

Lactation Biology: Conjugated Linoleic Acid

279 Direct assessment of the conversion of trans-vaccenic acid (TVA) to cis-9, trans-11 conjugated linoleic acid (CLA) in lactating dairy cattle. E. Mosley* and M. McGuire, *University of Idaho, Moscow.*

The utilization of ^{13}C TVA by the lactating dairy cow was investigated in Holstein cows ($n = 3$) through a bolus infusion into the abomasum of 1.5 g of trans-vaccenic- $1\text{-}^{13}\text{C}$ acid (TVA). Blood samples were taken at -24, -18, -12, -6, 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, 24, 36, 48, 60, 72, and 84 h, and milk samples were taken at -24, -18, -12, -6, 0, 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, and 84 h relative to TVA infusion. Milk and plasma lipid were extracted using chloroform:methanol. Plasma lipid was separated into triacylglycerol (TG), cholesterol ester (CE), phospholipid (PL), free fatty acid (FFA), and mono- and diacylglycerol (MDG) fractions. Lipid was methylated, converted to dimethyl disulfide and Diels-Alder adducts, and analyzed by GCMS. Enrichment was determined using a t-test for each sample time post infusion compared to samples taken at -24 h with significance declared at $P < 0.05$. Enrichment of ^{13}C in TVA of milk fat was detected at 4 (3.0%), 8 (8.3%), 12 (4.1%), 16 (2.2%), and 20 h (0.8%). Enrichment was also detected in TVA for plasma TG, FFA, PL, and MDG. In plasma TG, TVA was enriched with ^{13}C at 1 (21.2%), 1.5 (53.8%), 2 (45.1%), 3 (18.0%), and 4 h (5.7%) post infusion. Enrichment was detected in TVA at 1.5 (22.9%), 2 (21.1%), and 3 h (11.9%) in plasma FFA. TVA in plasma MDG was enriched only at 3 h (5.1%), however, plasma PL enrichment was detected from 2 h (0.5%) until 84 h (0.3%) with maximum enrichment detected at 8 h (2.7%). Enrichment of ^{13}C in cis-9, trans-11 CLA, the desaturase product of TVA, in milk fat was detected at 4 (2.6%), 8 (6.6%), 12 (3.4%), 16 (1.7%), and 24 h (0.7%). Enrichment was not detected in CLA for any plasma lipid fraction. Conversion of dietary TVA to CLA endogenously was confirmed with almost all of the conversion occurring in the mammary gland.

Acknowledgements: This project was supported by United Dairymen of Idaho and National Research Initiative Competitive Grant no. 2003-35206-13669 from the USDA Cooperative State Research, Education, and Extension Service.

Key Words: Trans-Vaccenic Acid, cis-9, trans-11 Conjugated Linoleic acid, Desaturase

280 Quantitative importance of endogenous cis-9, trans-11 conjugated linoleic acid synthesis in dairy cows. K. Shingfield*, S. Ahvenjärvi, V. Toivonen, A. Vanhatalo, and P. Huhtanen, *MTT Agrifood Research Finland, Jokioinen, Finland.*

Based on the potential benefits of conjugated linoleic acid (CLA) for human health there is a need to develop effective strategies for enhancing milk fat cis-9, trans-11 CLA concentrations. Cis-9, trans-11 CLA is derived from ruminal biohydrogenation of C18:2 n-6 and endogenous conversion of trans-11 C18:1 in the mammary gland. Most evidence to date suggests that endogenous synthesis is the main source of cis-9, trans-11 CLA, but the extent of trans-11 C18:1 bioconversion remains largely unclear. Four lactating cows were used in two sequential 4 x 4 Latin squares with 7 d experimental periods to examine milk fatty acid composition responses to abomasal infusions of CLA and C18:1 preparations enriched (g/100g fatty acids (FA)) with cis-9, trans-11 CLA (88.8) and trans-11 C18:1 (29.4). Experimental periods comprised of a 4 d infusion and 3 d interval between infusions. Treatments consisted of 0, 3, 6 and 12 g cis-9, trans-11 CLA/d (Expt. 1) and 0, 7.5, 15 and 30 g trans-11 C18:1/d (Expt. 2). Infusions of cis-9, trans-11 CLA increased linearly ($P < 0.001$) the concentration of this isomer in milk from 0.69 to 1.44 g/100g FA associated with a mean transfer efficiency of 0.389. Abomasal infusions of trans-11 C18:1 increased linearly ($P < 0.001$) milk trans-11 C18:1 and cis-9, trans-11 CLA concentrations from 1.25 to 2.82 and 0.63 to 1.26 g/100 g FA, respectively. Proportionately 0.081 and 0.214 of infused trans-11 C18:1 was recovered in milk as cis-9, trans-11 CLA and trans-11 C18:1, respectively. The relationship between the output of cis-9, trans-11 CLA with the sum of trans-11 C18:1 and cis-9, trans-11 CLA in milk of cows receiving trans-11 C18:1 infusions indicated that proportionately 0.278 of trans-11 C18:1 available to the mammary gland was bioconverted. In conclusion the relative biological value of trans-11 C18:1 avail-

able at the small intestine for incorporation and biosynthesis of cis-9, trans-11 CLA in milk fat was equivalent to proportionately 0.21 of the response to cis-9, trans-11 CLA infusions.

Acknowledgements: This work was funded by the European Union funded BIOCLA project QLK1-2002-02362.

Key Words: Conjugated Linoleic Acid, Trans Fatty Acids, Desaturase

281 Trans-10, cis-12 conjugated linoleic acid reduces milk fat synthesis in lactating sheep. A. L. Lock¹, J. W. Perfield II^{*1}, B. M. Teles², D. E. Bauman¹, and L. A. Sinclair², ¹*Cornell University, Ithaca, NY*, ²*Harper Adams University College, Newport, Shropshire, UK.*

The efficacy of trans-10, cis-12 conjugated linoleic acid (CLA) in reducing milk fat synthesis in dairy cows has been well documented. To date, there has been no examination of the effects of trans-10, cis-12 CLA on milk fat synthesis in lactating sheep. The current study was therefore designed to determine if trans-10, cis-12 CLA would inhibit milk fat synthesis in lactating sheep. Twenty multiparous milking ewes (56 ± 6.2 kg) in early lactation were blocked and randomly allocated to two treatments; grass hay plus concentrate either unsupplemented (Control) or supplemented with lipid-encapsulated trans-10, cis-12 CLA (LE-CLA; BASF AG, Ludwigshafen, Germany) at the rate of 25 g/d, providing 2.4 g/d of trans-10, cis-12 CLA. The experimental design was a 2 period crossover with 10 d treatment periods separated by a 10 d interval. Milk protein content and DMI were unaffected by treatment. Compared to the Control, the LE-CLA supplement reduced milk fat content from 6.4 to 4.9% ($P < 0.001$) and fat yield from 95 to 80 g/d ($P < 0.001$), but increased milk yield from 1471 to 1611 g/d ($P < 0.01$) and protein yield from 68 to 73 g/d ($P < 0.05$). The temporal pattern for milk fat content and yield demonstrated a progressive reduction for sheep receiving the LE-CLA supplement, reaching nadir by day 8. The reduction in milk fat yield was due to decreases in both de novo fatty acid synthesis and uptake of preformed fatty acids. Milk fat content of trans-10, cis-12 CLA was < 0.01 and 0.12 g/100 g of fatty acids for the Control and LE-CLA treatments, respectively. The transfer efficiency of trans-10, cis-12 CLA from the LE-CLA supplement into milk fat was 3.8%. In conclusion, the results of the present study demonstrate that trans-10, cis-12 CLA reduces milk fat synthesis in lactating sheep in a manner similar to that observed in lactating dairy cows. Furthermore, the energy spared by the reduction in milk fat corresponded to an increase in milk and milk protein yield. Further studies are required to verify and extend these results and to elucidate the mechanism of action for the effects observed with trans-10, cis-12 CLA supplementation.

Key Words: CLA, Milk Fat, Sheep

282 A comparison of trans-10, cis-12 CLA effectiveness at inducing milk fat depression (MFD) in early vs. established lactation. C. Moore, J. Kay, R. Rhoads, and L. Baumgard*, *University of Arizona, Tucson.*

Abomasal and close-arterial infusion of mixed CLA isomers reduces milk fat synthesis in established lactation. Long-term experiments utilizing rumen-inert (RI) CLA supplements indicate CLA induces MFD when fed to TMR or pasture fed cows during established lactation. However, similar amounts of RI-CLA appear ineffective at inducing MFD during the periparturient period and the mammary gland is insensitive to CLA until approximately the 3-4th week of lactation. We have recently demonstrated that large quantities of RI-CLA decrease milk fat synthesis immediately postpartum in both TMR and pasture fed cows. We close-arterial infused 7 g/d of purified trans-10, cis-12 CLA to investigate the differences in mammary sensitivity to CLA between established lactation and immediately postpartum. Transitioning multiparous Holstein cows ($n = 8$) fitted with indwelling jugular catheters were blocked by predicted calving date and randomly assigned to either IV CLA or control infusion (Intralipid) for the first 5 d of lactation. Transition cows were simultaneously pair infused

with mid lactation cows (n=8; 102 ± 25 DIM), and infusions started the day transition cows calved. In established lactation, CLA infusion did not alter DMI but tended to decrease (14%) milk yield and neither of these parameters were affected by CLA in early lactation. CLA infusion had little or no effect on milk protein or lactose variables in either established or early lactation. In established lactation, CLA decreased (P<0.01) milk fat content and yield (34 and 45%, respectively), but in early lactation CLA did not alter these parameters. Compared to control, milk fat *trans*-10, *cis*-12 CLA content increased during infusion but levels were similar (1.85 mg/g) between stages of lactation. In established lactation, CLA decreased (26%) and increased (24%) the milk fat content of *de novo* and preformed derived fatty acids, respectively. CLA did not alter the milk fatty acid origin in early lactation. The mammary glands fat synthesizing machinery is much less sensitive to *trans*-10, *cis*-12 CLA immediately postpartum.

Key Words: CLA, Milk Fat, Transition

283 The effect of conjugated linoleic acid on cell growth and glucose transport in bovine mammary cells. A. F. Keating^{*1,2}, F. Q. Zhao², R. J. Weselake¹, and J. J. Kennelly¹, ¹Dairy Research Group, Agricultural, Food and Nutritional sciences, University of Alberta, Edmonton, Canada., ²Lactation and Mammary Gland Biology Group, Department of animal science, University of Vermont, Burlington, VT.

Conjugated Linoleic Acid (CLA) is a naturally occurring lipid of ruminant milk and meat and is an attractive functional food due to its proposed beneficial health properties such as reducing risks for cancer, atherosclerosis and diabetes. It is, thus, a great interest to increase milk CLA levels by feeding CLA to lactating dairy animals. However, a previous study by this group had indicated that feeding certain CLA isoforms may affect mammary cell growth and induce cell apoptosis (Bell and Kennelly, 2003). For this reason, the effect of fatty acid treatment on both growth and membrane function of a bovine mammary cell line, Mac-T, was investigated. Fatty acids examined were *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, Linoleic acid, Linolenic acid and Vaccenic acid at concentrations of 15, 20, 25, 30, 35 and 37.5 µM/ml each. Results were analysed by SAS and showed that significant differences in cell growth occurred at 30 µM concentrations and above between the CLA isomers and other fatty acids, with cell death occurring due to CLA treatment (Table 1). The effect of fatty acid treatment on glucose transport in Mac-T cells showed that the CLA isomers showed increases in rates of transport compared to vaccenic acid.

Treatment	BSA	c9,t11		t10,c12		Linoleic	Linolenic	Vaccenic	p-value
		CLA	CLA	CLA	CLA				
15	2.21a	1.84a	2a	2.06a	1.8a	1.75a	0.91		
20	9.86a	6.33a	6.13a	8.08a	8.16a	11.08a	0.11		
25	7.5a	6.16a	5a	12.3a	10.33a	11.33a	0.44		
30	5.5a	2.56b	1.91b	7.75a	6.91a	8.16a	0.0008		
35	8.8a	1.33b	1.75b	6.73a	6.5a	9.33a	0.002		
37.5	5.15a	0b	0b	5.9a	5.85a	5.9a	0.0001		

Acknowledgements: This work was supported by the Dairy Farmers of Canada and the CLA Network

Key Words: Conjugated Linoleic Acid, Glucose Transport, Cell Growth

284 *Trans*-9, *cis*-11 conjugated linoleic acid (CLA) reduces milk fat synthesis in lactating dairy cows. J. W. Perfield II^{*1}, A. L. Lock¹, A. Sæbø², J. M. Grünari³, and D. E. Bauman¹, ¹Cornell University, Ithaca, NY, ²Natural ASA, Hovdebygd, Norway, ³Clanet Ltd, Espoo, Finland.

Previous investigations have established that *trans*-10, *cis*-12 conjugated linoleic acid (CLA) is a potent inhibitor of milk fat synthesis in dairy cows whereas *cis*-9, *trans*-11 CLA has no effect. However, in some cases of diet-induced milk fat depression (MFD), the formation of *trans*-10, *cis*-12 CLA in the rumen is inadequate to completely explain the observed effects. An increase in *trans*-9, *cis*-11 CLA has been observed in some instances of diet-induced MFD and our objective was to test the effect of a CLA mixture enriched in this isomer. Four rumen-fistulated lactating Holstein cows (149±18 DIM) were randomly assigned in a 4 X 4 Latin square experiment. Treatments were abomasal infusions of 1) ethanol (control), 2) *trans*-10, *cis*-12 CLA supplement (positive control), 3) *trans*-9, *trans*-11 CLA supplement, and 4) *trans*-9, *cis*-11 CLA supplement. The *trans*-10, *cis*-12 and *trans*-9, *trans*-11 CLA supplements were of high purity (>90%), whereas the *trans*-9, *cis*-11 CLA supplement consisted mainly of 3 CLA isomers: *trans*-9, *cis*-11 (32%), *cis*-9, *trans*-11 (29%) and *trans*-9, *trans*-11 (17%). CLA supplements supplied 5 g/d of the CLA isomer of interest and the daily dose was provided by infusion at 6 h intervals. Treatment periods were 5 d in length with a 7 d washout interval. Milk yield and DMI were unaffected by treatment (P > 0.05). Milk fat yield was reduced 27% by the *trans*-10, *cis*-12 CLA treatment and 15% by the *trans*-9, *cis*-11 CLA treatment, while the *trans*-9, *trans*-11 CLA treatment had no effect (P < 0.001). Milk protein content and yield were reduced by the *trans*-9, *trans*-11 CLA treatment only (P < 0.01). The transfer efficiency of specific CLA isomers within respective treatment groups was 22% for *trans*-10, *cis*-12 CLA, 21% for *trans*-9, *trans*-11 CLA and 46% for *trans*-9, *cis*-11 CLA (P < 0.001). Overall, abomasal infusion of *trans*-9, *cis*-11 CLA reduced milk fat synthesis, but to a lesser extent than *trans*-10, *cis*-12 CLA. This indicates that *trans*-9, *cis*-11 CLA may be responsible for a portion of the decreased milk fat production in some situations of diet-induced-MFD.

Key Words: Conjugated Linoleic Acid, Milk Fat Depression, Dairy Cow

285 Effects of dietary CLA on thermogenesis and body temperature indices in lactating dairy cows. M. Rhoads, R. Rhoads, L. Odens, R. Burgos, S. Baker, B. Pollard, C. Moore, J. Kay, M. VanBaale, R. Collier, and L. Baumgard^{*}, The University of Arizona, Tucson.

Dietary CLA markedly reduces milk fat synthesis without decreasing caloric intake or circulating NEFA levels in lactating dairy cows. During CLA-induced milk fat depression (MFD), bioenergetic alternatives for spared energy include increased milk synthesis, increased adipose lipogenesis or increased thermogenesis and heat loss, which was previously reported in non-lactating rodent calorimetry trials. Lactating Holstein cows (n=9) were housed in environmentally controlled chambers maintained at thermoneutral conditions (19°C, 39% relative humidity) and fed either a control diet or a CLA supplemented diet in a crossover design (two 6-d periods separated by a 5-d washout period). Diets (alfalfa-based TMR) were isoenergetic and provided either 290 g/d of a rumen inert palm oil supplement or 300 g/d of a rumen inert CLA supplement (containing several CLA isomers, including *trans*-10,*cis*-12). Temperature probes attached to CIDR devices and inserted into the vagina provided continuous (once every 5 min) core body temperature (BT) measurements during treatment periods. Shoulder, rump and tail head skin temperatures were determined twice daily. Skin and core BT are reported for the last 3 d of each period when MFD was maximized. Rectal temperatures were obtained on d 6 of each treatment period. CLA supplementation did not affect feed intake but increased milk yield (38.2 vs. 36.2 kg/d, P<0.02). CLA decreased milk fat content and yield by 34% (P<0.01) and 31% (P<0.01), respectively, but had little or no effect on milk protein and lactose parameters. Feeding CLA did not alter rectal temperature (38.5°C) or skin temperatures at the shoulder (33.8°C), rump (34.2°C) and tail head (33.4°C). Core (vaginal) BT did not differ between treatments (38.9°C). In conclusion, cows exhibiting CLA-induced MFD remained euthermic during CLA supplementation, and spared energy may have been partitioned towards enhanced milk synthesis.

Key Words: CLA, Body Temperature, Dairy Cow