Effects of Dietary Sources of Vegetable Oils on Performance of High-Yielding Lactating Cows and Conjugated Linoleic Acids in Milk

H. C. Zheng,^{1,†} J. X. Liu,¹ J. H. Yao,² Q. Yuan,² H. W. Ye,³ J. A. Ye,¹ and Y. M. Wu¹

¹Institute of Dairy Science, Zhejiang University, Hangzhou 310029, China

²Hangzhou Station for Detection of Agricultural Products, Hangzhou 310020, China

³Hangzhou Zhengxing Animal Industry Company, Hangzhou 311301, China

ABSTRACT

This study was conducted to examine the effects of dietary supplementation with vegetable oils on performance of high-yielding lactating cows and conjugated linoleic acid (CLA) content in milk fat. Twelve lactating Holstein cows in early lactation (30 to 45 d postpartum) were used in a triple 4 × 4 Latin square design. In each period, the cows in each group were fed the same basal diet and received one of the following treatments: 1) control (without oil), 2) 500 g of cottonseed oil, 3) 500 g of soybean oil, and 4) 500 g of corn oil. Each experimental period lasted for 3 wk, with the first 2 wk used for adaptation to the diet. Supplementation with vegetable oils tended to increase milk yield, with the highest milk yield in the cottonseed oil group (35.0 kg/d), compared with the control (34.4 kg/d). Milk fat percentage was decreased, but there were few effects on percentage and yield of milk protein as well as milk fat yield. The cows fed added soybean oil produced milk with the highest content of *trans*-11 C_{18:1} (23.8 mg/g of fat), which was twice that of the control (12.6 mg/g of fat). Content of *cis*-9, *trans*-11 CLA in milk fat increased from 3.5 mg/g in the control to 6.0, 7.1, and 10.3 mg/g for the cows fed oils from cottonseed, corn, and soybean, respectively. A significant linear relationship existed between *trans*-11 C_{18:1} and *cis*-9, *trans*-11 CLA. Supplementation with oils doubled the content of total fatty acids in blood plasma, with little difference between different vegetable oil sources. Octadecenoic acid content was significantly higher in blood plasma of animals fed added oils from cottonseed and soybean than those fed with corn oil and control. The plasma *trans*-11 C_{18:1} content was significantly higher in the oil-added animals than in control. Supplementation of vegetable oils tended to

improve milk production of lactating cows, and the CLA content in milk fat was significantly increased. Soybean oil seemed to be the optimal source to increase CLA production.

(Key words: vegetable oil, milk performance, conjugated linoleic acid, lactating cow)

Abbreviation key: CLA = conjugated linoleic acid.

INTRODUCTION

Conjugated linoleic acid (CLA) represents a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. The CLA are effective as anticarcinogenic, antidiabetic, and antilipogenic agents in the diet of laboratory animals (see review by Pariza et al., 2001). Recently, it has been demonstrated that *trans*-10, *cis*-12 C_{18:2} is responsible for reduced lipogenesis in the rodent (Pariza et al., 2001). In contrast, milk fat-derived *cis*-9, *trans*-11 C_{18:2} prevented growth of human mammary cancer cells more effectively than did synthetic *trans*-10, *cis*-12 C_{18:2} (O'Shea et al., 2000).

Ruminant meat and milk are the predominant natural sources of the CLA. *Cis*-9, *trans*-11 CLA, however, accounts for nearly 90% of total CLA in milk fat from cows fed typical diets (see review by Bauman et al., 1999). *Trans*-10, *cis*-12 C_{18:2} represented less than 2% of total CLA (Piperova et al., 2000). The *cis*-9, *trans*-11 CLA can be formed as a result of incomplete biohydrogenation of dietary fatty acids and by desaturase action on *trans*-11 C_{18:1} in the rumen. It can also arise from isomerization via *cis*-12, *trans*-11 isomerase produced by rumen bacteria (Kepler and Tove, 1967). In the bovine mammary gland (Bauman et al., 1999) or human tissues (Pariza et al., 2001), *trans*-11 C_{18:1} (another intermediate of biohydrogenation) can be a source for endogenous synthesis of *cis*-9, *trans*-11 CLA via Δ^9 -desaturase.

Typically, milk fat contains between 3 and 6 mg of CLA/g of fat, but the levels of CLA in milk can vary widely among herds (Kelly and Bauman, 1996). The substantial variation in content of CLA in milk fat between herds suggests that diet has a major influence.

Received December 20, 2004.

Accepted February 23, 2005.

Corresponding author: Jian-Xin Liu; e-mail: liujx@zju.edu.cn.

*Research supported in part by Ministry of Science and Technology of China and by Zhejiang Provincial Department of Science and Technology, China.

†Current address: Institute of Animal Science & Veterinary Medicine, ZAAS, Hangzhou 310021, China.

Table 1. Ingredient and composition of the basal diet.

Ingredient, % (DM basis)	
Corn silage	18.8
Ryegrass silage	6.3
Long alfalfa hay	27.5
Sugar beet pulp pellet	5.8
Ground corn grain	31.0
Full-fat cottonseed	2.0
Soybean meal	6.0
CaHPO ₄ ·2H ₂ O	1.2
Mineral-vitamin mix ¹	1.4
Composition (% of DM)	
NDF	33.7
ADF	15.6
CP	18.0
Ether extract	4.7
Crude ash	7.7
Ca	0.9
P	0.35
NE _L , MJ/kg of DM	7.42

¹Composition (per kilogram): NaCl, 178.6 g; NaHCO₃, 500 g; Mg, 20 g; Fe, 3 g; Cu, 1 g; Mn, 4 g; Zn, 4 g; Co, 20 mg; I, 80 mg; vitamin A, 1,250,000 IU; vitamin D, 150,000 IU; and vitamin E, 1000 IU.

Kelly et al. (1998) demonstrated that dietary supplementation of vegetable oils high in linoleic acid gave the greatest response, and there is a clear dose-dependent increase in milk fat content of CLA. The objective of this study was to compare the effect of dietary supplementation of different plant oils on CLA content in milk fat. The vegetable oils examined were similar in linoleic acid content but varied in proportions of C₁₈ mono-, di-, and trienes.

MATERIALS AND METHODS

Animals and Diets

Twelve multiparous Holstein cows in early lactation (between 30 and 45 d postpartum) were used in a triple 4 × 4 Latin square design to evaluate responses to supplementary vegetable oils. Treatments included a control diet and diets supplemented with oil from cottonseed, soybean, or corn. The vegetable oils were acquired from a local supermarket. Each experimental period lasted 1 wk, and was preceded by a 2-wk period of adaptation to the diet. Net energy content of diets was calculated from the ingredient composition. Diets were formulated to meet energy and protein requirements (NRC, 2001) of lactating cows producing 35 kg of milk and consuming 24 kg of DM/d (Table 1). Oils were added at 500 g/d per head, i.e., at a level of about 2% of dietary DM, resulting in a dietary ether extract content of 6.7%. The oil was added to the concentrate daily by pouring it on and mixing at feeding to ensure its equal distribution in the ration. The cows were fed and milked 3 times a day.

Sampling, Measurements, and Analyses

Forages and concentrates were sampled on d 1, 3, and 5 of each experimental period. Samples were immediately dried in a forced-air oven at 60°C, and stored in sealed plastic containers at room temperature until analyzed. In preparation for analyses, dried forages and concentrates were ground first through a 2-mm screen (Wiley; Arthur H. Thomas, Philadelphia, PA), and then through a 1-mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Forages and concentrates were analyzed for ADF (Robertson and Van Soest, 1981), NDF (Van Soest et al., 1991), and total N (AOAC, 1990; method no. 988.05).

Four 50-mL aliquots of milk were collected at each milking (0700, 1400, and 2100 h) on d 1, 3, 5, and 7 of each experimental period. One aliquot containing Bromopol (milk preservative; D&F Control Systems, San Ramon, CA) was stored at 4°C until analyzed for fat, protein, and lactose by infrared analysis (Laporte and Paquin, 1999) with a 4-channel spectrophotometer (MilkoScan, Foss Electric, Hillerød, Denmark). The second aliquot without Bromopol was stored at -20°C until the end of the experiment, then thawed and centrifuged at 10,000 × *g* for 1 h to harvest milk fat for fatty acid analysis.

For fatty acid analysis in total plasma, blood samples (10 mL) were obtained from the coccygeal artery immediately after collection of milk samples on d 7. Blood was transferred to tubes containing 286 IU of heparin in 100 µL of sterile saline and centrifuged at 3000 × *g* for 15 min to harvest plasma. Plasma was stored at -20°C until lipid extraction and fatty acid analysis.

Total lipids from plasma (2 mL) were extracted with chloroform:methanol (2:1, vol/vol). Fatty acids in vegetable oils, milk fat, and blood plasma were methylated by in situ transesterification with 0.5 *N* methanolic NaOH followed by 14% boron trifluoride in methanol (Lor and Herbein, 2001). Samples were injected by autosampler into a Hewlett-Packard 6890A gas chromatograph equipped with a flame-ionization detector (Hewlett-Packard, Sunnyvale, CA). Methyl esters from all samples were separated on a 0.32 mm × 30 m × 0.25 µm i.d. fused silica capillary column (HP-Innowax, Hewlett-Packard). Pure methyl ester standards (Sigma, St. Louis, MO) were used to identify peaks, and determine correction factors for individual fatty acids. For vegetable oils and milk fatty acid analysis (1 µL of methyl esters in hexane injected at a 1:5 split ratio), the injector temperature was maintained at 220°C and the detector temperature maintained at 225°C. For fatty acids in vegetable oils, the oven temperature was 200°C (held for 20 min); for fatty acids in milk fat, the oven temperature was 50°C (held for 1 min), and then

Table 2. Contents of medium- and long-chain fatty acids in the vegetable oils.

Fatty acids	Vegetable oils from		
	Cottonseed	Soybean	Corn
	mg/g of oil		
C14:0	6	1	0
C16:0	219	94	106
C18:0	22	24	30
C18:1	138	169	270
C18:2	496	471	452
C18:3	...	68	...

increased at 20°C/min to 220°C (held for 10 min). Ultrapure nitrogen was used as the carrier gas. All fatty acids eluted at a flow of 2.5 (for vegetable oils) and 1.9 mL/min (for milk fat).

Analysis of fatty acids in total plasma and lipid fractions required injection of 1 µL of methyl-esters in hexane with split ratio of 1:20. The injector temperature was maintained at 225°C and the detector temperature at 250°C. The initial column temperature was 205°C (held for 5 min), and then increased at 2°C/min to 220°C (held for 10 min). Ultrapure nitrogen was used as the carrier gas. All fatty acids eluted at a flow of 0.8 mL/min.

Statistical Analyses

Data were processed using the GLM procedure of SPSS (2001) using a linear model with the effects of diet, square, period within square, and cow within square. The interaction between square and treatment was tested, found to be nonsignificant and then removed from the model. Main effects (diet) were tested using Duncan's multiple range procedure using the GLM procedure of SPSS (2001).

RESULTS

The 3 vegetable oils used in the study were chosen because they had similar linoleic acid content although they differed in C_{16:0}, C_{18:1}, and C_{18:3} (Table 2). The DM intake was relatively constant during each period and did not differ among the 4 treatments ($P > 0.1$). Across all treatments, DM intake averaged 23.93 kg/d.

Milk yields were not different for type of oil fed (Table 3), although they were relatively higher in cows fed vegetable oil diets ($P > 0.05$). Milk fat percentage was lowest when cows received the soybean oil diet, significantly different from the other diets (3.05 vs. 3.44, $P < 0.05$), whereas milk fat yield showed no significant differences between the 4 diets. Contents and yield of protein and lactose remained unchanged for all treatments.

Myristic and palmitic acids contents were decreased ($P < 0.05$) by supplementation with vegetable oils (Table 4). These fatty acid contents in milk fat did not differ due to oil sources. Stearic acid was higher when supplemented with oil from cottonseed or corn ($P < 0.05$). Oleic acid was significantly increased ($P < 0.05$) and was relatively higher in response to soybean oil. The cows supplemented with soybean oil produced milk with highest content of *trans*-11 C_{18:1}, twice that in the control, followed by the animals on corn and cottonseed oil diets. A similar response was observed for the *cis*-9, *trans*-11 CLA in milk fat, which increased to 10.3, 7.1, and 6.0 mg/g of fat for the animals supplemented with soybean oil, corn oil, and cottonseed oil, respectively, from 3.5 mg/g for the control. A significant linear relationship existed between *trans*-11 C_{18:1} and *cis*-9, *trans*-11 CLA ($R^2 = 0.664$, $P < 0.05$) (Figure 1).

Supplementation with vegetable oils almost tripled the content of fatty acids in blood plasma ($P < 0.01$), with little difference between different oil sources (Table 5).

Table 3. Dry matter intake and milk production, composition, and component yields by Holstein cows influenced by supplementation of different vegetable oils.

Variable	Control	Oils from			SEM
		Cottonseed	Soybean	Corn	
DM intake, kg/d	24.1	23.8	24.0	23.9	0.15
Milk production, kg/d	34.4	35.0	34.9	34.8	0.55
Composition, %					
Fat	3.44 ^a	3.34 ^{ab}	3.05 ^c	3.18 ^b	0.081
Protein ¹	3.11	3.14	3.09	3.11	0.058
Lactose	5.04	5.03	5.00	4.89	0.033
Yield, kg/d					
Fat	1.20	1.17	1.06	1.10	0.047
Protein ¹	1.07	1.10	1.08	1.08	0.025
Lactose	1.73	1.76	1.74	1.70	0.027

^{a,b,c}Means within row with different superscripts differ ($P < 0.01$).

¹Total protein.

Table 4. Contents of medium- and long-chain fatty acids in milk fat from Holstein dairy cows influenced by supplementation of different vegetable oils.

Fatty acids	Control	Oils from			SEM
		Cottonseed	Soybean	Corn	
		mg/g of fatty acids			
C _{14:0}	162.7 ^a	123.7 ^b	136.2 ^b	135.8 ^b	4.08
C _{16:0}	461.4 ^a	431.9 ^b	413.7 ^c	422.6 ^{bc}	4.08
C _{18:0}	244.6 ^{bc}	260.6 ^{ab}	241.3 ^c	267.4 ^a	5.45
C _{18:1 cis-9}	85.8 ^c	118.1 ^{ab}	128.9 ^a	111.6 ^b	5.27
C _{18:1 trans-11}	11.8 ^C	19.9 ^B	23.9 ^A	20.3 ^B	0.74
C _{18:1 (others)}	4.1 ^c	5.3 ^b	7.1 ^a	5.8 ^b	0.33
C _{18:2}	26.0 ^b	34.3 ^a	38.7 ^a	29.5 ^b	1.61
CLA <i>cis</i> -9, <i>trans</i> -11	3.5 ^c	6.0 ^b	10.2 ^a	6.9 ^b	0.56
CLA <i>trans</i> -10, <i>cis</i> -12	0.1	0.2	0.1	0.1	0.07

^{A,B,C}Means within row with different uppercase superscripts differ ($P < 0.01$).

^{a,b,c}Means within row with different lowercase superscripts differ ($P < 0.05$).

When cows received the cottonseed oil, palmitic acid content in blood plasma was significantly higher (237.3 mg/g of fat, $P < 0.05$), whereas content of C_{18:2} was lower (410.6 mg/g of fat, $P < 0.05$) than for the other treatments. Content of *trans*-11 C_{18:1} was higher ($P < 0.05$) for oil-supplemented diets than for the control, with higher content for the soybean oil diet than for the cottonseed oil diet ($P < 0.05$). The content of oleic acid in blood plasma for cottonseed oil or soybean oil was significantly higher than that for corn oil and control, with no difference between the latter 2 groups.

DISCUSSION

Our experiment evaluated the quantitative significance of ruminal availability of oleic or linoleic acid in

secretion of conjugated linoleic acid in milk. Fatty acid profiles in blood plasma and milk fat were used to assess changes in the production of hydrogenation intermediates in the rumen.

Contents of C_{14:0} and C_{16:0} in milk fat were significantly lower in response to supplemented vegetable oils. Milk fat contains fatty acids derived from *de novo* synthesis by the mammary gland (C_{4:0} to C_{14:0} plus a portion of C_{16:0}) and from mammary uptake of preformed fatty acid (a portion of C_{16:0} and all longer chain fatty acids). Exogenous fatty acids compete for esterification with newly synthesized short-chain fatty acids in mammary cells, and could lead to feedback inhibition of lipogenic enzymes (Palmquist et al., 1993). Supplemental *cis*-9 C_{18:1} was preferentially incorporated into the sn-2 position of the milk fat triglyceride at the expense of C_{16:0} (DePeters et al., 2001), effectively lowering its content and increasing *cis*-9 C_{18:1}. Comparable responses in milk triglyceride composition have been observed in cows fed on high linoleic oil (Christie, 1981; Palmquist et al., 1993). Thus, greater uptake and secretion of dietary and rumen-derived fatty acids may account for the majority of the reduction in *de novo* synthesis in cows fed unsaturated oils (Palmquist et al., 1993).

All of the vegetable oil diets resulted in milk fat contents of *cis*-9, *trans*-11 C_{18:2} that were 2- to 3-fold higher than that in the control. Content in the soybean oil treatment was significantly higher than for the other treatments (Table 3). Previous work has suggested that the biohydrogenation sequence of linoleic acid can lead to an increase in CLA levels in milk fat (McGuire et al., 1996). The 3 vegetable oils used in this study have similar contents of linoleic acid, but resulted in different CLA contents in milk fat. This result indicated that other fatty acids might contribute to CLA production. A strong linear relationship between *trans*-11 C_{18:1} and CLA in milk fat was found in our study ($R^2 = 0.664$, P

Figure 1. Linear relationship between concentrations of *trans*-11 C_{18:1} and *cis*-9, *trans*-11 conjugated linoleic acids (CLA) in milk fat from Holstein dairy cows.

Table 5. Contents of medium- and long-chain fatty acids in blood plasma of Holstein dairy cows influenced by supplementation with vegetable oils.

Fatty acids ¹	Control	Oils from			SEM
		Cottonseed	Soybean	Corn	
Total, µg/mL	1185 ^B	2821 ^A	2867 ^A	2841 ^A	365
Composition, mg/g of fatty acids					
C _{14:0}	40.6	44.7	46.2	43.6	1.06
C _{16:0}	212.9 ^b	237.3 ^a	215.6 ^b	217.2 ^b	7.35
C _{18:0}	216.9	225.1	212.8	206.0	6.84
C _{18:1 cis-9}	59.1 ^b	71.2 ^a	71.9 ^a	59.6 ^b	3.82
C _{18:1 trans-11}	8.5 ^c	11.1 ^b	14.2 ^a	12.5 ^{ab}	0.76
C _{18:2}	462.0 ^a	410.6 ^b	439.3 ^a	462.5 ^a	8.65

^{A,B}Means within row with different uppercase superscripts differ ($P < 0.01$).

^{a,b,c}Means within row with different lowercase superscripts differ ($P < 0.05$).

¹The total means the sum of C_{14:0}, C_{16:0}, C_{18:0}, C_{18:1 cis-9}, C_{18:1 trans-11}, and C_{18:2}, and the composition indicates the proportion of the individual fatty acid to the sum.

< 0.05), which agreed with other studies (Lawless et al., 1998; Griinari and Bauman, 1999; Secchiari et al., 2003), suggesting that CLA is predominantly synthesized in the mammary cells from *trans*-11 C_{18:1}.

Linolenic acid (*cis*-9, *cis*-12, *cis*-15 C_{18:3}) has been shown to be converted to *cis*-9, *trans*-11, *cis*-15 conjugated triene, then to *trans*-11, *cis*-15 C_{18:2}, and finally to an octadecenoic acid that is either *trans*-11, *trans*-15, or *cis*-15 (Harfoot and Hazelwood, 1988). Harfoot and Hazelwood (1988) confirmed that biohydrogenation of oleic acid by mixed ruminal microbes involves the formation of several positional isomers of *trans* monoenes (including *trans*-11 C_{18:1}) rather than only direct biohydrogenation to form stearic acid. Consequently, *trans*-11 C_{18:1} is a common intermediate in the biohydrogenation of linoleic acid, α -linolenic acid, and oleic acid. *Cis*-9, *trans*-11 CLA content in milk fat was highest ($P < 0.05$) in response to soybean oil (Table 4), although soybean oil had moderate C_{18:2} and C_{18:1}, compared with the other 2 vegetable oils used in this study (Table 2). The fact that soybean oil was rich in C_{18:3} may explain this observation. The C_{18:2} content in corn oil was 44 mg/g of fat lower than that in cottonseed oil (Table 2), but the *cis*-9, *trans*-11 CLA content in milk fat was slightly higher in response to corn oil than to cottonseed oil (Table 4), which may be due to the higher C_{18:1} content in corn oil than in cottonseed oil.

Dietary supplementation with vegetable oils may alter rumen fermentation, with decreased acetic acid production (Onetti et al., 2001), which could cause the milk fat depression syndrome. Several mechanisms have been proposed to explain how lipids interfere with ruminal fermentation (Jenkins, 1993). The lipid "coating" theory and direct antimicrobial effects have received the most attention. Antimicrobial effects of lipids in the rumen have many similarities to cytotoxic effects of fatty acids on membrane function of eukaryotic cells,

such as uncoupling of oxidative phosphorylation. Long-chain unsaturated fatty acids may readily attach to lipid bilayers in biological membranes because of their hydrophobic and amphiphilic nature. The longer the chains and the more double bonds that fatty acids have, the easier it is for them to attach and destroy the membrane of bacteria. This is most likely why the milk fat percentage is the lowest in response to soybean oil.

CONCLUSIONS

Secretion of unsaturated fatty acids and rumen-derived *trans* C_{18:1} in milk may be increased by inclusion of high-oil feed ingredients in dairy diets. *Trans*-11 C_{18:1} is a common intermediate in the biohydrogenation of linoleic acid, α -linolenic acid, and oleic acid. *Cis*-9, *trans*-11 CLA in milk fat seemed predominantly synthesized in the mammary gland from *trans*-11 C_{18:1}. The transfer ratio of C_{18:1}, C_{18:2}, C_{18:3} to *trans*-11 C_{18:1} should be studied further.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Hu Zhan-li, Lin Gui-you, and Zhong Xiao-sheng for their assistance in animal feeding and care.

REFERENCES

- AOAC. 1990. Official Methods of Analysis. Vol. I, 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 1999. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci. Online. Available <http://www.asas.org/jas/symposia/proceedings/0937.pdf>. Accessed July 1, 2003.
- Christie, W. W. 1981. The effect of diet and other factors on the lipid composition of ruminant tissues and milk. Pages 193–226 in Lipid Metabolism in Ruminant Animals. W. W. Christie, ed. Pergamon Press, Oxford, UK.
- DePeters, E. J., J. B. German, S. J. Taylor, S. J. Essex, and H. Perez-Monti. 2001. Fatty acid and triglyceride composition of milk fat

- from lactating Holstein cows in response to supplemental canola oil. *J. Dairy Sci.* 84:929–936.
- Griinari, J. M., and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Pages 180–200 in *Advances in Conjugated Linoleic Acid Research*, Vol. 1. M. P. Yurawecz, ed. AOCS Press, Champaign, IL.
- Harfoot, C. G., and G. P. Hazelwood. 1988. Lipid metabolism in the rumen. Pages 285–322 in *The Rumen Microbial Ecosystem*. P. N. Hobson, ed. Elsevier Applied Science Publishers, London, UK.
- Jenkins, T. C. 1993. Symposium: Advances in ruminant lipid metabolism. *J. Dairy Sci.* 76:3851–3863.
- Kelly, M. L., and D. E. Bauman. 1996. Conjugated linoleic acid: A powerful anticarcinogen found in milk fat. Pages 124–133 in *Proc. Cornell Nutr. Conf.*, Cornell University, Ithaca, NY.
- Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburgh, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J. Nutr.* 128:881–885.
- Kepler, C. R., and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids. III. Purification and properties of a linoleate Δ^{12} *cis*-, Δ^{11} *trans*-isomerase from *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* 242:5686–5692.
- Laporte, M.-F., and P. Paquin. 1999. Near-infrared analysis of fat, protein, and casein in cow's milk. *J. Agric. Food Chem.* 47:2600–2605.
- Lawless, F., J. J. Murphy, D. Harrington, R. Devery, and C. Stanton. 1998. Elevation of conjugated *cis*-9, *trans*-11 octadecadienoic acid in bovine milk because of dietary supplementation. *J. Dairy Sci.* 81:3259–3267.
- Loor, J. J., and J. H. Herbein. 2001. Alterations in blood plasma and milk fatty acid profiles of lactating Holstein cows in response to ruminal infusion of a conjugate linoleic acid mixture. *Anim. Res.* 51:119–134.
- McGuire, M. A., M. K. McGuire, M. A. Guy, W. K. Sanchez, T. D. Shultz, L. Y. Harrison, D. E. Bauman, and J. M. Griinari. 1996. Effect of dietary lipid concentration on content of conjugated linoleic acid (CLA) in milk from dairy cattle. *J. Anim. Sci.* 74 (Suppl. 1):266. (Abstr.)
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academy Science Press, Washington, DC.
- Onetti, S. G., R. D. Shaver, M. A. McGuire, and R. R. Grummer. 2001. Effect of type and level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. *J. Dairy Sci.* 84:2751–2759.
- O'Shea, M., R. Devery, F. Lawless, J. J. Murphy, and C. Stanton. 2000. Milk fat conjugated linoleic acid (CLA) inhibits growth of human mammary MCF-7 cancer cells. *Anticancer Res.* 20:3591–3601.
- Palmquist, D., A. Beaulieu, and D. Barbano. 1993. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* 76:1753–1771.
- Pariza, M., Y. Park, and M. Cook. 2001. The biological active isomers of conjugated linoleic acid. *Prog. Lipid Res.* 40:283–298.
- Pipero, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, trans fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. *J. Nutr.* 130:2568–2574.
- Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in *The Analysis of Dietary Fibers in Foods*. W. P. T. James and O. Theander, ed. Marcel Dekker, New York, NY.
- Secchiari, P., M. Antongiovanni, M. Mele, A. Serra, A. Buccioni, G. Ferruzzi, F. Paoletti, and F. Petacchi. 2003. Effect of kind of dietary fat on the quality of milk fat from Italian Friesian cows. *Livest. Prod. Sci.* 83:43–52.
- SPSS. 2001. *Statistics*. Version 10.0 ed. SPSS Inc., Chicago, IL.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.