.75 µg/ml in selected *E. coli*. The antibacterial activities of LFM1 and LFM2 were much better than the bLF20-29. The potent

antibacterial property of these peptides could be useful in further study and field application.

**Key Words:** lactoferrin, lactoferricin, antibacterial peptide

**1300 Local expression of IGF-1 and IGFBP-3 mRNA in mammary tissue of prepubertal heifers after treatment with growth hormone.** P.M. Jobst\*<sup>1</sup>, S.D. Berry<sup>1</sup>, M.L. McGilliard<sup>1</sup>, D. Ayares<sup>2</sup>, D.A. Henderson<sup>1</sup>, W.E. Beal<sup>1</sup>, and R.M. Akers<sup>1</sup>, <sup>1</sup>Virginia Polytechnic Institute and State University, <sup>2</sup>PPL Therapeutics Inc.

Two experiments were conducted to determine the effects of bovine growth hormone (GH) and estradiol (E<sub>2</sub>) on mRNA expression of insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 (IGFBP-3) in mammary tissue of prepubertal heifers. In experiment one seven heifers treated were with GH for 7d. Mammary parenchyma and stroma were collected by surgical biopsy before and after GH. Explants of parenchyma and stroma were cultured for 36h in media without or with GH (1µg/ml), E<sub>2</sub> (20pg/ml), GH + E<sub>2</sub>, or IGF-1 (100ng/ml). RIA indicated serum IGF-1 and GH levels were elevated following GH treatment. Expression of IGF-1 and IGFBP-3 mRNA was not affected by GH treatment or hormones in culture. However, expression of IGF-1 mRNA was greater in stromal than parenchymal tissue. In experiment two, nine prepubertal heifers were administered placebo or GH. GH (Posilac<sup>®</sup>) was given every 14 days for three months. Blood samples were collected weekly. Heifers were sacrificed and mammary tissue collected. Stroma and parenchyma explants were cultured and analyzed for expression of mRNA as described above. Serum IGF-1 and GH levels were elevated following Posilac<sup>®</sup> treatment. Prior treatment of heifers with Posilac<sup>®</sup> did not affect the response of tissue explants in culture. However, in both parenchymal and stromal explants, IGF-1 increased (24%) expression of IGFBP-3 compared with explants in the absence of hormones. Stroma produced 27% more IGFBP-3 mRNA than parenchyma. Stroma explants cultured in E<sub>2</sub> or E<sub>2</sub> + GH produced 60% and 54% more IGF-1 mRNA respectively, compared to explants without hormones. Overall stromal explants produced 2.4-fold more IGF-1 mRNA than parenchyma explants. These data indicate that E<sub>2</sub> and IGF-1 elicit acute changes in the local IGF-1 axis of the bovine mammary gland.

**Key Words:** IGF-1, Growth hormone, Mammary gland

**1301 Milk yield and constituents of Fleckvieh cattle in Bavaria:1-First lactation.** Kamal Marzouk\*<sup>#</sup>, <sup>#</sup>Minia Univ..

Data from 3814 first lactation of Fleckvieh cattle was collected from 29 herds. This data came from milk recording organization in Bavaria, Germany. The aim of this study was to evaluate the milk yield and constituents of first lactation to erect a selection programme. The means of kg milk, 100-days for different traits of milk production and constituents were 1823.68 kg,72.40 kg,3.96%, 57.9 kg, 3.20%, 1.24%,0.75% and 2.14 MJ for milk yield, kg fat,%F,kg protein,%protein, Fat/Protein (F/P)ratio, index fP=(% fat-%protein)and energy in milk[milk energy yield (MJ)=milk yield\* (0.37 \* %fat+0.21\* %P + 0.95)+milk yield \* 0.07]], resp. The same traits at kg-milk, 200-days were 3395.42 kg, 134.14 kg, 3.95%, 111.01 kg,3.27%, 1.30%,0.67% and 2.16 MJ, resp. Also, at kg milk, 305-days the previous traits were 4553.44 kg, 183.50 kg, 4.07%, 152.4 kg, 3.32%, 1.68%, 0.76% and 2.21 MJ,resp. Effect of herd-years was significant on all milk yield and constituents traits at different periods except on fat/protein ratio at kg milk, 200-days. Seasons at calving had a significant effect on all traits at kg milk, 100-days except index fp,% fat, index fp at kg milk; 200 days and %fat, f/p ratio, index fp and energy in milk at kg milk, 305-days. On the other hand,the means of persistency=[(milk yield days 101-200/milk yield days 1-100)\*100] was 82% and not affected by seasons of calving.

**Key Words:** Milk yield and constituents, Milk energy, Persistency

## ASAS/ADSA Extension Education and ASAS/ADSA Teaching Undergraduate and Graduate Education

**1302 Dairy farm HACCP: PMO bulk tank temperature and wash cycle compliance on 10 Minnesota dairies.** S. Nagel and J. K. Reneau\*, University of Minnesota, St. Paul, MN, USA.

Bulk tank temperatures should be a critical control point in a dairy farm HACCP plan. This study used temperature recording data loggers to

observe bulk tank temperature patterns on 12 Minnesota dairies. From a list of potential cooperators supplied by the Minnesota Department of Agriculture, 12 dairies were selected by geographical distribution and herd size. Onset Computers' HOB0<sup>®</sup> temperature recording data loggers were placed inside bulk tanks near the outlet. The thermometers