groups for energy level (High=10.5 and Low=8.3 ME/kg DM). The animals from each group were slaughtered at 8 months of age. Samples of longissimus thoracis (Lt) and semimembranosus (Sm) muscles were removed from the carcasses 4h after slaughter and stored at -80° C until they were analyzed. The aminoacids (Aspartic acid, Glutamic acid, Serine, Threonine, Alanine, Arginine, Proline, Phenylalanine, Leucine and Isoleucine, Valine, Methionine, Histidine, Lysine, Tryptophan and Glycine) were quantified by means of chromatographic technique and the protein was determined by Kjeldahl nitrogen analysis. Data were tested following the SAS programme using the General Linear Models procedure. In general, data presented similar profiles in both muscles, in the two treatment groups, with only a few significant differences. Lt muscle of animals fed in group H had higher values for glutamic acid ($P< 0.01$) and aspartic acid ($P <0.05$). Lt contained a higher amount of ether extract and protein when compared to Sm. The results of this study indicate that the levels of energy of the diet as used in this trial did not influence the profile of the amino acids in the two muscles at this age.

Key Words: Beef, Chemical composition, Diet

688 Fiber type (Myosin Heavy Chain I) and biochemical traits of *Longissimus thoracis* from three European beef breed types. M. Gil*[1], X. Serra[1], M.A. Oliver[1], C. Sa'udo[2], J.L. Olleta[2], M.D. Garcia-Cachn[3], M.C. Olivn[3], M.M. Lopez-Parra[4], R. Quintanilla[5], and J. Piedrafita[5], [1]*IRTA- CTC, Monells, Spain,* [2]*Univ. Zaragoza, Zaragoza, Spain,* [3]*ETC, Guijuelo y CIATA, Villaviciosa, Spain,* [4]*SIAEX, Badajoz, Spain,* [5]*UAB, Barcelona, Spain.*

The effect of the breed type on some chemical and biochemical traits (intramuscular fat (IMF), haem pigment, myosin heavy chain I (MHC-I), lactate dehydrogenase and isocitrate dehydrogenase (ICDH) activities) of muscle *longissimus thoracis* (LT) from seven European beef breeds was studied. The animals (478) were fattened with concentrate *ad libitum* and straw from 7 to 12-18 months depending upon breed-production system. Breed-production systems were grouped into three types: 1) Fast growth rate (FG) (n=199, carcass weight, CW, 312.9 ±36.7 kg), 2) double muscle condition (DM) (n=70, CW, 324±21.8 kg) and 3) rustic characteristics (RC) (n=208, CW, 265.1±26.7 kg). MHC-I was determined by enzyme-linked immunosorbent assay using a specific MHC-I monoclonal antibody. A least squares analysis including the effect of breed type was performed using the GLM procedure of the SAS. The breed type influenced all the muscle characteristics studied. The three groups showed different average daily gains (ADG) (LSM±SE, 1.64±0.02 (FG), 1.41±0.04 (DM) and 1.30±0.02 (RC) kg/day). The animals from the DM group had the highest lean percentage (76±0.6%) and the lowest IMF percentage (0.99±0.14%) whereas the RC breeds showed the opposite (65.5±0.35% and 2.64±0.08% for lean and IMF, respectively). The most oxidative traits were found in the RC group (MHC-I, 35.3±0.9%). ADG was negatively correlated with pigment content and ICDH in the FG group of animals, but this was not observed in the other groups. MHC-I presented a positive and significant correlation with respect to pigment content in the three groups studied, indicating that muscles with a higher MHC-I content had a higher myoglobin content and, consequently, were more oxidative. The differences in muscle characteristics found in the three breed types suggest differences in the technological and sensorial characteristics of their meat quality.

Key Words: Breed type, Myosin I, Intramuscular fat

MILK SYNTHESIS

689 Estrogen treatments to initiate dryoff in dairy cows. M.L. Schairer*, K.C. Bachman, M.J. Hayen, and H.H. Head, *University of Florida Gainesville.*

Mammary involution in dairy cows is accelerated by exogenous estrogen. Estradiol-17β (E_2) and estradiol-17β cypionate (ECP®) were evaluated to determine their effectiveness in promoting mammary involution as monitored by the decline in daily milk yield. Response of pregnant, lactating dairy cows (n=5) to 4 consecutive once-daily injections of E_2 (E4x15; 15mg/d, s.c.) served as the positive control for intramuscular ECP treatments of 20 and 30 mg administered via single injection (ECP1x30, n=4; ECP1x20, n=5) or two once-daily injections (ECP2x15, n=6; ECP2x10, n=5). Cows were milked thrice daily before and after the injections which were begun at the onset of projected 60d dry periods. Milk production (kg/d) for E4x15 and ECP treatments (1x30, 2x15, 1x20, 2x10) before injection and on day 7 post-first injection were: 19.9, 21.0, 20.0, 21.6, 17.8 and 7.8, 8.4, 8.0, 10.6, 12.7. Thus, daily milk yields declined 60.9, 60.0, 60.1, 51.0, and 29.0 percent despite the thrice daily milkings. Average daily high ambient temperature and minimum relative humidity were 32.8°C and 57%. This heat stress plus the physical stress and oxytocin stimulation associated with the milk removal activity, as well as, stage of gestation at estrogen treatment, were considered possible contributors to the abortion events that occurred. Days post-injection at which 6 cows aborted were: E4x15, n=2 (7,8); ECP1x30, n=1 (15); ECP1x20, n=1 (12); and ECP2x10, n=2 (9,11). During a second experiment, at final milk removal, thirty pregnant, lactating cows projected to have 30d dry periods received one i.m. injection of ECP: (cottonseed excipient, n=10; 30 mg, n=10; or 20 mg, n=10). Abortion did not occur. In contrast to the previous cows, these cows received ECP later in gestation (250vs220 d pregnant) and were not milked post-injection. Also, they experienced less heat stress, in that, both temperature (26.2°C) and humidity (56%) were lower. Results suggest that ECP® administered at dryoff might allow the length of the non-income producing dry period to be shortened profitably.

Key Words: Estrogen, Dry period, Involution

690 Exogenous *trans*10,*cis*12-18:2 reduces *de novo* synthesis and desaturation of milk fatty acids in cows fed diets supplemented with high-oleic or high-linoleic oil. J. J. Loor* and J. H. Herbein, *Virginia Polytechnic Institute & State University, Blacksburg.*

To determine the effects of an elevated supply of *c*9,*t*11-18:2 (CLA9/11) or *t*10,*c*12-18:2 (CLA10/12) on milk fat concentration and fatty acid profiles, four Holstein cows fed oil (2.5% of DM) were infused (0.625 g/h) with CLA9/11 (90% pure) or CLA10/12 (95% pure) for 48 h via the abomasum. High-oleic safflower (HO; 70% *c*9-18:1) or high-linoleic safflower oil (HL; 65% *c*9,*c*12-18:2) were used as oil supplements. Treatments were assigned in a 2 × 2 factorial design. Cows were fed the assigned diets for 11 d prior to each 48-h infusion period. During the 7-d transitions between periods, all cows were fed a diet supplemented (2.5% of DM) with equal proportions of HO and HL. Milk samples were obtained at -12 and 0 h before infusion, at 12, 24, 36, and 48 h during infusion, and at 60, 72, 84, and 96 h after infusion. Milk yield and DMI were not affected by treatment. Percentages and yields of protein, lactose, and SNF in milk also were not affected by treatment. Milk fat percentages from 24 to 96 h were lower in response to CLA10/12 (2.7, 2.4, 2.3, 2.2, 2.1, 2.5, and 2.7%) compared with CLA9/11 (3.3, 3.2, 3.4, 3.5, 3.4, 3.5, and 3.4%) regardless of diet. Fat yield also was lower in response to CLA10/12. Concentration of *c*9,*t*11-18:2 in milk fat before infusion was higher when HL (9 mg/g total fatty acids) was fed compared with HO (6 mg/g), and it increased to 15 or 11 mg/g by 48 h of CLA9/11 infusion in cows fed HL or HO. Although *t*10,*c*12-18:2 was not detectable in milk fat before infusion, it accounted for 6 mg/g by 48 h of CLA10/12 infusion. Regardless of diet, concentration of saturated fatty acids with 4 to 16 carbons decreased from 410 mg/g at 0 h to 350 mg/g at 48 h of CLA10/12 infusion, whereas concentration of 18:0 increased from 144 mg/g at 0 h to 210 mg/g at 48 h. Concentration of *t*11-18:1 in milk fat (9 or 16 mg/g for HO or HL) before an infusion was greater when HL was fed, and infusion of CLA10/12 caused a further increase in concentration by 48 h (15 or 18 mg/g for HO or HL). Ratios of 18:0 to *c*9-18:1, *t*11-18:1 to *c*9,*t*11-18:2, and *c*9,*c*12-18:2 to 20:4 in milk fat increased from 0.5, 1.7, and 18 at 0 h to 0.8, 2.7, and 25 at 48 h of CLA10/12 infusion regardless of diet. Increased *t*10,*c*12-18:2 availability and uptake by the mammary gland reduced *de novo* fatty acid synthesis and desaturation of 18-carbon fatty acids.

Key Words: CLA, Trans fatty acids, Milk fat

691 Effect of conjugated linoleic acids (CLA) on lipid metabolism in lactating dairy cows. L.H. Baumgard*, B.A. Corl, D.A. Dwyer, T.R. Mackle, and D.E. Bauman, *Cornell Univ., Ithaca, NY.*

CLA have been shown to affect a large number of biological processes including lipid metabolism. Administration of CLA to lactating dairy cows causes milk fat depression, and we demonstrated this is specific for the *trans*-10,*cis*-12 isomer (Baumgard et al., Am. J. Physiol. 278:R179). In the present study we evaluated the effects of CLA isomers on lipid fractions and verified effects on milk fat synthesis and composition. Three Holstein cows (183 ± 8 DIM) were utilized in a 3x3 Latin square design. Treatments consisted of 5-d abomasal infusion of 1) control, 4L of skim milk/d, 2) 9,11 CLA, 10 g/d of *cis*-9,*trans*-11 CLA isomer and 3) 10,12 CLA, 10 g/d of *trans*-10,*cis*-12 CLA isomer. Blood samples were obtained on d 5 of each period and performance data represent d 4 and 5 of infusion. Treatments had no effect on DMI (26 kg/d), milk yield (32 kg/d), milk protein yield (923 g/d) or protein percentage (2.94). Compared to control and 9,11 CLA treatments, the 10,12 CLA resulted in a 31% reduction in milk fat yield (1034^a, 1106^a, 742^b g/d) and a 29% decrease in milk fat percentage (3.22^a, 3.44^a, 2.36^b) (P<0.05). Relative to control, infusion of the 9,11 CLA increased the *cis*-9,*trans*-11 concentration in plasma phospholipids (0.3^a vs. 2.5^b mg/g) and plasma triglycerides (1.4^a vs. 3.8^b mg/g). Likewise *trans*-10,*cis*-12 levels were undetectable in controls but infusion of 10,12 CLA increased levels to 1.8 mg/g in plasma phospholipids and 2.7 mg/g in plasma triglycerides. These CLA isomers were not detected in other plasma lipid fractions (<0.1 mg/g). An examination of milk fat composition indicated de-novo synthesized fatty acids accounted for 69% of the total reduction in milk fat yield (molar basis) observed with the 10,12 CLA treatment. In addition, ratios of $C_{14:0}/C_{14:1}$, $C_{16:0}/C_{16:1}$, $C_{18:0}/c9$-$C_{18:1}$ and $t11$-$C_{18:1}/c9$,$t11$-CLA were significantly increased during the 10,12 CLA treatment. We have verified the inhibitory effects on milk fat synthesis are unique to the *trans*-10,*cis*-12 isomer and that both CLA isomers were incorporated into plasma phospholipid and trigylceride fractions.

Key Words: CLA

692 Conjugated linoleic acids as free fatty acids or triglycerides cause milk fat depression. T. W. Hanson*[1], J. G. Giesy[1], M. A. McGuire[1], E. L. Annen[1], D. E. Bauman[2], A. Saebo[3], and M. K. McGuire[4], [1]*University of Idaho, Moscow,* [2]*Cornell University, Ithaca NY,* [3]*Natural Lipids, Norway,* [4]*Washington State University, Pullman.*

Conjugated linoleic acids (CLA) have been shown to be potent inhibitors of cancer in many animal models. Further, commercial mixtures of CLA isomers have been found to depress milk fat synthesis in cows, humans and pigs. These commercial mixtures contain free fatty acids rather than triglycerides predominately found in nature. Thus, we conducted the current study to examine whether CLA infused as a triglyceride would depress milk fat and enrich milk with CLA similarly to CLA as free fatty acids. Lactating Holstein cows (n = 4) were used in a cross over design. Periods lasted 6 d with 2 d of baseline followed by 4 d of CLA infusion in which 50 g/d of CLA (CLA-60, Natural Lipids, Norway) was delivered in a free fatty acid or triglyceride form. The CLA mixture contained (as a percentage of CLA isomers) *cis*-9, *trans*-11 (23.7%), *trans*-10, *cis*-12 (34.5%) and other CLAs (41.8%). Lipid was homogenized with skim milk and infused directly into the abomasum. Seven d wash out existed between periods. No changes in feed intake, milk yield, or milk protein percentage were detected (P > 0.05) during CLA infusions. Milk fat content was decreased (P < 0.05) from 4.14 to 2.37% and from 3.95 to 2.64% for cows infused with CLA as free fatty acids or triglyceride, respectively. No effect of CLA form was detected. For both lipid types, reductions in milk fat percentage appear to be a result of decreased (P < 0.05) de novo fatty acid synthesis, specifically C6:0 (35.1%), C8:0 (36.6%), C10:0 (39.1%), C12:0 (33.5%) and C14:0 (16.3%). Milk fat CLA isomer concentrations increased (P < 0.05) from 0.67 to 1.16% for *cis*-9 *trans*-11 and from non-detectable levels (less than 0.01%) to 0.32% for *trans*-10 *cis*-12, with no detectable difference between treatments for either isomer. Commercial mixtures of CLA are potent inhibitors of milk fat synthesis and enhance milk CLA concentrations when infused abomasally in either the form of free fatty acids or triglycerides.

Key Words: Conjugated Linoleic Acid, Milk Fat Depression

693 Effect of dose of calcium salts of conjugated linoleic acid (CLA) on milk yield, fat and CLA content of milk fat in Holstein cows early in lactation. J.G. Giesy*[1], T.W. Hanson[1], H.C. Hafliger[1], M.A. McGuire[1], C.H. Skarie[2], and K. Cummings[3], [1]*University of Idaho, Moscow,* [2]*Conlinco, Detroit Lakes, MN,* [3]*Church & Dwight Co. Inc., Princeton, NJ.*

Previous research in our laboratory demonstrated milk fat depression in early and mid lactation cows fed calcium salts of CLA isomers while enhancing CLA content of milk fat. Reduced milk fat synthesis occurred when cows were fed 50 g CLA-60 (Conlinco) as a topdress supplement. Objective of the current study was to evaluate a dose response of CLA feeding on milk fat percentage. Five multiparous Holstein cows averaging 93 ± 5 d in milk and 46.5 ± 3.0 kg/d milk yield were used in a 5 x 5 Latin square design. Each period consisted of 5 d treatment phase followed by 9 d rest. Cows received injection of bST 3 d prior to initial treatment of each period. Treatments consisted of 0, 10, 25, 50 or 100 g CLA from CLA-60 in calcium salt form (Church & Dwight). The CLA-60 mixture contained (as a percentage of CLA isomers) $c9$, $t11$ (23.7%), $t10$, $c12$ (34.5%) and other CLAs (41.8%). Supplements were made isolipid with addition of Megalac® and were fed once daily (0700 h) as a topdress. After consuming supplement, cows were fed a TMR containing 19% CP, 20% ADF, 39.8% NFC and 5% lipid. Milk was sampled at each milking and analyzed for components and fatty acid profile. Mean dry matter intake (25.8 ± 0.3 kg/d) and milk yield (43.5 ± 0.4 kg/d) were not affected ($P > 0.05$) by dose of CLA. On d 5 of supplementation, milk fat percentage decreased linearly ($P < 0.05$) with increasing dose of CLA (3.08, 2.85, 2.83, 2.46 and 2.29% for 0, 10, 25, 50 or 100 g CLA, respectively). Milk fat depression was associated with a linear ($P < 0.01$) reduction in C6:0, 8:0, 10:0 and 12:0 fatty acids. Milk fat content of $c9$, $t11$ and $t10$, $c12$ isomers of CLA increased linearly (4.9 to 6.4 mg/g fat; $P < 0.01$) and quadratically (0.3 to 1.3 mg/g fat; $P < 0.05$), respectively, with increasing dose. Linear effect of dose on milk fat and quadratic effect of dose on $t10$, $c12$ CLA suggest maximal milk fat depression was not achieved by feeding 100 g (21.1g $t10$, $c12$) of CLA.

Key Words: Conjugated Linoleic Acid, Milk Fat Depression

694 Response of milk fat to intravenous administration of the trans-10, cis-12 isomer of conjugated linoleic acid (CLA). S. Viswanadha*, T. W. Hanson, J. G. Giesy, and M. A. McGuire, *University of Idaho, Moscow.*

Abomasal infusion of commercial mixtures of CLA has been shown to decrease milk fat synthesis. Ten g/d of the trans-10, cis-12 isomer of CLA has been shown to cause milk fat depression when infused abomasally. Our objective was to determine the dose dependent response to intravenous administration of the trans-10, cis-12 isomer of CLA. Four multiparous Holstein cows averaging 123 ± 30 DIM were randomly assigned to treatments in a 4 × 4 Latin square design. Catheters were inserted into the jugular vein for infusions and blood sampling. Treatments consisted of intravenous infusions of 0, 2, 4 and 6 g/d CLA (>95% trans-10, cis-12 CLA, Natural Lipids, Norway). Infusates contained 72 g/d 10 % Intralipid (Baxter; Deerfield, IL), saline and CLA to 90 ml. They were divided into six equal parts and infused every 4 h. Periods were of 5 d duration with a 7 d wash out. Milk samples were obtained twice daily and analyzed for fat, protein and CLA. Dry matter intake (23 ± 2.4 kg/d), milk yield (29.5 ± 3.3 kg/d) and protein percent (3.26 ± 0.084%) were not affected (P>0.05) by treatment. However, milk fat percent was reduced (P<0.05) and the response was linear to the dose of CLA infused. Milk fat percent was 4.02, 3.86, 3.29 and 2.92% on d 5 for treatments 0, 2, 4 and 6 g/d CLA. Concentrations of cis-9, trans-11 (3.66 mg/g fat) and trans-10, cis-12 CLA in milk fat were not affected by treatment. Milk fat contained 0.86, 0.95, 0.93 and 2.03 mg of trans-10, cis-12 CLA per gram of fat (SE=0.065) for treatments 0, 2, 4 and 6 g/d CLA respectively. Efficiency of incorporation of trans-10, cis-12 isomer of CLA into milk fat was calculated to be 28.5% for the 6 g/d treatment. Intravenous infusion of the trans-10, cis-12 isomer of CLA depressed milk fat in a linear manner over the range of infusion studied. However, concentration of CLA in milk fat was unaffected by treatment.

Key Words: Conjugated linoleic acid, Milk fat, Trans-10, cis-12 CLA

695 The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA and other Δ^9 desaturated fatty acids in milk fat. B. A. Corl*[1], L. H. Baumgard[1], D. A. Dwyer[1], J. M. Griinari[2], B. S. Phillips[3], and D. E. Bauman[1], [1]*Cornell Univ., Ithaca, NY,* [2]*Helsinki Univ., Finland,* [3]*NCAUR, ARS/USDA, Peoria, IL.*

Biomedical studies with animal models have shown *cis*-9, *trans*-11 conjugated linoleic acid (CLA), the predominant isomer in milk fat, has anticarcinogenic effects. We recently demonstrated that a portion of milk fat CLA originates by endogenous synthesis from *trans*-11 $C_{18:1}$ by Δ^9-desaturase (Corl et al., J. Anim. Sci. 77 (Suppl. 1):118). Our present objective was to examine the contribution of endogenous synthesis of CLA by inhibiting Δ^9-desaturase with sterculic oil (SO) and by increasing exogenous supply of substrate with addition of partially hydrogenated vegetable oil (PHVO). In a 4 x 4 Latin square design with lactating dairy cows (115 $\pm$ 9 DIM, mean $\pm$ s.d.), treatments were 1) control (C), 2) SO (10 g/d), 3) PHVO (250 g/d) and 4) SO+PHVO. Treatment periods were 4 d and both SO, prepared as an emulsion in skim milk, and PHVO were abomasally infused (4x/d). Treatments had no effect on milk yield (38.7 kg/d), milk protein yield (1.07 kg/d) or DMI (26.4 kg/d), but milk fat yield was decreased 14.3% by SO and PHVO treatments. As expected, milk fatty acid composition was altered. The PHVO treatment increased milk fat concentration of *trans*-11 $C_{18:1}$ (39.0%) and CLA (17.3%) compared to C. Addition of sterculic oil caused the opposite result; CLA was reduced by 65.3% for the SO vs C treatments and by 61.1% for the SO+PHVO vs PHVO treatments; this represents a minimal estimate of the endogenous contribution. The inhibition of Δ^9-desaturase was incomplete and using a correction factor derived from $C_{14:1}$ indicated a maximum of 78% of the milk fat CLA was of endogenous origin. Overall, data clearly demonstrate that the majority of CLA in milk fat arises from endogenous synthesis via Δ^9-desaturase. Data also indicate that Δ^9-desaturase is involved in the formation of other milk fatty acids including *cis*-monoenes from saturated fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$) and conjugated and non-conjugated dienes from *trans* isomers of $C_{18:1}$.

Key Words: milk fatty acids, desaturase

696 Effects of duodenal infusion of graded amounts of Leu on mammary uptake and metabolism in dairy cows. H. Rulquin*[1] and P.M. Pisulewski[2], [1]*UMRPL INRA, St Gilles, France,* [2]*Agricultural Univ., Cracow, Poland.*

Leu is one of the proposed limiting amino acids for dairy cow. However, its requirement and the dynamic of its metabolism in the mammary gland are poorly documented. A 4×4 Latin square was realized to study effects of duodenal infusion of graded amounts of Leu (0, 40, 80, and 120 g/d) during 4 days on mammary uptake and milk secretion in Holstein cows. Diet covered 100 and 75% of energy and protein requirements. An extra duodenal infusion of 430 g/d of free Leu mixture of Met, Lys, Thr, Phe, Trp, Val, Ile, His, Arg, Tyr and Glu was used to meet 110% of protein requirements. Supply of Leu provided 75, 100, 125, and 150% of the expected requirements for the 4 treatments respectively. Cows equipped with a duodenal cannula and an ultrasonic blood flow probe fitted around a pudic artery were sampled for arterial and mammary venous blood 6 times during 12 hours. Milk yield was unaffected by infusions. Milk protein secretion increased significantly to a maximum between the second and the third doses (570, 652, 648, and 624 g/d). Arterial concentrations of Leu in plasma increased linearly with amounts infused (0.55, 1.40, 1.88, and 2.12 mg/100 ml). Mammary extraction rate linearly decreased (72, 47, 44, and 36%), whereas mammary uptake increased (13.7, 19.4, 26.8, and 23.4 mg/min for a half udder). Uptake of other essential amino acids followed that of Leu. Ratio of Leu plasma uptake to milk output increased to a maximum for the third dose (1.05, 1.31, 1.87, and 1.66). It was concluded that 1) mammary uptake of Leu is closely related to arterial level, 2) maximum mammary protein synthesis is achieved when the uptake of Leu is 1.31 times the Leu milk output, 3) optimum of dietary Leu concentration is located between 8.87 and 11.1% of Leu truly digested in the small intestine (LeuDI) in proteins truly digested in the small intestine (PDI).

Key Words: Leu, Mammary metabolism, Dairy cow

697 Effects of duodenal infusion of graded amounts of His on mammary uptake and metabolism in dairy cows. H. Rulquin*[1] and P.M. Pisulewski[2], [1]*UMRPL INRA, St Gilles, France,* [2]*Agricultural Univ., Cracow, Poland.*

Histidine is proposed as the third limiting amino acid for dairy cows after Met and Lys. However, its requirement and the dynamic of its metabolism in the mammary gland are poorly documented. A 4×4 Latin square was used to study effects of duodenal infusion of graded amounts of L-His-HCl-H_2O (0, 36, 55, and 74 g/d) during 4 days on mammary uptake and milk secretion in Holstein cows. Diet covered 100 and 75% of energy and protein requirements respectively. An extra duodenal infusion of 430 g/d of free His mixture of Met, Lys, Thr, Leu, Trp, Val, Ile, Phe, Arg, Tyr and Glu was used to meet 110% of protein requirements. Supply of His provided 75, 100, 125, and 150% of the expected requirements for the 4 treatments respectively. Cows equipped with a duodenal cannula and an ultrasonic blood flow probe fitted around a pudic artery were sampled for arterial (carotid) and venous blood (subcutaneous) 6 times during 12 hours. Milk yield was unaffected by infusions, but protein yield increased linearly (610, 675, 694, and 720 g/d). True protein concentration of milk increased significantly to a plateau at the second dose infused (2.80, 3.07, 2.97, and 3.03%). Arterial concentrations of His in plasma increased linearly (0.17, 1.27, 1.25, and 1.50 mg/100 ml). Mammary extraction rate decreased dramatically to a minimum at the second dose infused (55, 16, 15, and 15%) whereas mammary uptakes increased linearly (5.51, 11.64, 11.88, and 14.03 mg/min for a half udder). Ratio of plasma uptake of His to milk increased linearly (0.90, 1.12, 1.18, and 1.24). Mammary flow of plasma decreased linearly (7.19, 4.93, 4.69, and 4.77 L/min for a half udder). It is concluded that 1) mammary uptakes of His is related to arterial level of His, 2) optimum milk protein yield is obtained when mammary uptake is 1.2 times the milk output, 3) optimum of dietary His concentration is located between 3.4 and 5.6% of His truly digested in the small intestine (HisDI) in proteins truly digested in the small intestine (PDI)

Key Words: His, Mammary metabolism, Dairy cow

698 Compensatory nutrition regulation of β-casein gene expression in HC11 mammary epithelial cells. J.W. Schroeder*, W.K. Keller, and C.S. Park, *North Dakota State University, Fargo.*

Energy restriction and realimentation imposed during developmental stages enhances mammary growth and differentiation. Objectives were to develop an understanding of cellular mechanisms that modulate proliferation and differentiation in HC11 murine mammary epithelial cells in culture. In two separate studies, cells were seeded and grown in a humidified environment at 37°C and 5% CO_2, until confluent, in medium consisting of RPMI-1640 with L-glutamine, 10% fetal bovine serum, 5 μg/mL insulin, 10 ng/mL epidermal growth factor and 50 μg/mL gentamicin. Cells were then preincubated for 24 h, but without epidermal growth factor. The subsequent induction medium was growth medium without epidermal growth factor, plus 0.1 μM dexamethasone and 5 μg/mL prolactin. Control medium contained 2 mg/mL glucose, treatment medium contained 0.5 mg/mL glucose, and realimentation medium contained 3 mg/mL glucose. In Study One, glucose was restricted during the induction phase. Cell proliferation of treatment cells was reduced 32% following energy restriction, while treatment cells had 33% greater proliferation than that of the control following refeeding. Protein synthesis was not different for the treatment cells after restriction, but was 6.5% greater following realimentation. Protein secretion levels were greater for the control after 96 h. Gene expression of β-casein and poly(ADP-ribose) polymerase was not affected by glucose level during cell induction. In Study Two, glucose levels were modified during logarithmic growth. Treatment group cell proliferation tended to be higher than that of the control at the onset, but the means were not different. Protein synthesis tended to be higher in treated cells than in control cells, but protein secretion levels were not different. Transcript levels of poly(ADP-ribose) polymerase, γ-glutamyl transpeptidase, and xanthine oxidoreductase were different at 48 h of induction only; ornithine decarboxylase displayed similar trends in gene expression, but levels were not different. Results indicate that energy restriction and realimentation during the induction phase altered cell proliferation and protein synthesis. When energy modulation was employed during rapid cell growth, subsequent alteration in cell proliferation and protein synthesis and secretion were more subtle.

Key Words: HC11 cell line, Proliferation, Differentiation, Compensatory nutrition

699 Linoleic acid upregulates expression of mFABP and CD36 in bovine mammary cells. R.C. Gorewit *[1], [1] *Cornell University, Ithaca, NY.*

Fatty acid binding proteins (FABPs)are involved in fatty acid metabolism, and growth and differentiation of a variety of tissues. CD36 is a glycoprotein cell adhesion molecule (CAM) thought to be involved in the proliferation and differentiation of a variety of cells. It is in very high concentrations in milk fat from lactating cows. Expression of FABP and CD 36 are upregulated by certain fatty acids, termed peroxisome proliferators in liver and adipose tissues. The purpose of our study was to determine if linoleic acid would up regulate the expression of mFABP and CD36 in cultured bovine mammary cells. Bovine mammary cells, isolated from lactating cows and cloned as epithelial cells by micromanipulation, were used. They were grown to confluency in Dulbecco's modified Eagles medium containing 10% horse serum. After confluency, cells were exposed to the following: 20ul of 40 % ethanol- 60% medium, linoleic acid 20 ul (400nM). Dishes were incubated for 12 hrs. After incubation, cells were washed with medium, and collected using a rubber policeman, centrifuged in a microcentrifuge and solubilized in Triton X-100, buffered at pH 7.4, and subjected to SDS- PAGE electrophoresis and Western immunoblotting. Total mRNA was isolated from cells for Northern blot analysis. An increase in FABP and CD36 protein and mRNA expression were seen from cells incubated with linoleic acid compared to controls. These data indicate that linoleic acid upregulates expression of mFABP and CD36, two factors that are involved in growth and differentiation of mammary cells.

Key Words: Linoleic acid, FABP, CD36

700 Immunoreactive BRCA1 is localized in bovine mammary epithelium, milk and milk fat globule membranes. Y Chung[1], V.L. Spitsberg[2], and R. C. Gorewit*[1], [1] *Cornell University, Ithaca, NY,* [2] *BioVita Technologies.*

BRCA1 is the first breast cancer susceptibility gene that has been isolated, and structural defects of the gene increase early onset breast and/ or ovarian cancer. There is controversy with regard to the localization of BRCA1 within the cell and whether or not it is a secreted protein. Therefore, we determined if immunoreative BRCA1 was secreted into human and cows milk and examined its cellular localization in bovine mammary tissue. Milk fat globule membranes were isolated from fresh bovine and human milk. Proteins were isolated by SDS PAGE , transferred to nitrocellulose membranes and incubated with mouse monoclonal anti-BRCA1 antibody and subsequently incubated with secondary antibody. For immunolocalization, mammary tissues from lactating and involuting cows were fixed and parafin embedded. Eight micron serial sections were prepared. Slides were deparafinized and incubated with human specific anti-BRCA1 antibodies. Immunoreactive BRCA1 was detected in MFGM from human and cow milk. The size of bovine BRCA1, was identical to the human. It was a full length 220 kDa protein. Immunohistochemistry showed the protein localized in the nucleus, cytosol, milk and milk fat globule membrane. Staining was specific for epithelial cells. These results strongly suggest that BRCA1 is secreted into milk as a component of the MFGM and is located specificly in the mammary epithelial cell.

Key Words: BRCA1, Epithelial cells, MFGM

701 Bovine mammary BRCA1 is differentially expressed through various physiological stages. Y Chung[1] and R.C. Gorewit*[1], [1] *Cornell University, Ithaca, NY.*

BRCA1 is necessary for the growth of various tissues and appears to be involved in tumor suppression. The ability of the BRCA1 C-terminus domain to stimulate p21 provides direct evidence of its role as a transcriptional activating factor. Cross talking with other proteins, such; as Rad51 and BARD1 implies that it takes part in DNA repair during the S-phase. The role of BRCA1 in normal mammary development is not clear. We examined BRCA1 gene expression in bovine mammary tissues from heifers during pregnancy and cows in early, mid and late lactation and involution. Mammary tissue biopsies were collected and immediately frozen in liquid nitrogen for total RNA isolation. Total RNAs were isolated by Tri reagent and assayed for expression by RNase Protection Assay (RPA). BRCA1 transcript expression increased during pregnancy and reached its peak during terminal mammary differentiation in late pregnancy. In cows, levels of expression increased as lactation advanced and the levels remained high until mid-lactation. The expression levels dramatically decreased in late lactation and remained low during involution. Our data suggests that BRCA1 is differentialy expressed during pregnancy and lactation in cattle and that the protein is likely involved in normal bovine mammary growth and differentiation.

Key Words: BRCA1, Lactation, Pregnancy, Involution, mRNA

702 Lipid synthesis by primary cultures of bovine mammary epithelial cells. E. Matitashvili*[1] and D. Bauman[1], [1] *Cornell University, Ithaca, NY.*

Lipid synthesis by primary mammary epithelial cells isolated from lactating cows was studied in different culture conditions. The cells were cultured in serum-free medium containing insulin, prolactin and hydrocortisone using attached or floating calf tail collagen, or complex of extracellular matrix proteins (ECM) isolated from lactating bovine mammary gland. The rate of lipid synthesis assessed by ^{14}C-acetate incorporation had approximate ratio 1: 1.7 : 2 for cells cultured on attached collagen : floating collagen : ECM. Overall, the degree of cellular differentiation had a major impact on the percent of secreted lipids, on the distribution of labeled acetate among different lipid classes and on the level of expression of mRNA for acetyl-CoA carboxylase, glycerol phosphate acyltransferase, acylglycerol phosphate acyltransferase, stearoyl-CoA desaturase and fatty acid-binding protein. The percent of secreted lipids ranged from 22 to 64%, being lowest for cells cultured on attached collagen gels. Thin layer chromatography showed that triglycerides were predominant lipid class in cells cultured on all substrata but their proportion increased from 59 to 79% in more differentiated cells. The rate of ^{14}C acetate incorporation in cells cultured on floating collagen gels was stable for 5 days, although percent of secreted lipids decreased slightly at the end of culture. The expression of all above mentioned proteins was higher in more differentiated cells and was stable for at least 6 days in cells cultured on floating collagen gels and on ECM.

Key Words: Mammary, Culture, Lipid

703 Effect of different isomers of C18:1 and C18:2 fatty acids on lipogenesis in bovine mammary epithelial cells.. E. Matitashvili*[1] and D. Bauman[1], [1] *Cornell University, Ithaca, NY.*

Primary bovine mammary epithelial cells isolated from an early lactating cow were plated on collagen gels and incubated in medium containing 10 % fetal bovine serum for 24 h. The cells were cultured in serum-free and fatty acid-free medium for another 24 h. Treatments were then added with a fresh portion of the same medium. Treatments consisted of C18:1,c9; C18:1,c11; C18:1,t11; C18:2,c9t11; C18:2,t10c12 fatty acid isomers (200 μM) complexed to BSA (3:1). Treatments continued for either 6 h or for 72 h. C18;2,t10c12 inhibited ^{14}C-acetate incorporation into cellular lipids by about 60 % mimicking the inhibition of milk fat synthesis seen in dairy cows abomasally infused with this isomer. Some of the key enzymes involved in milk lipid synthesis: acetyl-CoA carboxylase (ACC), glycerol phosphate acyltransferase (GPAT) and acylglycerol phosphate acyltransferase (AGPAT) were not greatly affected by any of the treatments including C18:2,t10c12. Therefore, it seems likely that fatty acid synthetase (FAS) could be the major site of alterations in the biochemical pathway of milk lipid synthesis in the cells treated with this isomer, although it remains to be confirmed. C18:1,t11 stimulated expression of mRNA for stearoyl-CoA desaturase (SCD) almost threefold. This could potentially lead to increased synthesis of C18:2,c9t11 in treated cells. Surprisingly, C18:1,c11 had no effect on SCD expression. None of the treatments altered expression of the κ-casein gene.

Key Words: Fatty acid, Mammary, Culture

704 Effects of insulin and insulin-like growth factor (IGF)-I on casein and IGF-binding protein production in primary cultures of bovine mammary cells. M. D. Hanigan*[1], F. Cheli[2], J. C. Chow[1], W. Y. Kim[1], D. M. Carlson[1], C. C. Calvert[1], and R. L. Baldwin[1], [1] *University of California, Davis,* [2] *University of Milan, Italy.*

Insulin and IGF-I have been observed to stimulate milk protein synthesis. They also stimulate mammary blood flow thereby delivering more metabolites to the udder. It is possible that these endocrine effects

are mediated entirely by changes in substrate supply. The objective of this work was to test whether these endocrines would stimulate α-casein secretion rates in the absence of a change in metabolite supply. Two experiments were conducted, one with tissue slices taken from 2 lactating cows and the second using isolated alveoli derived from 4 lactating cows. When plated on rat-tail collagen gels, these cells secreted α-casein into media (ELISA) for a minimum of 17 d. There were no detectable differences in ^{3}H-proline incorporation into total protein when tissue slices were exposed for 2 h to 5 ng insulin/ml, 5 ng insulin plus 50 ng IGF-I/ml, or 1 μg insulin/ml. In the 2nd experiment, there were no detectable differences in α-casein secretion among treatments when confluent cells were treated for 2 d with 1 ng insulin/ml (LI), 1 ng insulin plus 200 ng insulin-like growth factor-I/ml (IGF), or 1 μg insulin/ml (HI). α-Casein secretion was not found to be correlated with DNA mass. No significant differences in DNA mass per well were observed. The IGF treatment resulted in significantly greater ^{3}H-proline incorporation into secretory protein as compared to HI (P=.06) but not as compared to LI. Production of insulin-like growth factor binding protein (IGFBP)-2 and the 40K form of IGFBP-3 (ligand blot assay) was significantly enhanced by IGF (P=.07) as compared to HI but not LI. Production of IGFBP-2 was significantly enhanced by HI as compared to LI (P=.10). Insulin and IGF-I appear to play a role in regulating IGFBP production and total secretory protein synthesis in mammary tissue. There were no detectable effects on rates of α-casein secretion.

	LI	IGF	HI	SE
α-casein (μg/well/d)	.79	1.13	1.03	.21
^{3}H-Proline (cpm/well/d)	$4881^{x,y}$	6736^{x}	3397^{y}	1307
DNA (μg/well)	2.26	1.39	1.69	.74
IGFBP-2[b]	$.19^{x}$	$.30^{x,y}$	$.41^{y}$	.11
IGFBP-3 (40K)[b]	$.021^{x,y}$	$.034^{x}$	$.016^{y}$	.008
IGFBP-3 (43K)[b]	.077	.061	.069	.038

[a] LSM [b] Scanning densitometry units [x,y]Means in the same row with different superscripts differ (P<.10)

Key Words: Milk Protein, Insulin, Insulin-like Growth Factor-I

705 An ultrasonographic method for estimating mammary cistern volume after different milking intervals in dairy cows.

M. Ayadi[1], G. Caja*[1], X. Such[1], and C.H. Knight[2], [1]*Universitat Autonoma de Barcelona, Bellaterra, Spain* , [2]*Hannah Research Institute, Ayr, UK*.

Four lactating Holstein cows (3 multiparous and 1 primiparous; average milk yield: 20±3 l/d) were used to define a methodology for the estimation of the area of udder cisterns (*Sinus lactiferi*) by B-mode real time ultrasonography and to study the evolution of milk stored in the gland at different milking intervals. A sectorial transducer probe (5MHz, 80° scanning angle) placed in close parallel to the teats and towards the udder was used to obtain vertical cut pictures in two perpendicular planes (A, 0°; and B, 90°). Udder scans A and B in duplicate for each udder quarter were taken randomly at 4, 8, 12, 16, 20 and 24h intervals. After scans, cisternal milk was evacuated by using a teat cannula after the injection of an oxytocin receptor blocking agent (10mg/kgBW Atosiban®). Alveolar milk from each quarter was obtained by machine milking using oxytocin. Scans were analyzed using specialized software. Glandular parenchyma (echogenic) and lumen full of milk of the cisterns (anechoic) were in evidence in the scans. Measured areas (3423 to 42274 pixels) and evacuated milk (0.09 to 2.14 l) in the cistern of each quarter increased in parallel with time showing a curvilinear evolution with saturation after 16h. No differences were observed between A and B scans which were highly correlated (r=0.92, P<0.01) and were, therefore, averaged for each quarter. Correlation between cisternal milk and area showed the highest values in the 8 to 12 h milking interval (r=0.84 to 0.88; P<0.01) as a consequence of milk storage pattern. Alveolar milk (0.62 to 3.26 l) increased rapidly between 4 and 16 h and thereafter was steadily constant. We conclude that, the proposed ultrasonographic methodology gives a satisfactory estimation of cisternal milk between 8 and 12 h after milking and it can be used as a non-invasive method in the dynamic studies of dairy cow mammary glands.

Key Words: Ultrasonography, Cisternal Milk, Udder

706 Interbreed differences in cisternal and alveolar milk partitioning in the udder according to yield in dairy sheep.

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With the aim of studying the interbreed differences in machine milkability, a sample of 10 Manchega (MN, 0.93 l/d) and 10 Lacaune (LC, 1.87 l/d) ewes were used to compare the milk partitioning in the udder at mid lactation (90 DIM). Milk fractions and cisternal area were measured in the milking parlor at the p.m. milking, with (ATO) or without (CON) a previous i.v. injection of an oxytocin receptor blocking agent (Atosiban®; 10 μg/kgBW) when animals were in the stalls, to evaluate the spontaneous milk ejection reflex. Cisternal area was estimated by ultrasonography using a sectorial transducer (5MHz, 80°). Cisternal milk was drained by using a teat cannula. Finally, alveolar milk was machine milked after an i.v. injection of extra oxytocin (4IU) in order to empty the udder. According to differences in daily milk yield, both groups differed (P<0.001) in cisternal milk (LC, 269ml; MN, 120ml) and cisternal size (LC, 24cm^2; MN, 13cm^2) but not in alveolar milk (LC, 102ml; Mn, 95ml; P>0.05). Correlation of estimated areas in the scans and cisternal milk was r=0.79 (MN, 0.82; LC, 0.50). Atosiban showed differences in milk partitioning depending on the breed. Cisternal values were similar in MN ewes (CON, 12.4cm^2 and 122ml; ATO, 13.1cm^2 and 118ml; P>0.05), but differed in LC (CON, 24.0cm^2 and 299ml; ATO, 23.3cm^2 and 239ml; P<0.01), for area and drained milk, respectively. Consequently, alveolar milk was similar in MN ewes (CON, 86ml; ATO, 104ml; P>0.05) and different in LC (CON, 89ml; ATO, 115ml; P<0.001). We conclude that milk partitioning can be done in MN ewes without the use of a blocking agent, but not in LC ewes, which showed spontaneous milk ejection in the milking parlor. Despite the differences in milk yield, alveolar milk was the same in both breeds, indicating the main role of the cisterns in milk secretion of dairy sheep. Values of milk partitioning were in accordance to the known milkability of each breed, the LC giving higher machine milking fraction.

Key Words: Dairy Ewes, Oxytocin, Milk Ejection

707 Postweaning changes in the porcine mammary gland parenchymal wet weight.

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The objectives were to characterize the change in parenchymal wet weight of mammary tissue in sows which occurs following weaning and to determine the effects of administration of estrogen on postweaning mammary gland regression. Forty-five primiparous sows were used in this study. Litter size was set to 10 piglets on d 1 of lactation by cross-fostering. Estrogen vehicle or estrogen vehicle plus estrogen (.25 mg / kg BW / day) was administered to sows twice daily in equal doses beginning at the time of weaning on d 21 of lactation and continuing until slaughter of the sow. Sows were slaughtered on d 0 (d 21 of lactation), 2, 3, 4, 5, or 7 of involution. Functional and nonsuckled teats were identified before mammary glands were collected at slaughter. Mammary glands were trimmed of skin and extraneous fat pad, and each gland was individually weighed. Only glands which had been suckled throughout lactation were included in this analysis. Mammary gland wet weight decreased (P < .01) from weaning (485.7 ± 8.2 g at d 0 of involution) through 7 d postweaning (159.6 ± 9.5 g at d 7 of involution). Rate of decline in mammary wet weight for the 7 d postweaning period was 50.5 ± 1.9 g / d for the nonestrogen treated sows (P < .0001) and 45.9 ± 1.9 g / d for estrogen treated sows (P < .0001). Decline in mammary wet weight during involution was slower for estrogen-treated sows compared with nonestrogen treated sows (P < .02). The major difference between mammary wet weights of suckled glands of estrogen-treated (397.5 ± 13.2 g) vs. nonestrogen-treated sows (316.5 ± 12.8 g) was observed at d 2 of involution (P < .01). Mammary parenchymal wet weight decreases by about two-thirds during the initial 7 d postweaning. The rate of regression is decreased by treatment with estrogen, particularly in the period from weaning to d 2 of involution. Mammary involution in sows after weaning is an important part of the normal cycle of mammary growth, lactation and regression.

Key Words: Sow, Mammary Gland, Involution

708 Milk composition in early lactation is affected by expression of a bovine α-lactalbumin transgene in sows. M. S. Noble*, G. T. Bleck, J. B. Cook, M. B. Wheeler, and W. L. Hurley, *Department of Animal Sciences, University of Illinois, Urbana*.

The objective was to determine the effect of bovine α-lactalbumin (α-LA) in transgenic sows on milk composition in early lactation. Colostrum and milk samples were collected from first parity α-LA transgenic sows (n = 8; transgenic) and their non-transgenic full-sibling littermates (n = 10; control). Colostrum and milk samples were analyzed for concentrations of lactose, total solids, protein and fat. Lactose concentration was greater (P = 0.05) in milk of transgenic sows during the first week of lactation, when compared with milk from control sows. Lactose concentration was not different between transgenic and control sows after d 7 postpartum. Total solids, protein and fat concentrations of colostrum from transgenic sows were present at lower concentrations (P < 0.1) as compared with colostrum from control sows. By 48 hours postpartum, total solids, protein and fat concentrations were no longer different in milk of transgenic and control sows. Increased expression of α-LA in the peripartum period of transgenic sows may result in increased lactose concentration and decreased concentration of the other major components of colostrum and milk during early lactation. Although lactose is the major osmole in milk, other osmoles also contribute to the osmotic balance, particularly in colostrum which has a high protein concentration. Increased synthesis of lactose in the peripartum period may increase the contribution of lactose to the osmotic balance of colostrum. This in turn may lead to greater water content of colostrum, resulting in dilution of other colostral solids. Expression of the α-LA transgene in first parity sows has a significant impact on milk composition early in lactation.

Key Words: milk composition, transgenic, swine

709 Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected canola seeds. C. E. Ahnadi[1], L. Delbecchi*[1], J. J. Kennelly[2], and P. Lacasse[1], [1]*Dairy and Swine R&D Center, Lennoxville, QC, Canada,* [2]*University of Alberta, Edmonton, AB, Canada*.

Six Holstein cows in mid-lactation were fed a total mix ration supplemented with either 6% canola meal (control), or 4.5% unprotected canola seeds plus 1.5% canola meal (UCS), or 6% formaldehyde-protected canola seeds (PCS). The control, UCS, and PCS supplements contained respectively 6.7%, 45.4%, and 25.5% of lipid, mainly composed (60%) of C18:1. Treatments were applied according to a double 3X3 Latin square design with 3-week periods. Blood and milk samples and mammary biopsies were harvested during the last week of each period. Feeding UCS and PCS supplements slightly decreased (P<0.05) milk production, milk protein content, and milk protein yield, without affecting milk fat content and yield (P>0.1). Blood concentrations of C18:0 and C18:1t11 were increased by UCS (P<0.05) but not by PCS (P>0.25), suggesting that formaldehyde protection against ruminal biohydrogenation was efficient. Feeding canola seeds increased C18:1c9 in blood (P<0.01) and this increase was greater with PCS (P<0.05). NEFA content in serum was significantly affected by the type of supplement used, with values of 71.4 μEq/l, 77.4 μEq/l, and 85.0 μEq/l for control, UCS, and PCS, respectively (P<0.05). UCS and PCS treatments decreased the proportion in milk fat of short chain fatty acids (P<0.01) and increased that of C18:1c9 (P<0.01) which averaged 22.7%, 26.6% and 27.6% for control, UCS and PCS, respectively. UCS enriched milk fat in C18:0 (P<0.05) which averaged 13.4%, 15.4% and 13.7% for control, UCS and PCS, respectively. Accordingly, a higher C18:1c9 : C18:0 ratio was observed in milk fat during PCS feeding (P<0.01). PCS resulted in a higher milk fat content of polyunsaturated fatty acids (P<0.01). Analysis of acetyl-CoA carboxylase (ACC) gene expression in the mammary gland using semi-quantitative RT-PCR showed an higher ACC expression (P<0.05) with PCS than with UCS. RT-PCR analysis of delta-9 stearoyl-CoA desaturase (SCD) expression showed that PCS induced significantly (P<0.05) higher levels of SCD expression than control. This enhanced SCD expression, together with the dietary effect of the supplement itself, could explain the higher C18:1c9 : C18:0 ratio obtained with PCS. Project supported by Dairy Farmers of Canada.

Key Words: Milk fat, Lipogenic enzymes, Canola

710 Influence of conjugated linoleic acid (CLA) enriched cheese on body composition. T. R. Dhiman*[1], I. S. MacQueen[1], D. J. McMahon[1], J. L. Walters[1], and M. W. Pariza[2], [1]*Utah State University, Logan,* [2]*University of Wisconsin, Madison*.

A study was conducted to determine the influence of feeding CLA enriched cheese on body composition. Thirty-six four week old ICR female mice in four treatments (9 mice per treatment) were fed diets containing either low CLA cheese, medium CLA cheese, high CLA cheese, or low CLA cheese plus synthetic CLA (synthetic CLA contained: 41% *cis*-9,*trans*-11 isomer and 44% *trans*-10,*cis*-12 isomer). The CLA contents of the diets were 0.12, 0.33, 0.53, and 0.53% of total fat in low, medium, high and synthetic CLA treatments, respectively. Low, medium and high CLA cheese were prepared from milk of cows fed diets containing forage and grain in 50:50 ratio, grass hay, or grazing pasture, respectively. Experimental period was 35 days. Animals were housed in cages (3 mice/cage) and fed ad libitum. Animal weights were recorded twice a week. On day 35 of the experiment animals were sacrificed and empty carcass weights were recorded. Freeze dried carcasses were analyzed for fat, protein, ash, and fatty acid profile. Average feed intake per cage was 8.8, 9.2, 9.1, and 8.9 g/day on DM basis in low, medium, high, and synthetic CLA treatments. Weight gain during the experiment was 4.2, 7.0, 5.5, and 3.0 g in low, medium, high, and synthetic CLA treatments, respectively. The *cis*-9,*trans*-11 isomer of CLA in empty carcass was 1.1^c, 2.7^b, 3.4^a, and 1.2^c% of fat in low, medium, high and synthetic CLA treatments, respectively. The moisture, protein, fat and ash were 65.8, 17.7, 12.5, and 4.0% of empty carcass weight in high CLA treatment versus 69.4, 20.3, 5.7, and 4.6% in synthetic CLA treatment, respectively. Results suggest that feeding CLA enriched cheese did not influence the body composition in this study. However, feeding synthetic CLA to mice reduced (P >0.01) body fat (by 54%) compared with mice fed naturally enriched high CLA cheese.

Key Words: CLA, Milk, Fat

711 Mammary responses to short term close arterial infusions of selected amino acid profiles and acetate. N.G. Purdie*[1], D.R. Trout[2], J.P. Cant[2], and D.P. Poppi[1], [1]*The University of Queensland, Brisbane, Queensland, Australia,* [2]*University of Guelph, Ontario, Canada*.

A 10-hour arterial infusion of 300g amino acid, having the same profile as milk protein, increased protein and decreased fat contents of milk in lactating dairy cows (Cant *et al.* J.Dairy Sci. Vol. 80 suppl. 1,153). To test whether different responses would be obtained with an infusion of a microbial amino acid profile or with an additional energy supply, 6 multiparous cows were infused arterially with a control infusion of 0.9% saline (S), a microbial profile (M) and a milk protein profile (C), with or without 500g of acetate, in a 3x2 factorial design. Cows were fitted with left and right external iliac artery catheters and subcutaneous abdominal vein catheters. Arterial and venous blood samples were collected at hourly intervals during the last three hours of infusion. Blood flow in the iliac artery was not significantly (P<.05) different across treatments. Milk protein percentages were significantly higher in the amino acid treatments than on the control, 3.72(M), 3.70(C), 3.54(S). Milk yield was increased under amino acid regimes. Acetate infusions showed a significant reduction in milk yield (514 vs 558 g/h) and significant increases in protein, fat and lactose percentages. Under acetate infusions, local arterial concentrations of acetate increased by 119%, but extraction by the mammary glands only decreased from 76.4% to 71.3%, so the acetate uptake approximately doubled. The addition of acetate to the microbial profile caused increases in protein and lactose percentages while its addition to the milk protein profile had no measurable effects. There are no significant differences in the ability of the infused amino acid profiles to ameliorate milk component production or mammary uptake of milk precursors from the blood. Milk protein production was not energy-limited at a mammary level.

Key Words: Amino acid, Acetate, Arterial infusion

712 Mammary metabolism in cows fed graded supply of rumen-protected methionine (RPM) added to a methionine imbalanced diet. M.C. Thivierge*[1], R. Berthiaume[2], J.F. Bernier[1], and H. Lapierre[2], [1]*Universite Laval, QC, Canada*, [2]*Dairy and Swine R & D Center, QC, Canada*.

To determine the effect of RPM on the efficiency of utilization of amino acids (AA) for milk protein synthesis, three 1st and three 2nd lactation cows were used in a replicated 3 x 3 Latin square with 14-d experimental periods. Addition of 0, 36, 72 g/d of RPM (Mepron®-M85, Degussa Hüls Inc.) to the diet resulted in an estimated ratio of methionine/essential AA at the duodenum averaging 80, 136 and 192% of the recommended ratio of 0.051. Cows were fed in 12 equal meals daily at 95% of their previous ad libitum intake (17.5 kg DMI; SE 0.08). Six hourly blood samples were collected simultaneously from the artery and the mammary vein on d 14 of each period. Mammary plasma flow was estimated using the Fick principle, using Phe and Tyr concentration in arterial and venous plasma and in milk. Milk yield (32.8 kg; SE 1.14) and mammary plasma flow (499 l/h; SE 25) were not affected by RPM ($P > 0.10$). Milk CP content increased in 1st lactation cows but not in 2nd lactation cows. Methionine net flux remained constant, but as arterial concentration of Met increased, Met extraction rate by the mammary gland decreased. Uptake to output ratios indicate that the net uptake of Met and His, two AA that are not extensively oxidized in the mammary gland, remained proportional to their secretion in milk. Adding RPM did not improve significantly the utilization of BCAA by the mammary gland, as for other essential AA. Globally, the udder response to Met supply was limited. The financial support of Novalait is acknowledged.

RPM; g/d:	0	36	72	SEM	[1]Lin.	Quad.
Milk CP; g/kg						
1st lact.	30.68	32.98	34.87	0.71	0.01	0.94*[2]
2nd lact.	32.11	32.24	31.76			
Met net flux; mmol/h	5.97	5.27	6.42	0.26	0.25	0.15
Met arterial; μM	17.0	21.0	32.0	1.2	$\leq$0.01	0.04
Met extraction; %	65.5	56.3	43.2	2.3	$\leq$0.01	0.54
Met uptake:output	0.98	1.03	0.94	0.02	0.19	0.08
His uptake:output	1.09	1.19	1.14	0.06	0.57	0.42
BCAA[3] uptake:output	1.81	1.56	1.50	0.08	0.25	0.52

[1]Linear & Quadratic contrasts; [2]treatment*lactation interaction; [3]Val + Ile + Leu

Key Words: Cows, Methionine, Rumen-protected

713 Effect of plasma insulin concentrations on milk protein yield in dairy cows. A. L'Esperance*[1], J.F. Bernier[1], D.E. Bauman[2], and P.Y. Chouinard[1], [1]*Laval University, QC, Canada*, [2]*Cornell University, Ithaca, NY*.

Elevated circulating insulin (5 times normal) in combination with abomasal infusion of casein stimulates milk protein yield in dairy cows. The objective of our study was to examine the dose-response of insulin on milk protein yield using the hyperinsulinemic-euglycemic clamp technique. The experiment involved 4 rumen-fistulated cows (61 $\pm$ 24 DIM) fed for ad libitum intake. The diet was formulated to meet NRC (1989) requirements, and cows were abomasally infused with casein (500 g/d) to ensure an adequate supply of amino acids. Cows received intravenous infusion of insulin at two different rates: 0.5 (LI) and 1 μg/kg BW/h (HI) during 4 d according to a crossover design. Data were analyzed using the mixed procedure of SAS. Plasma insulin concentrations were increased 2.4 and 5.3 times over the baseline when cows received LI and HI, respectively ($P < 0.01$). Glucose infusion rates required to maintain euglycemia ($\pm$10% of baseline) were 1.9 times higher for cows on HI as compared with LI ($P = 0.03$). Milk protein content increased gradually during the insulin infusion, and this increase was of a similar magnitude for both treatments. Milk yield increased during the first 3 d of infusion in cows receiving HI, then fell in day 4. Milk protein yield followed a similar pattern. Milk yield for cows receiving LI remained constant over the 4 d of infusion and milk protein yield increased slightly. Increasing plasma insulin 5 times over normal increased milk protein yield, but this level of insulin had negative impact on milk yield when maintained more than 3 d under our experimental conditions. Project supported by Novalait Inc. and FCAR Fund.

Item	Trt	D 0	D 1	D 2	D 3	D 4	Tm. Lin	Tm. Qd.	Tm. x Tm.-L	Tm. x Tm.-Q
Milk	Li	35.8	35.3	35.1	35.2	34.5	0.13	0.01	0.69	0.01
(kg/d)	HI	35.3	35.8	37.9	36.1	33.0				
Protein	LI	3.34	3.35	3.42	3.45	3.53	<0.01	0.02	0.64	0.33
(%)	HI	3.29	3.30	3.29	3.45	3.52				
Protein	LI	1193	1180	1200	1214	1217	0.73	0.09	0.77	0.03
(g/d)	HI	1152	1169	1235	1233	1154				

Key Words: Dairy cows, Milk portein, Insulin

714 Effect of plasma insulin concentrations on milk fatty acid profile in dairy cows. A. L'Esperance*[1], J.F. Bernier[1], D.E. Bauman[2], B.A. Corl[2], and P.Y. Chouinard[1], [1]*Laval University, QC, Canada*, [2]*Cornell University, Ithaca, NY*.

Recent work demonstrated that insulin regulated Δ-9-desaturase gene expression in sheep adipose tissue (Ward et al.,1998. Biochim Biophys Acta, 1391:145). The Δ-9-desaturase reaction introduces a cis-double bond between carbons 9 and 10 of fatty acids. A large portion of oleic acid (cis-C18:1) secreted in milk fat is synthesized by the action of Δ-9-desaturase on stearic acid (C18:0) in bovine mammary gland. The objective of our study was to examine the dose-response of insulin on milk fatty acid profile using the hyperinsulinemic-euglycemic clamp technique. The experiment involved 4 rumen-fistulated cows (61 $\pm$ 24 DIM) fed for ad libitum intake. The diet was formulated to meet NRC (1989) requirements, and cows were abomasally infused with casein (500 g/d) to ensure an adequate supply of amino acids. Cows received intravenous infusion of insulin at two different rates: 0.5 (LI) and 1 μg/kg BW/h (HI) during 4 d according to a crossover design. Data were analyzed using the mixed procedure of SAS. Plasma insulin concentrations were increased 2.4 and 5.3 times over the baseline when cows received LI and HI, respectively ($P < 0.01$). Glucose infusion rates required to maintain euglycemia ($\pm$10% of baseline) were 1.9 times higher for cows on HI as compared with LI ($P = 0.03$). Insulin infusions increased milk fat content of short- and medium-chain fatty acids, and decreased the proportions C18:0 and cis-C18:1 in milk fat ($P < 0.01$). The decrease in milk fat content of C18:0 was greater when cows received HI as compared with LI ($P < 0.04$). Insulin infusions decreased the ratio of C18:0 to cis-C18:1, but the decrease was of a lower magnitude when cows received LI as compared with HI ($P < 0.04$). Insulin shifted the balance between de novo and preformed fatty acids toward de novo, and stimulated the activity of Δ-9-desaturase as evidenced by the decreased ratio of C18:0 to cis-C18:1. Project supported by Novalait, Inc. and FCAR Fund.

Key Words: Milk fatty acids, Insulin, Δ-9-desaturase

715 Feeding strategies aimed at regulating fatty acid synthesis in the dairy cow. N.S. Beswick and J.J. Kennelly*, *University of Alberta, Edmonton, AB, Canada*.

Our objective was to identify dietary treatments capable of lowering milk fat percentage. We identified four dietary treatments for this purpose. Four multiparous animals were fed diets in a 4 x 4 latin square design. Mammary and adipose tissue biosies were collected. The diets were: control (50:50); high concentrate (75:25); 5 % fish oil; or 5 % safflower oil. We hypothesized that each of the treatments would result in a reduction in fatty acid synthesis. We did not observe changes in milk yield, or in the percentage of milk fat. However, the yield of milk fat was significantly reduced by fish oil treatment ($p<0.05$). There was also a trend toward milk fat yield reduction ($p<0.1$) caused by the high concentrate treatment. We analysed mRNA abundance of four key lipogenic enzymes acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LpL) and stearoyl-CoA desaturase (SCD), protein abundance for ACC and FAS, and activity for ACC and FAS in both adipose tissue and mammary gland. No significant treatment effects were observed. There were milk fatty acid compositional changes. Fish and safflower oil treatments significantly reduced the short and medium chain fatty acids. This finding demonstrates that the oils negatively affected de novo synthesis in the mammary gland. Fish oil also resulted in significant elevations in total CLA along with specific isomers. Trans fatty acids were elevated by fish oil. Milk protein was significantly reduced by the fish oil treatment. The high concentrate diet resulted in elevation of trans fatty acids. In conclusion, we demonstrated the de

novo synthesis was reduced by both the fish oil and safflower oil treatments. While there no significant changes in the lipogenic enzymes, it is possible that the changes to de novo synthesis were not large enough to be measured. Overall, the results demonstrate that there was significant potential to alter milk fat composition.

Key Words: CLA, Lipogenesis, Milk Fat Depression

716 Role of stage of lactation in the regulation of fatty acid synthesis in the dairy cow. N.S. Beswick and J.J. Kennelly*, *University of Alberta, Edmonton, AB, Canada*.

Our objective was to determine the influence of stage of lactation on fatty acid synthesis in adipose tissue and the mammary gland of the dairy cow. We examined four multiparous animals at four time points in their lactation corresponding to early (35+7d), mid (110+1d), and late lactation (270+1d), along with 30 d into the dry period. Mammary and adipose tissue biopsies were collected at each of these time points. We measured four key lipogenic enzymes: acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LpL), and stearoyl-CoA desaturase (SCD). We hypothesized that the enzymes would decrease in abundance or activity in the mammary gland and would increase in adipose tissue over the course of the lactation. We analysed the mRNA abundance of LpL and SCD, and the protein abundance and activity of ACC and FAS. The mRNA abundance of LpL in the mammary gland was not significantly affected. However, in adipose tissue this value was significantly higher in the dry period than in early lactation ($p<0.01$). With SCD, the mid lactation value for mammary gland was highest and was significantly higher than that of the dry value ($p<0.01$). In adipose tissue, the mRNA abundance was highest in late lactation and was significantly higher than in early lactation ($p<0.01$). Acetyl-CoA carboxylase activity was unaffected by stage of lactation in the mammary gland, but protein abundance was significantly higher in early lactation than in the dry period ($p<0.05$). In adipose tissue, protein abundance was not significantly influenced, but was significantly higher in the late and dry period than in the early period ($p<0.05$). Fatty acid synthase activity demonstrated no significant effect due to stage of lactation, but this was more likely a result of the very high SEM of the activity assay. Adipose protein abundance was significantly lower in early lactation than in the dry period. Milk yield and the percentage of its components were not affected by stage of lactation. However, fatty acid composition analysis revealed that de novo synthesis of fatty acids was lower during early lactation than mid and late lactation. In conclusion, we demonstrated that stage of lactation is significant in the regulation of lipogenesis in the dairy cow.

Key Words: Stage of lactation, lipogenesis

717 The effect of abomasal amino acid imbalances on milk composition in lactating dairy cattle. T.L. Weekes* and J.P. Cant, *University of Guelph, Ontario, Canada*.

Milk production responses to rumen-protected methionine and lysine supplementation of dairy rations suggest that cows often experience an amino acid imbalance. The purpose of this experiment was to define the milk composition response to an intentionally large amino acid imbalance. The experimental design consisted of a 6x6 latin square balanced for carry-over effects. Each period was five days in length. All cows were fed a diet formulated to have 8.0% crude protein and 1.6 Mcal/Kg NE_L. The six isotonic solutions infused abomasally at 8 L/d for 5 days were: 1) 3.0% saline; 2) 15% free amino acids having the profile of milk protein as a positive control; 3) positive control minus methionine; 4) minus lysine; 5) minus histidine; 6) minus leucine, isoleucine, and valine. The amino acid infusions were calculated to provide approximately one-third of the total duodenal amino acid flow. Milk protein yield was increased from 585 g/d on the saline control to 698 g/d with infusion of the complete amino acid solution. Removal of lysine, methionine, and histidine each reduced protein yield back to control levels; branched-chain amino acid removal had no effect. Milk fat yield was increased significantly from 728 g/d on the saline control to 986 and 1048 g/d on the lysine and histidine imbalances, respectively. Likewise, milk fat percentages increased from 3.4% with saline to 4.6% with the lysine imbalance and 5.3% with the histidine imbalance. Protein:fat ratios were .58 and .50 on these two treatments relative to .84 on the control. A postruminal essential amino acid imbalance decreases milk protein percentage and yield and causes a pronounced increase in milk fat secretion. Histidine removal caused the greatest imbalance effect, followed by lysine then methionine. Effects on milk composition of the amino acid infusate without branched-chain amino acids were the same as those for the positive control. Branched-chain amino acids do not limit milk synthesis.

Key Words: Amino Acid Imbalance, Milk Composition

718 The effect of long-term supplementation of conjugated linoleic acid (CLA) to dairy cows grazing tropical pasture. S. R. Medeiros[1], D. E. Oliveira[1], L.J.M. Aroeira[2], M. A. McGuire[3], D. E. Bauman[4], and D.P.D. Lanna*[1], [1]*ESALQ, São Paulo, Brazil,* [2]*CNPGL-EMBRAPA, Minas Gerais, Brazil,* [3]*University of Idaho, Moscow,* [4]*Cornell University, Ithaca, NY*.

The objective of this study was to evaluate the effects of long-term treatment with CLA on lactating cows. Twenty Zebu X Holstein cows were rotationally grazed during the summer on stargrass (Cynodon nlenfuensis var. nlenfuensis) plus 4kg/d of a high-protein supplement formulated with corn, soybean meal, wheat middlings and fishmeal to provide 110% of estimated metabolizable protein requirements. Supplement was fed from the 4th to the 9th week of lactation, twice a day, and each treatment (n=10) received either 150g/head/day of Megalac (Control) or the same amount of a Ca-protected CLA mixture (60% CLA, Church & Dwight). Below are the averages for the 4th to the 9th week of lactation. Milk production was unchanged, despite a small increase in CLA treated cows. As a result, the differences observed in yields of milk constituents are in accordance with their contents in milk. CLA decreased milk fat content by 25% and yield by 21%. This effect was observed within two days of treatment when cows were with less than 30 days in milk, which may be related to the source of substrates for fat synthesis in the mammary gland of these low producing cows. Protected CLA greatly increased protein content and yield (+13%). Because milk production was unchanged while energy secretion decrease ($P<0.05$) these results suggest Control cows could achieve their potential energy output under the conditions of this experiment.

	Control	CLA	Probability	CV(%)
Milk Yield, kg/day	16,6	17,0	0.516	8,9
Milk Fat, %	2,80	2,11	0.001	12,1
Milk Protein, %	2,79	3,07	0.025	7,9
Milk Lactose, %	4,62	4,50	0.239	4,7
Milk Solids, %	10,87	10,43	0.138	5,8
Milk Fat, g/day	458	361	0.025	19,7
Milk Protein, g/day	457	517	0.031	11,0
Milk Lactose, g/day	795	766	0.407	9,4
Milk Total Solids, g/day	1783	1769	0.901	12,8
Log SSC	5,18	5,70	0.477	19,8
Body Condition Score	3,5	3,5	0.867	7,5