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Supplementation of a rumen-protected conjugated linoleic acid mixture (*cis*-9, *trans*-11; *trans*-10, *cis*-12) to early lactation dairy cows – effects on feed intake and performance

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MEINER FAMILIE

Supplementation of a rumen-protected conjugated linoleic acid mixture (*cis*-9, *trans*-11; *trans*-10, *cis*-12) to early lactation dairy cows – effects on feed intake and performance

Conjugated linoleic acids (CLA) are geometric and positional isomers of linoleic acid with conjugated double bonds. *Cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA are the two most intensively studied isomers. The *cis*-9, *trans*-11 isomer has been mainly studied with respect to improved human diet and health issues, whereas studies considering *trans*-10, *cis*-12 CLA have mainly investigated the effects on ruminant nutrition because of its dose-dependent milk fat depressing properties. Most studies have been performed in the United States of America; however, all of them were time-limited, and did not follow the effects of a restricted CLA feeding period throughout lactation. This has led to one main objective of this thesis, which includes a study where a rumen-protected mixed CLA source (3.22 g/d and 3.36 g/d *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA, respectively) was fed for a) 80 d and b) 120 d starting 6 d after parturition. The total observation period was 240 d. Furthermore, a study was performed investigating the effects of supplementation with 4.96 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA as a rumen-protected mixed CLA product starting during the transition period and early lactation (d 18 a.p. to d 80 p.p.). Performance in milk was observed for 100 days.

The results reveal a tendency for depressed milk fat content across the entire lactation period in the long term (100 d) by transition feeding with CLA. The milk yield tended to increase with CLA feeding across the lactation period, depending on the duration of supplementation. These results, however, are only valid for multiparous cows. It seems as if primiparous cows react differently upon CLA-induced milk fat depression. No effects on milk fat were visible in this case. Dairy cows fed CLA during the transition period showed a quicker reduction in milk fat content after parturition than if feeding started after parturition. It seems as if the period of lactation at which the CLA supplementation is terminated has an influence on the recovery of milk fat content. The energy balance could have an effect, since animals in positive energy balance mobilize fatty acids that were retained beforehand. No differences between the American studies and the present study could be found. No effects of CLA product supplementation were detected on the amount of energy corrected milk yield, dry matter intake, live weight, energy balance, ketosis indices.

Verabreichung einer pansengeschützen Zulage von konjugierten Linolsäuren (*cis*-9, *trans*-11; *trans*-10, *cis*-12) an frühlaktierende Milchkühe – Auswirkungen auf Futteraufnahme und Leistung

Konjugierte Linolsäuren (CLA) sind geometrische und positionelle Isomere der Linolsäure, bei denen die Doppelbindungen konjugiert sind. Den am häufigsten untersuchten Isomeren *cis*-9, *trans*-11 CLA und *trans*-10, *cis*-12 CLA werden verschiedene Effekte zugewiesen. Während *cis*-9, *trans*-11 CLA hauptsächlich in Bezug auf die Humanernährung und –gesundheit untersucht wird, erlangt *trans*-10, *cis*-12 CLA Bedeutung in der Wiederkäuerernährung durch die Dosis abhängige Milchfettgehalt absenkende Wirkung. Die meisten Studien zu dessen Wirkungsweise wurden bisher in den USA durchgeführt. Diese waren jedoch zeitlich begrenzt, weshalb in der vorliegenden Thesis eine Studie präsentiert wird, die die Effekte einer Verfütterung von pansenstabilen CLA-Produkt (jeweils 3.22 g/d bis 3.36 g/d *trans*-10, *cis*-12 CLA und *cis*-9, *trans*-11 CLA) beginnend nach dem Abkalben für jeweils 80 oder 120 Tage, über die Supplementierungsdauer (240 Tage insgesamt) hinaus untersucht wird. Eine weitere Studie befasste sich mit der Verabreichung von CLA-Produkt (4.96 g/d *trans*-10, *cis*-12 CLA sowie *cis*-9, *trans*-11 CLA) in der Transitphase (-18 bis 80 Tage in Bezug auf das Kalbedatum) und mögliche Unterschiede zu US Literaturdaten.

Die Ergebnisse zeigen, dass durch eine Verabreichung von CLA-Produkt bei Transitfütterung oder über 120 Laktationstage, der Milchfettgehalt tendenziell über die Laktationsdauer absenkt werden kann, während die Milchmenge in der Tendenz über die Laktationsleistung, abhängig von der Supplementierungsdauer, gesteigert ist. Diese Ergebnisse gelten nur für mehrkalbige Kühe. Färsen reagieren anders auf eine CLA induzierte Milchfettdepression, denn der Milchfettgehalt war nur unzulänglich abgesenkt. Es waren keine Effekte über die Laktationsdauer zu erkennen. Tiere, die während der Transitphase CLA-Produkt erhielten, reagierten nach Laktationsbeginn schneller mit einer Milchfettdepression, als Tiere die erst mit der Kalbung CLA erhielten. Der Absetzzeitpunkt des CLA Produktes wirkt sich auf die Dauer der Regeneration des Milchfettgehaltes aus. Der Energiestatus der Tiere könnte hierauf einen Einfluss haben, da bei negativer Energiebilanz CLA aus retenierten Körperreserven freigesetzt wird. Unterschiede zwischen US Studien und der vorliegenden Studie konnten nicht ermittelt werden. Die CLA-Supplementierungen wirken sich nicht auf die Menge an energiekorrigierter Milch, Trockenmasseaufnahme, Lebendmasse, Energiebilanz, Ketosevariablen und die Fruchtbarkeit der Tiere aus.

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ABBREVIATIONS

ACC	:	Acetyl CoA carboxylase
ADF	:	Acid detergent fiber
BFT	:	Back fat thickness
BHBA	:	Betahydroxybutyrate
CF	:	Crude fiber
CL	:	Crude lipid
CLA	:	Conjugated linoleic acid
CLA-ME	:	Conjugated linoleic acid-methylesters
CON	:	Control
DIM	:	Days in milk
DM	:	Dry matter
DMI	:	Dry matter intake
EB	:	Energy balance
ECM	:	Energy corrected milk
FAS	:	Fatty acid synthase
FFA	:	Free fatty acid
FM	:	Fresh matter
HPLC	:	High-performance liquid chromatography
IGF-1	:	Insulin like growth factor- 1
LCFA	:	Long chain fatty acid
LKV	:	Landeskontrollverein
LPL	:	Lipoprotein lipase
LSM	:	Least square mean
LUFA	:	Landwirtschaftliche Untersuchungs- und Forschungsanstalt
ME	:	Metabolizable energy
MFD	:	Milk fat depression
mRNA	:	Messenger ribonucleic acid
MUFA	:	Monounsaturated fatty acid
uCP	:	Utilizable crude protein
NDF	:	Neutral detergent fiber
NEFA	:	Non-esterified fatty acid
NEL	:	Net energy lactation
NRW	:	North Rhine Westphalia
PAR	:	Parity
PUFA	:	Polyunsaturated fatty acid

RNB	:	Ruminal nitrogen balance
SAS	:	Statistical analysis system
SCC	:	Somatic cell count
SCD	:	Stearoyl-CoA desaturase
SCFA	:	Short chain fatty acid
SE	:	Standard error
SEM	:	Standard error of the mean
SFA	:	Saturated fatty acid
SD	:	Standard deviation
TRT	:	Treatment
TMR	:	Total mixed ration
UDP	:	Undegradable protein
VFA	:	Volatile fatty acid
VLDL	:	Very low density lipoprotein

1 INTRODUCTION

Scientific interest in conjugated linoleic acid (CLA) has grown steadily over the past two decades and various studies have been published in diverse fields of expertise. Generally, two isomers are addressed with respect to CLA, the *cis*-9, *trans*-11 CLA and the *trans*-10, *cis*-12 CLA. However, a broad number of other CLA isomers exist, but the various beneficial physiological and health-promoting effects have been assigned to the two isomers mentioned above. These issues reach from human health and nutritional benefits to profits in animal performance and health. These actions are *inter alia* enforced by the anti-atherogenic properties (McLeod et al., 2004; Terpestra, 2004), anti-carcinogenic activities for a wide range of cancer types (Banni et al., 1999; Ip et al., 2000; Majumeder et al., 2002; Field and Shley, 2004; Lock et al., 2004) and lipid synthesis modifying effects in the body (Park et al., 1997; DeLany et al., 1999; Ostrowska et al., 1999; Azain et al., 2000; Brown et al., 2004; Terpestra, 2004; Chung et al., 2005) and specifically in mammary tissues (Chouinard et al., 1999; Baumgard et al., 2001). Beneficial effects to the immune (O'Shea et al., 2004; Tricon et al., 2004) and the reproductive systems (Castañeda-Gutiérrez et al., 2007, de Veth et al., 2009) have further been stated, mainly by the analysis of animal models dealing with rats, mice and rabbits, but also in livestock feeding trials.

In human nutrition, CLA is primarily promoted as a slimming agent with beneficial effects on health maintenance and disease prevention. Therefore, efforts have been made to enhance the CLA content in milk fatty acids. The main natural CLA present in milk fat is *cis*-9, *trans*-11 CLA, comprising 75-90% of the total CLA (Bauman and Grinarii, 2003; Parodi, 2003); it is attributed to the anti-carcinogenic and anti-atherogenic activities of CLA. Strategies enhancing the levels of *cis*-9, *trans*-11 CLA in milk fat elevate the nutraceutical value of milk and have to follow the rule that increasing amounts of *trans*-11 C18:1 in the rumen outflow is transformed by Δ9-destaurase to *cis*-9, *trans*-11 CLA. To increase rumen *trans*-11 C18:1 outflow, polyunsaturated fatty acids have to be available for rumen biohydrogenation. Anti-obesity effects have been attributed to the *trans*-10, *cis*-12 isomer of CLA, but it never represents more than 1 to 2% of the total CLA in milk fat. Food derived from ruminants cannot provide sufficient amounts of this isomer to have biological effects on body fat.

Within the last decade, the supplementation of CLA in livestock markets has been encouraged. Different outcomes are supported by supplementing animals with CLA products. In non-ruminant nutrition, CLA is mainly promoted as an agent to increase lean muscle mass, whereas in ruminants it is considered as a potent nutrient repartitioning agent with benefits to health and fertility. A milk fat depressing effect of *trans*-10, *cis*-12 CLA has been verified

(Chouinard et al., 1999; Baumgard et al., 2001; Viswanadha et al., 2003), and supplementing this isomer as a nutrient repartitioning agent during early lactation has become of interest in countries where the milk market is ruled by quota systems. However, in those markets ruled by a quota system, like the European Union and Canada, it could further be an instrument to optimize the quota in terms of short shipping the fat quota by reducing the milk fat content. However, the viability of this strategy is mainly dependent on the actual price paid for milk. Newer studies have provided information that *trans*-10, *cis*-12 CLA carries the additional benefit of providing a further increase in the farmer's profit. In some studies (Odens et al. 2007; Liermann et al., 2008), a positive influence of mixed CLA products on energy balance in early lactation dairy cows has been observed. Since reproductive performance is highly linked to the extent and the timing of the negative energy balance nadir (Lucy et al., 1992; Beam and Butler, 1999), benefits in this trait could possibly be linked to CLA supplementation; these claims have been supported by Castañeda-Gutiérrez et al. (2007).

Several studies have been performed to analyze the influence of *trans*-10, *cis*-12 CLA on milk production in dairy cows by abomasal infusion (Chouinard et al., 1999; Baumgard et al., 2001, 2002; Bell and Kennelly, 2003; Sæbø et al., 2005; Harvatine and Bauman, 2011) and oral supplementation (Perfield et al., 2002; Bernal-Santos et al., 2003; Gervais et al., 2005; Moallem et al., 2010; Sigl et al., 2010; Hutchinson et al., 2011). Earlier studies (1999 to 2005 as reported above) were mostly conducted in cows during established and late lactation and ended with the termination of CLA supplementation. With the exception of Hutchinson et al., 2011, recent studies (2010 to 2011 as reported above) were conducted in early lactation dairy cows and the observation of CLA´s influence continued with the cessation of CLA supplementation. Thus two studies were designed to investigate the development of milk composition and variables influencing the energy balance in cows fed a rumen-inert CLA product, containing *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, during early lactation and beyond the period of CLA supplementation. The focus of the first study was to investigate the impact of variable duration of supplementation with a CLA product on mammary synthesis after the cessation of supplementation. Supplementation effects were further studied in terms of energy balance, dry matter intake and health as well as fertility performance. The focus of the second study was to investigate how the same variables responded when a CLA product was fed throughout the transition period and early lactation, following animals up to the 100th day in milk. A further objective of this study was to determine if the impact of a mixed CLA product fed to dairy cows ingesting a typical Central European ration generates comparable effects as those found in the literature from American studies.

REFERENCES

Azain, M. J., D. B. Hausman, M. B. Sisk, W. P. Flatt, and D. E. Jewell. 2000. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J. Nutr. 30:1538-1554.

Banni, S., E. Angioni, V. Casu, M. P. Melis, G. Carta, F. P. Corongiu, H. Thompson, and C. Ip. 1999. Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. Carcinogenesis. 20:1019-1024.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition. 23:203-227.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769.

Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertility 54:411-424.

Bell, J. A., and J. J. Kennelly. 2003. Short communication: postruminal infusion of conjugated linoleic acids negatively impacts milk synthesis in Holstein cows. J. Dairy Sci. 86:1321-1324.

Bernal-Santos, G., J. W. Perfield II, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003: Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Brown, J.M., M. S. Boysen, S. Chung, O. Fabivi, R. F. Morrison, S. Mandrup, and M. K. McIntosh. 2004. Conjugated linoleic acid induces human adipocytes delipidation: autocrine/paracrine regulation of MEK/ERK signaling by adipocytokines. J. Biol. Chem. 279:26735-26747.

Castañeda-Gutiérrez, E., B. C. Beefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253-4264.

Chouinard, P. Y., L. Coreneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579-1584.

Chung, S., J. M. Brown, M. B. Sandberg, and M. McIntosh. 2005. Trans-10, cis-12 CLA increases adipocyte lipolysis and alters lipid droplet-associated proteins: role of mTOR and ERK signaling. J. Lipid Res. 46:885-895.

DeLany, J. P., F. Blohm, A. A. Truett, J. A. Scimeca, and D. B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 276:R1172-1179.

De Veth, M. J., D. E. Bauman, W. Koch, G. E. Mann, A. M. Pfeiffer, and W. R. Butler. 2009. Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows. J. Dairy Sci. 92:2662-2669.

Field, C. J., and P. D. Schley. 2004. Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n3 fatty acids. Am. J. Clin. Nutr. 79:1190S-1198S.

Gervais, R., R. Spratt, M. Líonard, and P. Y. Chouinard. 2005. Lactation response of cows to different levels of ruminally inert conjugated linoleic acids under commercial conditions. Can. J. Anim. Sci. 85:231-242.

Harvatine, K. J. and Baumann, D. E. 2011. Characterization of the acute lactational response to *trans*-10, *cis*-12 conjugated linoleic acid. J. Dairy Sci. 94:6047-6056.

Hutchinson, I., M. J. de Veth, C. Stanton, R. J. Dewhurst, P. Lonergan, A. C. O. Evans, and S. T. Butler. 2011. Effects of lipid-encapsulated conjugated linoleic acid supplementation on milk production, bioenergetic status and indicators of reproductive performance in lactating dairy cows. J. Dairy Res. 78 308-317.

Ip, C., M. M. Ip, T. Loftus, S. Shoemaker, and W. Shea-Eaton. 2000. Induction of apoptosis by conjugated linoleic acid in cultured mammary tumor cells and premalignant lesions of the rat mammary gland. Cancer Epidemiol. Biomarkers Prev. 9:689-696.

Lock, A. L., B. Corl, D. M. Barbano, D. E. Bauman, and C. Ip. 2004. The anticarcinogenic effect of trans-11 18:1 is dependent on its conversion to cie-9, trans-11 CLA by Δ9-desaturase in Rats. J. Nutr. 134:2698-2704.

Liermann, T., A.-M. Pfeiffer, and F. J. Schwarz. 2008. Effects and post-effects on performance and metabolic parameters of early lactation dairy cows to dietary rumen-protected CLA and rumen protected fat. Proc. Soc. Nutr. Physiol. 17:30.

Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry matter, milk production and energy balance on rime of postpartum ovulation and fertility in dairy cows. Anim. Prod. 54:323-331.

Majumer, B., K. W. J. Wahle, S. Moir, A. Schofield, S.-N. Choe, A. Farquharson, I. Grant, and S. D. Heys. 2002. Conjugated linoleic acids (CLAs) regulate the expression of key apoptotic genes in human breast cancer cells. FASEB J. 16:1447-1449.

McLeod, R. S., A. M. LeBlanc, M. A. Langille, P. L. Mitchell, and D. L. Currie. 2004. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. Am. J. Clin. Nutr. 79 (Suppl):1169S-1174S.

Moallem, U., H. Lehrer, M. Zachut, L. Livshitz, and S. Yacoby. 2010. Production performance and pattern of milk fat depression of high-yielding dairy cows supplemented with encapsulated conjugated linoleic acid. Animal. 4: 641-652

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. J. Dairy Sci. 90:293-305.

O'Shea, M., J. Bassaganya-Riera, and I. C. M. Mohede. 2004. Immunomodulatory properties of conjugated linoleic acid. Am. J. Clin. Nutr. 79 (Suppl) 1199S-1206S.

Ostrowska, E., M. Muralitharan, R. F. Cross, D. E. Bauman, and F. R. Dunshea. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J. Nutr. 129:2037-2042.

Park, Y., K. J. Albright, W. Liu, J. M. Storkson, M. E. Cook, and M. W. Pariza. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids 32:853-858.

Parodi. P. W. 2003. Conjugated Linoleic Acid in Food, in Advances of Conjugated Linoleic Acid Research. (Sébédio, J.-L,. Christie, W. W. and Adlof, R. O., eds.) Volume 2. pp. 101-122. AOCS Press. Champaign, Illinois.

Perfield, J. W. II, G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Sæbø, A, P.-C. Sæbø, J. M. Griinari, and K. J. Shingfield. 2005. Effect of abomasal infusions of geometric isomers of 10, 12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids 40:823-832.

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer and M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scand. 52:16

Terpestra, A. H. 2004. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. A review. Am. J. Clin. Nutr. 79:352-361.

Tricon, S., G. C. Burdge, S. Kew, T. Banerjee, J. J. Russell, R. F. Grimble, C. M. Williams, P. C. Calder, and P. Yaqoob. 2004. Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid on immune cell function in healthy humans. Am. J. Clin. Nutr. 80:1626-1633.

Viswanadha, S., J. G. Giesy, T. W. Hanson, and M. A. McGuire. 2003. Dose response of milk fat to intravenous administration of the trans-10, cis-12 isomer of conjugated linoleic acid. J. Dairy Sci. 86:3229-3236.

2 LITERATURE REVIEW

Chemistry of conjugated linoleic acid

The category of geometric and positional isomers of linoleic acid is called conjugated linoleic acid (**CLA**). Linoleic acid is a single molecule and an octadecadiene acid with two *cis* double bonds, which are positioned, counting from the carboxy group, at carbon atoms 9 and 12. This makes the linoleic acid an *omega*-6 fatty acid. Figure 1A provides the structure of this molecule. The conjugation of double bonds in linoleic acids is achieved by relocation, with the result that the double bonds only are separated by a single (methylene) bond. In the majority of cases, conjugated double bonds are positioned at carbon atoms 8 and 10, 9 and 11, 10 and 12 or 11 and 13 (Larsen et al., 2003). *Cis* and *trans* formation adds further diversity to CLA molecules and provides a great variety of molecules defined as CLA. The structures of two important CLA molecules, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, are shown in Figures 1B and 1C.

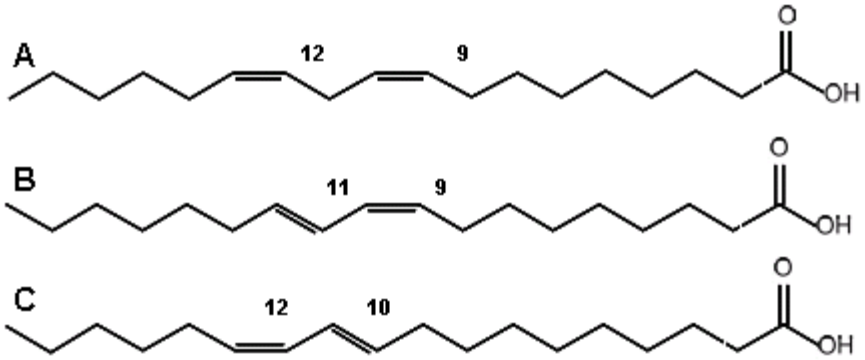

Figure 1. Structural formula of linoleic acid (A), *cis*-9, *trans*-11 CLA (B) and *trans*-10, *cis*-12 CLA (C).

CLA in ruminant products

Ruminants synthesize a variety of CLA isomers endogenously from polyunsaturated fatty acids (**PUFA**). Because *trans*-fatty acids naturally occur through the rumination process (Arab, 2003), ruminant products are a main dietary base for human CLA intake. The CLA synthesis of ruminants is based on two origins. Rumen biohydrogenation of PUFA represents the first pathway, whereas the second is based on tissue CLA synthesis with the help of bovine stearoyl-CoA desaturase (**SCD**) (Griinari and Baumann, 1999). These pathways have been shown by Chilliard and Ferlay (2004) to yield 75% of total CLA synthesis. A further way to increase the amount of single CLA isomers in ruminant products is the

supplementation of specific rumen-inert CLA isomers. This is a practicable method to increase the content of *trans*-10, *cis*-12 CLA in ruminant products, but it is a poor choice to influence the concentration of *cis*-9, *trans*-11 CLA in milk and meat, because its endogenous synthesis is great with respect to the intestinal absorption rate.

Endogenous rumen CLA synthesis

Rumen microbial lipid metabolism is characterized by the lipolysis of dietary lipids. Free fatty acids (**FFA**) are released, followed by biohydrogenation, which is also affected by rumen micro-organisms (Harfoot and Hazlewood, 1997). Biohydrogenation of dietary PUFA results in the accumulation of CLA or its precursors (Kelly, 2001) in the rumen. Free PUFA in rumen fluid are preferentially biohydrogenated by bacteria, while protozoa have a minor role in this process (Harfoot and Hazlewood, 1997). A series of bacterial species possess hydrating activities on PUFA. Kemp and Lander (1984) divided them into two groups, depending on the steps of biohydrogenation catalyzed by them and the resulting end products. Group A bacteria are capable of isomerizing and biohydrogenating linoleic and α-linolenic acid. The major end product of this process is *trans*-11 C18:1 (Ward et al., 1964). This is the substrate for the bacteria of group B, which then biohydrogenate the substrate further to the end product, stearic acid (C18:0). Figure 2 gives an overview of the biohydrogenation pathways of different bacterial groups in the rumen.

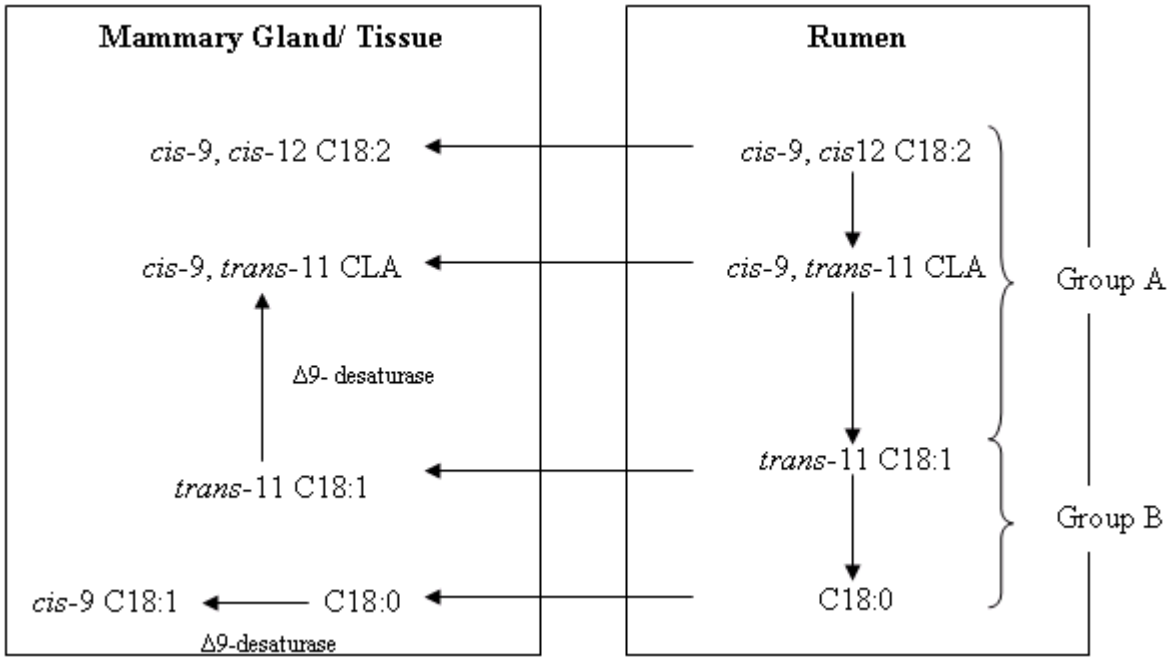

Figure 2. Endogenous synthesis of conjugated linoleic acid due to rumen biohydrogenation of dietary linoleic acid and to desaturation in tissue. Adapted from Bauman et al. (2000).

Rumen biohydrogenation of linoleic acid

The sequence of linoleic acid biohydrogenation is initiated by the isomerization of its *cis*-12 double bond. This is a very unusual process, because action in the middle of a molecule is uncommon, especially because no co-factors or other activating functional groups are required by the enzymes (Kepler and Tove, 1967). The specific enzyme transforming the *cis*-12 double bond into a *trans*-11 double bond is called linoleatisomerase or Δ12-*cis*, Δ11-*trans* isomerase. It is bound to the bacterial cell membrane and reacts specifically on *cis*-9, *cis*-12 dienes with a free carboxy group. The quick attachment of a proton to carbon atom 13 initiates the isomerisation of the *cis*-12 double bond. As soon as only one double bond is in the geometric *trans*-form or the double bond is relocated to one carbon atom, the activity of Δ12-*cis* Δ11-*trans* isomerase ceases (Kepler et al., 1970). The following step in biohydrogenation is the reduction of *cis*-9, *trans*-11 CLA to *trans*-11 C18:1. Because biohydrogenation of *trans*-11 C18:1 occurs more slowly than the previous step, *trans*-11 C18:1 accumulates (Singh and Hawke, 1979), resulting in greater flow into the gastrointestinal tract. Fellner et al. (1995) showed in an *in vitro* study that *trans*-isomers other than *trans*-11 C18:1 also originate from biohydrogenation. In most cases, these were *trans*-9 and *trans*-7 fatty acids. In addition to *trans*-11 C18:1, *trans*-10 C18:1 has been detected in the milk of dairy cows (Griinari et al. 1998). A micro-organism was isolated by Verhulst et al. (1987) that converts linoleic acid to *trans*-10, *cis*-12 CLA, so it is likely, by analogy to *trans*-11 C18:1, that *trans*-10 C18:1 may be formed in the rumen via microbial metabolism. However, the biohydrogenation of the *cis*-12 bond cannot be reversed, because mammals do not possess a Δ12-desaturase in tissue. This means that all *trans*-10, *cis*-12 CLA reported in ruminant tissues and milk originates solely from *trans*-10, *cis*-12 CLA absorbed in the gastrointestinal tract (Lawson et al., 2001). The general amount of *cis*-9, *trans*-11 CLA is higher in ruminant products than *trans*-10, *cis*-12 CLA because SCD is present in dairy cows tissues; this enzyme inserts a double bond into the *cis*-9 position of *trans*-11 C18:1.

Dietary unsaturated fatty acids are almost completely biohydrogenated in the rumen. The biohydrogenation of linoleic acid averages 80% under normal conditions. If the proportion of concentrate in total diet exceeds 70%, the rate of hydrogenation averages only 50% (Doreau and Ferlay, 1995). If the capacity of hydration and lipolysis is exceeded by dietary lipid ingestion, the rate of biohydrogenation is reduced and more unsaturated fatty acids reach the duodenum (Demeyer, 1973). The inhibition of SCD by high concentrations of linoleic and α-linolenic acid was reported by Kepler and Tove (1967). Increased rumen

concentrations of CLA and *trans*-C18:1 fatty acids indicate an inhibition of the reductase enzymes that convert CLA to *trans*-C18:1 acid and the latter to stearic acid.

Ruminal biohydrogenation of α-linolenic acid

The process of biohydrogenation is initiated by Δ12-*cis*, Δ11-t*rans* isomerase, which transforms α-linolenic acid into a *cis*-9, *trans*-11, *cis*-15 octadecatriene. This action is followed by hydration of the *cis*-9 double bond followed by hydration of the *cis*-15 double bond. Hence, it has been noted that the *cis*-9, *trans*-11 molecule is not an intermediate in the biohydrogenation of α-linolenic acid. However, *trans*-11 C18:1, but also *cis*-15 C18:1 and *trans*-15 C18:1, accumulate in the rumen (Harfoot and Hazlewood, 1997). Biohydrogenation of γ-linolenic acid enriches *trans*-11 C18:1 in the rumen fluid as well.

Alteration of rumen biohydrogenation

Substrate supply and the extent of biohydrogenation affect the types of intermediates and end-products of biohydrogenation, thus influencing the CLA content of ruminant milk (Kelly et al., 1998; Dhiman et al., 1999). Hence, the ingested amount of long chain fatty acids (**LCFA**) is crucial for the prospective amount of CLA in bovine milk fat, since they are either already CLA molecules or CLA is synthesized from them. Supplementing vegetable oil to bovine rations can substantially increase the amount of CLA in milk fat (Dhiman et al., 2000; Chouinard et al., 2001). High dietary oleic acid, linoleic acid and linolenic acid intakes increase *trans*-11 C18:1 uptake by tissues and hence the endogenous synthesis of *cis*-9, *trans*-11 CLA (AbuGhazaleh et al., 2003). Particularly, factors exhibiting negative action on B-group bacterial activity increase the amount of CLA synthesized from dietary lipids (Figure 2). Factors influencing rumen microbial activity, like ruminal pH, rumen turnover and the amount of soluble carbohydrates in the rumen, have an effect on the CLA content in milk fat (Kalscheur et al., 1997a, 1997b; Beam et al., 2000; Martin and Jenkins, 2002).

PUFA hydrating rumen bacteria are basically of a cellulolytic nature (Latham et al., 1972, Harfoot and Hazlewood, 1997). A drop in rumen pH inhibits the activity of bacteria and the lipase enzyme responsible for lipid breakdown. This explains, in part, the low degree of saturation in rumen and duodenal lipids of animals fed concentrated diets. Martin and Jenkins (2002) observed significantly lower rates of PUFA biohydrogenation *in vitro* when the pH decreased from 6.7 to 5.5, also leading to reduced synthesis of *trans*-C18:1. This effect was followed by increased digesta passage rates. Increasing amounts of *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 are synthesized in the rumen when high amounts of soluble carbohydrates are provided by low pH and a high passage rate (Martin and Jenkins, 2002). Griinari et al. (1998)

observed similar effects by feeding a ration that lowered the rumen pH and provided unsaturated lipids. *Trans*-10 C18:1 became the dominant isomer when the pH was reduced by the ration, whereas the total amount of *trans*-C18:1 fatty acids was not affected by rumen pH. The same phenomenon was shown by feeding a ration low in fiber and rich in fat (Griinari et al., 1998). Figure 3 shows the pathways of rumen biohydrogenation in an altered rumen environment.

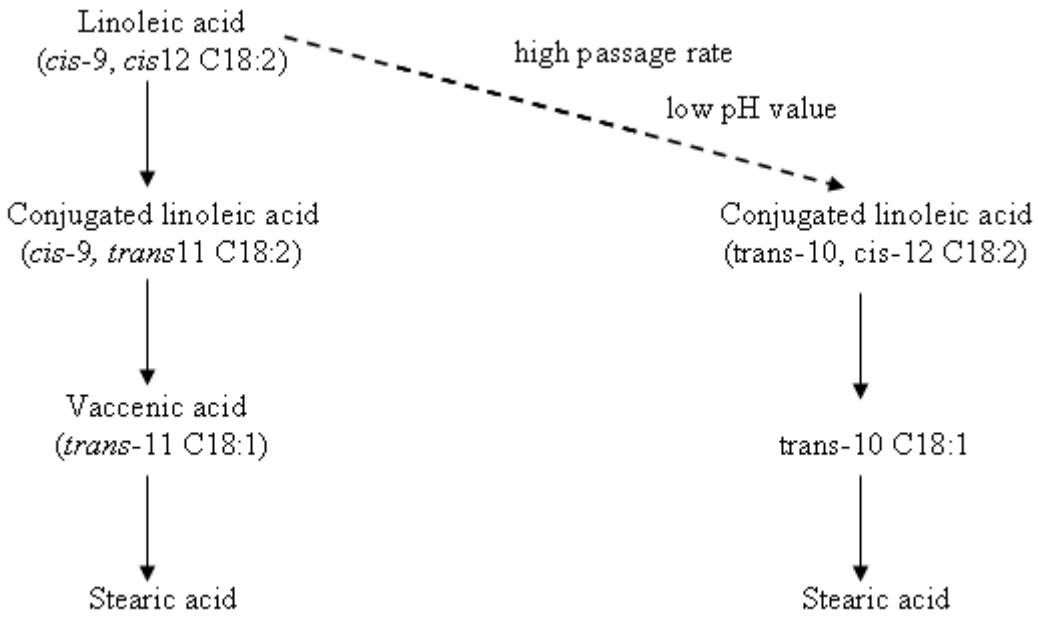

Figure 3. Pathways of linoleic acid biohydrogenation. Adapted from Griinari and Bauman (1999). The dashed line describes the shift that coincides with lowered rumen pH and higher passage rates. In conditions of diet-induced milk fat depression, this part is increased.

Unsaturated fatty acids fundamentally inhibit the activity of Δ12-*cis* Δ11-*trans* isomerase in the rumen environment (Kepler et al., 1970). High dietary amounts of PUFA reduce the rate of biohydrogenation and, consequently, the levels of CLA precursors. PUFA are more potent inhibitors than monounsaturated fatty acids (**MUFA**). Fatty acids with a chain length greater than 16 carbon atoms and with a double bond somewhere between the third and twelfth carbon atom are more potent than medium chain fatty acids (**MCFA**). The closer the double bond is positioned to the carboxy-end of the fatty acid, the greater the inhibiting effect (Kepler et al., 1970).

CLA in ruminant tissues

Chouinard et al. (1999a) reported that there appears to be some selectivity in uptake or incorporation of the *cis*-9, *trans*-11 CLA isomer compared with *trans*-10, *cis*-12 CLA in the tissues of dairy cows. There are differences between the isomers in the efficiency of the transfer of CLA to milk fat. Only about 10 g of 100 g dietary supplemented *trans*-10, *cis*-12 CLA was transferred into milk fat, whereas the *cis*-8, *trans*-10 CLA, *cis*-9, *trans*-11 CLA and *cis*-11, *trans*-13 CLA isomers are transferred into milk fat with more than double the efficiency (22.0-26.0 g/100 g dietary supplement). In an *in vivo* study, Mosley et al. (2006) infused *trans*-11 C18:1 into the abomasum of lactating cows and calculated the endogenous synthesis of *cis*-9, *trans*-11 CLA. They observed that about 83% of *cis*-9, *trans*-11 CLA in milk fat was derived from *trans*-11 C18:1 through desaturation by SCD. This equaled a daily amount of 4.3 g *cis*-9, *trans*-11 CLA in milk fat. A study by Corl et al. (2001) found a transformation rate of 78%. In grazing cows, 91% of the *cis*-9, *trans*-11 C18:2 originated from endogenous desaturation; this equals an amount of 9.5 g *cis*-9, *trans*-11 CLA in milk fat per day (Kay et al., 2004). The main activities of the SCD enzyme complex are in the mammary gland (McDonald and Kinsella, 1973; Mosley et al., 2006) and in adipose tissue (St. John et al., 1991; Page et al., 1997).

Bovine milk composition

The three main components of bovine milk are lactose, protein and fat. The composition of these components is influenced by many factors. These include nutrition, parity, stage of lactation and breed (Carroll et al., 2006). In particular, the lipid and N-fraction of milk can be modified, hence the milk lipid faction is the main fraction altered by diet. A major factor that impacts the concentration of milk fat and protein is milk yield. In ruminants, phenotypic and genetic correlations among fat and protein concentration and milk yield are negative (Emery, 1988). Genetic correlations between milk yield and its concentration of fat and protein are greater than environmental factors. The reduction in milk fat and protein content as milk yield increases is well-known. The amount of lactose usually increases to the same extent as milk yield, as it is an osmotic factor. Fat and protein synthesis generally increases at a slower rate (Emery, 1988).

DePeters and Cant (1992) pointed out that it is important to distinguish between responses that affect protein content versus responses affecting protein yield. Dietary treatments with positive effects on milk and protein yield often happen to cause negative

effects on the protein content. Hence, the purpose in influencing milk composition in most cases is to increase the protein or fat content, while maintaining or increasing milk yields. On the other hand, it is possible that a severe reduction in milk fat content can be overruled by an increased milk yield.

Alteration of milk components

An alteration in milk components could have great benefit in optimizing human nutrition, but a greater benefit could be seen in the regulation of dairy cows' energy expenditure during times of negative energy balance. The demand for low-fat products in human nutrition has increased recently. Consequently, this trend has been followed by the dairy industry, which has moved towards more personalized products. While milk protein is a very valuable component (i.e. for its cheese making properties), milk fat is becoming more and more redundant, which is partly expressed by changing prices for milk components.

For the dairy cow herself, alterations in milk components may play a major role during periods of negative energy balance (**EB**). Reduced yields of milk fat could help to overcome these situations more easily and with a reduced risk of developing a metabolic disorder, especially since milk fat is the component that contains the most energy and is also the most variable. Different influences provoke changes in milk composition. Genetics and nutrition play a major role; parity and the stage of lactation have an effect on milk composition as well, but they are dependent variables.

Genetics

Different breeds have different potential for milk component synthesis. Table 1 shows the differences between the most common breeds milked in Germany. Jerseys are the breed with the lowest protein-to-fat ratio, but with the highest contents of milk fat and protein, while lactose is lower than in the classical dairy cow breeds mentioned in Table 1.

Table 1. Milk yield and milk contents of different cattle breeds common in Germany (Landeskuratorium der Erzeugerringe in der Rinderzucht in Bayern, 2008).

Breed	Milk yield, kg	Fat, %	Protein, %	Protein/Fat	Lactose, %
German Holstein*	24.27	4.24	3.50	0.83	4.73
Brown Swiss	21.12	4.23	3.68	0.87	4.75
Simmental	16.76	4.01	3.51	0.88	4.78
Jersey	19.02	5.42	3.95	0.73	4.66

* German Holstein = Friesian Holstein and Red Holstein

The choice of breed has an impact on milk fat composition (Table 1). The early work of Krukovsky (1961) showed that the milk fat of Ayrshire cows had the highest iodine value, while milk fat from Jersey cows had the lowest, with Holstein and Brown Swiss cows at intermediate levels. These results suggest that there are differences in the fatty acid composition of milk fat between different dairy cow breeds. Stull and Brown (1964) later reported that the milk fat from Holstein cows is lower in C10:0, C12:0 and C16:0 and higher in C18:1 compared to the milk fat from either Jersey or Guernsey cows. The studies of White et al. (2001) and Carroll et al. (2006) showed that the milk of Holsteins is higher in C16:1 and C18:1 and lower in C6:0, C8:0, C10:0, C12:0 and C14:0 than the milk of Jerseys. Carroll et al. (2006) also reported that the milk of Brown Swiss have the highest proportion of monounsaturated *trans*-fatty acids in milk. Additionally, Jersey milk has the highest proportion of C4:0, C5:0, C6:0 and C7:0, regardless of the level of dietary fat. This implies increased *de novo* synthesis of fatty acids in Jersey cows, since fatty acids consisting of ≤14 C-atoms are typically synthesized *de novo* by the mammary gland (Storry, 1972).

Mele et al. (2009) demonstrated in a broad study with Italian Holstein cows that it is possible to improve milk fatty acid composition by genetic selection. By genetic selection for the bioactive isomer *cis*-9, *trans*-11 CLA, the content of pro-thrombotic and atherogenic fatty acids (C14:0 and C16:0) was decreased at the same time due to a negative correlation. Selection for C16:0 would improve overall milk fat yield, due to a positive correlation. Since

the correlation of *cis*-9, *trans*-11 CLA to C16:0 is negative, a decrease in this CLA isomer is implied if the milk fat yield is maximized.

Other important properties of milk fat are the positional arrangements of fatty acids esterified to milk triglycerides (**TG**). In the gastrointestinal tract, dietary TG is hydrolyzed, resulting in FFA and sn-3 monoacylglycerols (Bergström, 1964). The higher proportion of C18:3 in the sn-2 position of milk TG in Holstein and Jersey cows compared to Brown Swiss is important, since fatty acids esterified in this position are characterized by higher levels of intestinal absorption in humans (Innis et al., 1994; Kennedy et al., 1999).

The milk fat globule size may be directly related to the fatty acid composition; small globules have been shown to contain 5.9% less SCFA, 11.7% less C18:0 and 4.6% more C18:1 in TG, compared to large globules (Timmen and Patton, 1988). It has also been shown that fat globules tend to be larger in milk with a higher fat content (Wiking et al., 2003). Wiking et al. (2004) observed that the yield of milk fat was positively correlated with the diameter of the milk fat globule membrane, supporting an increase in milk fat globule size with increasing dietary fat, since milk fat yield is linearly increased with increasing dietary fat (Carroll et al., 2006). The milk fat globule size was larger in Jersey compared to Holstein and Brown Swiss (Banks et al., 1986; Carroll et al., 2006).

These results indicate that alterations in milk composition and the production of more favorable milk are possible by genetic selection. Yet, genetic selection is a time intensive method and requires short-term alterations for quick reactions on varying demands. Alterations in diet composition and supplementation of feed additives can meet these demands.

Dietary milk ingredient alteration

A sufficient supply of nutrients provided by feeding dairy cows with well-balanced rations leads to a high milk fat content. An adequate availability of milk fat precursors, acetate and butyrate, for *de novo* milk fat synthesis is of importance. Propionate has a negative impact on milk fat content. This clarifies why factors altering rumen microbial processes and thereby the ratio of volatile fatty acids (**VFA**) have an influence on the milk fat content. Factors included are the supply of structural fiber, the forage-concentrate ratio, the dietary crude lipid concentration and the level of feed intake.

Different kinds of dietary carbohydrates alter rumen fermentation and therefore the VFA profile. Cellulose and hemicellulose, for instance, are mainly degraded to acetate, whereas starch and sugar increase the production of propionate and butyrate. As fatty acids in the

mammary gland are synthesized mainly from acetate, a sufficient dietary supply of fiber is necessary to keep the milk fat content at a high level. Hence, fiber has a positive effect on maintaining a steady rumen pH and creating beneficial conditions for cellulolytic rumen bacteria. Sugar increases the milk fat content as well, since its main fermentative product is butyrate. However, an excess application of sugar has to be avoided, because of its negative effect on the rumen environment. High dietary starch intake accelerates the synthesis of propionate and lowers the amounts of acetate, resulting in low milk fat contents. Greater intake of soluble carbohydrates results in rumen acidosis, leading to drastic declines in milk fat synthesis. However, this method should not be a tool for lowering the milk fat content, since acidosis leads to a severe anticlimax in DM intake and decreases the health status of the cow.

The conclusion of the abovementioned observations is that a high intake of feed and above average milk production with a high milk fat percentage can be achieved if rations contain 50 to 60% concentrate and 40 to 50% roughage. A review by Sawal and Kurar (1998) gives a detailed overview of different ways by which varying the concentrate-forage ratio influences milk yield and composition, especially milk fat. However, feeding high concentrate rations often induces the phenomenon of diet-induced milk fat depression (**MFD**), which is a result of altered rumen microbial fermentation and isomerization patterns. The mechanisms of diet-induced MFD are discussed below (page 25).

The amount of dietary fat in a ration has a great impact on milk fat secretion. Under normal conditions, dietary ether extracts make up less than 3% of the ruminant diet and arise from forage, grains or seeds that are rich in linoleic or linolenic acids (Palmquist and Jenkins, 1980). A non-milk fat depressing ration should not exceed 4% unsaturated fatty acids and 6% total fatty acid in DM (Eastridge, 2006). Higher percentages of crude lipid intake have negative effects on rumen microbial activity with a reduced overall digestibility of organic matter (Doreau et al., 1997). Digestibility of fiber components, but not of starch components, is lowered by high dietary CL intake (Zinn, 1989). The competition between fat and bacteria to adsorb to particles reduces rumen fiber digestibility to a small extent (Harfoot et al., 1974). This explains why greater amounts of forage reduce the interaction between bacteria and fatty acids, and thereby minimize the digestibility reducing effects.

The greatest negative effect of dietary lipids on rumen digestibility is due to the direct influence of lipids on the microbial ecosystem in the rumen. *In vitro* studies have validated this statement by showing direct negative effects of lipids on rumen bacterial growth. At the

same time, the VFA ratio in the rumen is modified toward elevated amounts of propionate and lower amounts of acetate and butyrate with reduced methane production. All nutritional modifications on microbial activity appear at the same time and are dependent on the amount and nature of lipids fed (Palmquist and Jenkins, 1980; Jenkins, 1993), the general components of the diet (Ben Salem, 1993) and how much rumen soluble Ca is available, since Ca reduces the negative effects of lipids on carbohydrate digestibility (White et al., 1958; Galbraith et al., 1971; Maczulak et al., 1981). Palmquist et al. (1986) have suggested that PUFA and Ca could form salts, thus reducing the negative effect of PUFA. The negative effect of dietary PUFA on cellulolytic bacteria is greater than that of saturated fatty acids (**SFA**). PUFA have negative effects on protozoa as well. A nearly complete rumen defaunation is achieved by supplementing linseed oil, which is high in linolenic acid. Doreau and Ferlay (1995) gave a detailed overview of published studies, showing that concentrations of 4.5% linseed oil in total added fat can severely decrease the concentration of rumen protozoa.

In contrast to the abovementioned ways to influence the milk fat content, the supplementation of rumen-inert *trans*-10, *cis*-12 CLA is a safe and elegant way to reduce the milk fat content in a dose-dependent manner with a maximum of about a 50% reduction in the milk fat content (Baumgard et al., 2001). Therefore, the discussed negative effects of dietary lipid supplementation and other possible methods reducing milk fat content are avoided.

Milk fat synthesis

Intensive fat synthesis takes place in the mammary gland, meaning that up to 1.5 kg of fat are synthesized per day. For the synthesis of milk TG, the precursors, acyl-CoA bound fatty acids and glycerolphosphate, have to be available. The esterification of fatty acids to TG takes place at the surface of the endoplasmic reticulum. A peculiarity of ruminants is that C_4 and C_6 fatty acids, synthesized within the mammary epithelial cell, are nearly entirely synthesized into the sn-3 position of the glycerol (Breckenridge and Kuskis, 1969). Oleic acid produced *in situ* by microsomal desaturation of stearic acid derived from the blood shares a preference for this third position (Kinsella, 1972), although some may esterify to the sn-2 position (Bickerstaffe and Annison, 1970). The acylation of the sn-3 position seems to regulate the synthesis of milk TG (Askew et al., 1971; Bickerstaffe, 1971; Kinsella, 1972), but it cannot be the ultimate control point. It must be regulatory through feedback to prevent excessive formation of diglycerides. The acylation of diglycerides in order to form triglycerides contributes for this reason to the regulation of milk fat secretion, but only in cooperation with and not to the exclusion of other controls.

Around 50 to 60% of the glycerophosphate used for TG synthesis in ruminants is generated in the mammary gland from glucose through triosephosphate by glycolysis and through the pentose phosphate pathway. The fatty acids used for milk TG have two origins. In ruminants, the majority is synthesized *de novo* using the substrates acetate and betahydroxy butyric acid (**BHBA**). The rest is originated from very low density lipoproteins (**VLDL**) and chylomicrons. The uptake of non-esterified fatty acids (**NEFA**) from blood plasma into the mammary gland is dependent on their plasma concentration, which correlates very closely with the mobilization of adipose tissue (Chilliard et al., 1984). The amounts of NEFA from adipose tissue mobilization varies and is dependent on the stage of lactation, milk yield and many other factors (Palmquist and Mattos, 1978; Grummer, 1991).

The pool of fatty acids originating from *de novo* synthesis, direct uptake or desaturation comprises 97 to 98% of total milk fat. The rest is comprised of mono- or diacylglycerides. The physical properties of milk lipids are determined through the anomalous distribution of fatty acids at the glycerol molecule and through favored occupation of the sn-3 position by SCFA or oleic acid (Palmquist et al., 1993).

Lipid transfer between blood and mammocytes

About 50% of the fatty acids in milk originate from lipids transported in the blood (Baldwin and Smith, 1971; Bickerstaffe, 1971). At least 80% of blood TG is completely hydrolyzed during uptake by the mammary gland (West et al., 1972). As in adipocytes, the TG of plasma lipoproteins are hydrolyzed by lipoprotein lipase (**LPL**) in mammocytes. VLDL is believed to be the primary source for mammary LPL in ruminants. Their action increases the fatty acid concentration in the capillary fluid, ready for uptake by the mammary gland. Enzyme activity is high in lactating ruminants (Chilliard et al., 1986). With the initiation of lactation, the activity of mammary LPL increases eight-fold, while LPL activity in adipose tissue simultaneously decreases (Shirley et al., 1971). This is why mammary uptake of fatty acids is tightly correlated with the plasma concentration of LPL (Gaglistro et al., 1991). A limiting factor of mammocyte fatty acid uptake is the activity of LPL, which is inadequate if the TG concentration in plasma is extremely high, i.e. greater than 0.4 mM (Baldwin et al., 1980). Certain peptides act in an inhibitory fashion on LPL, while others are required cofactors for the hydrolysis of TG by the enzyme (Brown and Baginsky, 1972). Cholesterol esters and phospholipids also stimulate or inhibit LPL, depending on their concentration (Brumby, 1971). Thus, the activity of LPL can be modulated by the proportion

of lipids and the type of protein in its lipoprotein substrate. These modulating factors are affected by diet and hormones.

The specificity between lipoprotein peptides and LPL from specific organs provides a mechanism for the diversion of blood lipids between mammary and adipose tissues. This has been proven by separating lipoproteins with a preference for adipose LPL from those with a preference for mammary LPL by flotation from blood serum (Emery et al., 1972). The specificity of LPL for certain lipoproteins implies differences in enzymes native in different organs, and possible differences in the hormonal control of LPL among organs. Prolactin stimulates the formation or activation and release of LPL from explants of rat or bovine mammary tissue incubated for 4 hours in the presence of insulin (Emery, 1973). Prolactin has little or no effect on adipose tissue, but the LPL activity of adipose tissue is dependent on insulin during 24 hours of culture.

Mammary de novo synthesis of fatty acids

About 40% of fatty acids in milk fat TG originate from *de novo* synthesis. Acetate and BHBA circulate in the plasma and are substrates for *de novo* synthesis in the cytoplasm. The preferred chain initiator for fatty acid synthesis in mammary tissue is BHBA. Although some BHBA is converted to acetate, which can be oxidized or used for chain elongation, this amount does not readily equilibrate with acetate derived from the blood. The precursor BHBA delivers about 15% of the carbon necessary for *de novo* synthesized fatty acids; the rest is delivered by acetate. Nearly all fatty acids with a chain length of C4 to C14 and 60% of the C16 fatty acid originate from *de novo* synthesis (Waghorn and Baldwin, 1984). In general mammary gland tissue is not able to elongate C16 to C18, with the one exception of bovine mammary gland tissue (Moore and Christie, 1981).

Two key enzymes are essential for *de novo* synthesis of fatty acids. The first enzyme is acetyl-CoA carboxylase (**ACC**) that catalyzes the synthesis of malonyl-CoA from acetate. The second enzyme fatty acid synthase (**FAS**) catalyzes the condensation of malonyl-CoA with acetyl-CoA or butyryl-CoA originating from the acetate and BHBA pool in the cytosol (Barber *et al.,* 1997; Figure 4). The termination of chain length of the medium length fatty acids in most animal tissues is catalyzed by the intrinsic thioesterase activity of FAS (Knudsen and Grunnet, 1982). More cellular and molecular factors affecting the relationship between the different fatty acids to each other seem to exist, but they have not yet been identified.

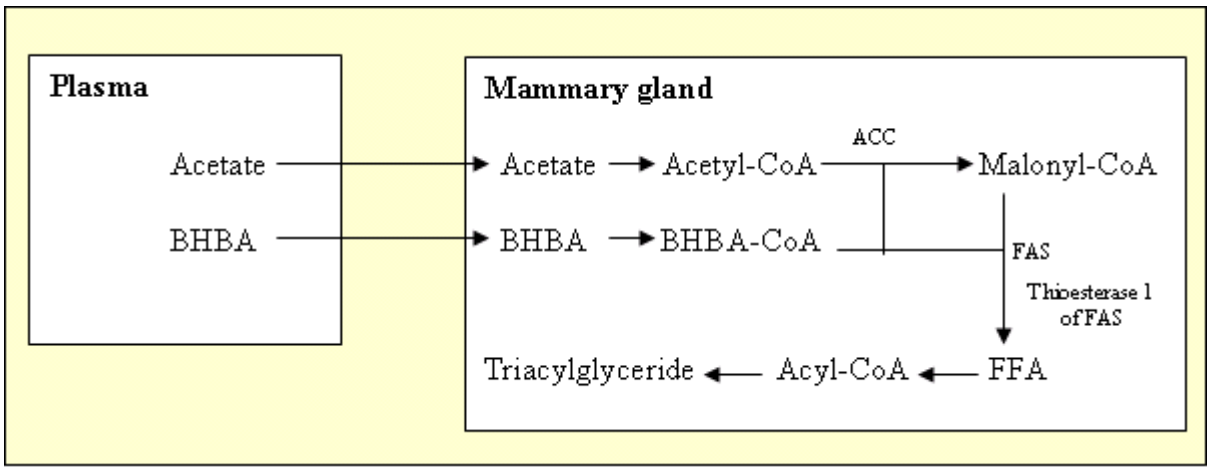

Figure 4. Schematic overview of *de novo* synthesis of fatty acid and their esterification to triglycerides in the mammary gland (Barber et al., 1997).

De novo synthesis is depressed by fat-supplemented diets. This leads to a decrease in the secretion and proportion of short and medium-chain fatty acids in milk. This is primarily due to the usual fat-induced increase in the proportion of propionate in the rumen at the expense of acetate and butyrate, and secondarily due to the inhibition of the synthesis of short- and especially medium-chain fatty acids by long-chain fatty acids (Chilliard et al., 1991), in particular by those with a *trans*-structure (Banks et al., 1984; Wonsil et al., 1994).

Milk fatty acid pattern

Moate et al. (2007) recently gave an overview of the general fatty acid composition of milk fat, provided in Table 2. They found dietary influences upon the fatty acid distribution between pasture- and total mixed ration (**TMR**)-based diets. Concentrations of total fatty acids, total *de novo* fatty acids, total C16 and total preformed fatty acids were numerically higher in milk of cows fed pasture-based diets, but the differences were not significant ($P > 0.05$). The concentration of C15, C17 and total *trans*-C18:1 fatty acids, however, was increased ($P < 0.05$) in cows fed pasture-based rations. On average, the concentration of *cis*-9, *trans*-11 CLA was not different between the dietary groups in the study by Moate et al. (2007). This was a bit surprising in light of the results of other researchers. However, it could have been that the concentrations of fatty acid concentrations in the TMR-based diets were overestimated due to supplemented fish oil (Moate et al., 2007).

Table 2. The proportion of 26 individual fatty acids in total milk fatty acids (mg/g) from milks described in 28 publications (adapted from Moate et al., 2007).

Fatty acid [1]	N [2]	Mean	SD
4:0	95	31.3	6.8
6:0	111	19.4	5.2
8:0	111	11.7	3.5
10:0	111	24.8	7.3
12:0	111	29.9	8.5
14:0	111	103.8	17.1
14:1*c*9	101	10.8	3.6
15:0	88	10.5	3.3
16:0	120	285.1	49.8
16:1*c*9	109	17.3	6.3
17:0	78	7.3	3.5
18:0	120	105.1	35.9
18:1*t*6-8	33	4.6	2.1
18:1*t*9	37	4.4	2
18:1*t*10	30	13.1	15.2
18:1*t*11	90	33.3	21.8
18:1*t*12	19	6.5	3.6
18:1*c*9	120	205	53.5
18:2*c*9,*c*12	120	31.3	21.1
18:2*c*9,*t*11	76	10.2	6
18:2*t*10,*c*12	35	0.4	0.3
18:2*c*11,*t*13	6	0.4	0.3
OCLA	25	1.5	1.4
18:3	114	5.9	3.6
20:0	30	1.5	0.6
20:5	39	1	1.1
22:6	31	0.7	0.7
Others	120	75.1	56.2
Total CLA	82	10.3	6.6
Total 18:1 *trans*	94	42.5	26.3
Total de novo	120	232.6	42.4
Total C16	120	300.9	52.7
Total preformed	120	466.5	75.8

[1] *c = cis; t = trans;* OCLA = other conjugated linoleic acids; CLA = conjugated linoleic acids, total 18:1 *trans*= sum of 18:*t*6-8, 18:1*t*9, 18:1*t*10, 18:1*t*11 and 18:1*t*12 isomers, total *de novo* = sum of 4:0 to 15:0 fatty acids, total C16 = sum of 16:0 and 16:1; total preformed = sum of all milk fatty acids with more than 17 carbon atoms

[2] N = total number of dietary treatments contributing to each mean

The pattern of fatty acids incorporated into milk fat TG has a crucial influence on the physical properties of milk lipids. Unsaturated fatty acids and SCFA make soft lipids. However, increasing amounts of double bonds incorporated into TG decrease the oxidative stability of the lipids. Hence, most fatty acids reaching the ruminant mammary gland are, to a great extent, LCFA with high melting points due to ruminal biohydrogenation. Ruminants have developed different methods of reducing the melting point of milk TG to stabilize fluidity at body temperature. In this procedure, the mammary gland tissue plays a central role, but it also occurs in adipose tissue. The different mechanisms were summed up by Chillard et al. (2000):

a) desaturation of LCFA by SCD (intestinal tissue, mammary gland tissue and adipose tissue);

b) synthesis of short and medium chain fatty acids;

c) the incapability of fatty acid elongation above C16;

d) the irregular esterification patterns of the different fatty acid molecules into milk TG in the secretory mammary gland cells.

PUFA lower the melting point as well. In the mammary gland, double bonds are only inserted by SCD, indicating the low rate of mammary PUFA synthesis. Typically, PUFA reach the mammary gland by lipoprotein transfer. Since PUFA originate from dietary fatty acids, the PUFA content in milk can be increased by reducing their biohydrogenation in rumen.

Fatty acid desaturation in mammary tissue

The activity of SCD, introducing a *cis*-9 double bond into LCFA, is high in mammary gland tissue. In fatty acids containing fewer than 18 carbon atoms, its activity is low; nevertheless, a small amount of C14 and C16 fatty acids is desaturated by the enzyme to C14:1 and C16:1 (Figure 5). Around 40% of mammary stearic acid is transformed into oleic acid (*cis*-9 C18:1), meaning that about 50% of secreted oleic acid in milk TG originates from desaturase activity (Bickerstaffe et al., 1974; Enjalbert et al., 1998). *Trans*-11 C18:1, the second fatty acid desaturated to a great extent by SCD, is desaturated to *cis*-9, *trans*-11 CLA, the preferentially secreted CLA isomer in milk fat (Griinari and Baumann, 1999). A study by Lock and Garnsworthy (2002) demonstrated that more than 80% of *cis*-9, *trans*-11 C18:2 in milk lipids originate from endogenous synthesis, but there seem to be differences in SCD activity between individual cows. Besides SCD, the enzymes Δ-5 and Δ-6 desaturase are

found in animal tissues. They are membrane-bound and insert *cis* double bonds to form PUFA (Nakamura and Nara, 2004). The overall production of *cis*-9, *trans*-11 CLA can be augmented by increasing the activity of SCD in the mammary gland rather than by increasing *cis*-9, *trans*-11 CLA production in the rumen or the overall intake of this isomer (Yang et al., 1999).

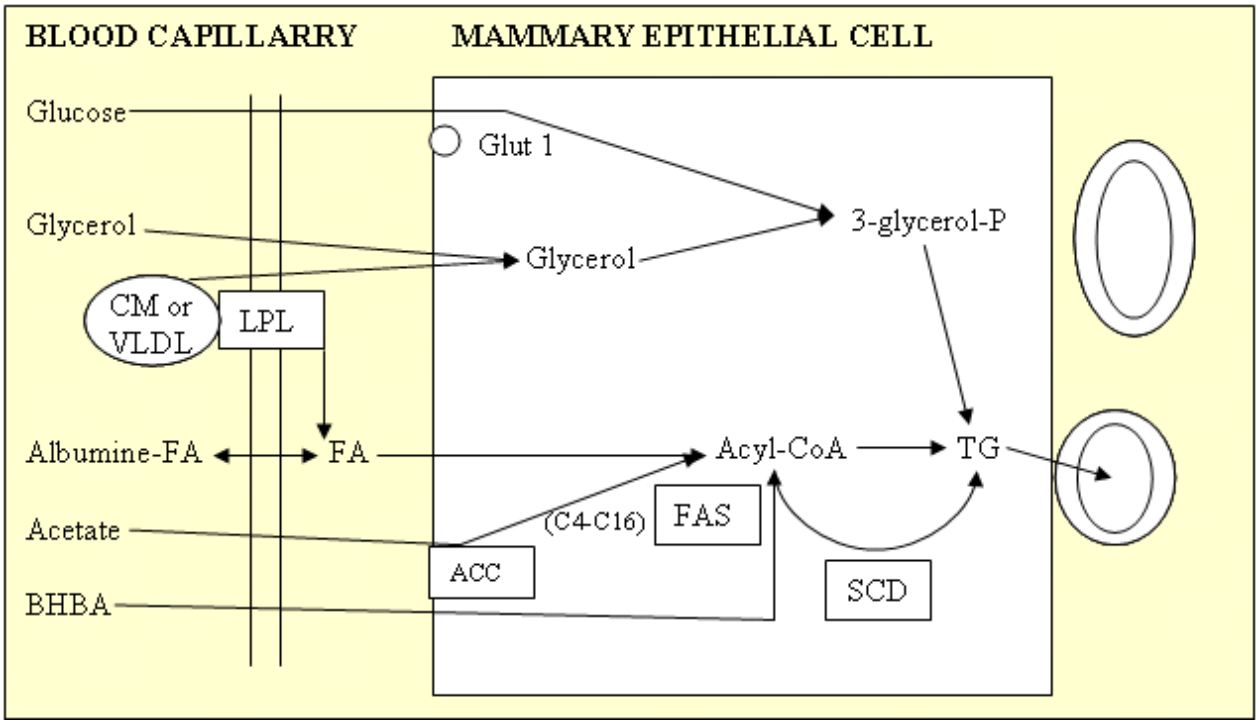

Figure 5. Milk fat synthesis and secretion in ruminants (Chilliard et al., 2000)

ACC = Acetyl-CoA carboxylase; CM = Chylomicron; SCD = Delta-9-desaturase; FA = Fatty acid; FAS = Fatty acid synthase; Glut 1 = Glucose transporter 1; LPL = Lipoprotein lipase; MFG = Milk fat globule; TG = Triglyceride; VLDL = Very low density lipoprotein.

Milk fat depression

Diet-induced low milk fat syndrome, also known as MFD, was first described about 150 years ago. It is characterized by a reduction in milk fat synthesis by up to 50%, with no changes in milk yield or the yield of other milk components (Bauman and Griinari, 2001). During the past two decades, research in this area has increased and is currently an active research area. Various theories trying to explain the depression in milk fat have been proposed, from reduced rumen acetate and butyrate production (Emery, 1988; Sutton, 1989) to the "glucogenic-insulin" and "*trans*-fatty acid" theories (McClymont and Vallance, 1962; Jenny et al, 1974; Annison, 1976; Davis and Brown, 1970; Astrup et al., 1976; Banks et al., 1984; Wonsil et al., 1994), to the most recent theory of biohydrogenation proposed by Bauman and Griinari (2001).

Historically, MFD due to dairy cow diet has been divided into two groups: the first group includes diets providing large amount of readily fermentable carbohydrates and low amounts of fiber, and the second group includes diets supplementing large amounts of oils high in PUFA (Davis and Brown, 1970). The recent viewpoint is that these two groups are not independent of each other in inducing MFD. Furthermore, it has been shown that diets rich in concentrate and low in fiber increase the content of *trans*-10, *cis*-12 CLA and *trans*-10 C18:1 fatty acids in milk. On the other hand, the milk fat content is not always reduced when plant oils are fed due to a simultaneous high roughage intake (Brown et al., 1962). Thus, two dietary conditions are required for MFD to occur: 1) an alteration in rumen microbial processes and 2) the presence of sufficient amounts of C18-PUFA in the diet. Rumen microbial processes can be altered by high grain/low fiber diets, feeding forage with reduced particle size, the use of marine oil supplements and by adding anti-methanogenic compounds to the feed. The presence of C18-PUFA from feeds has to be in a sufficient amount in order to deliver precursors for the formation of factors that result in MFD (Baldwin et al., 1969; Sutton et al., 1988; Palmquist and Schanbacher, 1991).

The *trans*-fatty acid theory proposes that *trans*-fatty acids are formed as intermediates during rumen biohydrogenation of unsaturated fatty acids. Harfoot and Hazlewood (1997) have shown that a complex mixture of *trans*-fatty acids is formed during rumen biohydrogenation. A major *trans*-fatty acid in milk fat, resulting from rumen biohydrogenation, is the isomer *trans*-11 C18:1 (Molkentin and Precht, 1995), but it has not been proven whether this isomer or the overall *trans*-C18:1 isomer level is responsible for MFD. Griinari et al. (1998) suggested that the amount of *trans*-10 C18:1 is accountable for the action on milk fat synthesis, and Bauman and Griinari (2003) observed that an increase in *trans*-10 C18:1 in milk fat is typical for diets causing MFD.

The role of CLA in MFD

The "*trans*-fatty acid theory" was vitiated by Lock et al. (2007), who could not induce MFD by abomasal infusion of 42.6 g of *trans*-10 C18:1/d into dairy cows; subsequently, Bauman and Grinarii (2001) proposed the "biohydrogenation theory". This theory is based on the concept that, under dietary conditions inducing MFD, the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates that are potent inhibitors of milk fat synthesis. Under normal conditions, Wallace et al. (2007) found that linoleic acid was biohydrogenated to different CLA isomers of which *cis*-9, *trans*-11 CLA, *trans*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were the major intermediates. Furthermore,

traces of *trans*-9, *cis*-11 CLA, *cis*-9, *cis*-11 CLA and *cis*-10, *cis*-12 CLA were found in the rumen digesta.

An alteration of biohydrogenation due to changed rumen environmental conditions is possible. The mechanisms would depend on an alteration in rumen microbial processes and a sufficient supply of unsaturated fatty acids. Under these conditions, *trans*-10 C18:1 and its intermediates can be synthesized in the rumen. It has been suggested that a decline in rumen pH provokes a shift in the biohydrogenation pathway of linoleic acid, as shown in Figure 3. The strain *Megasphera elsdenii* YJ-4 has been shown to produce *trans*-10, *cis*-12 CLA (Kim et al., 2000). Kim et al. (2002) showed that strains of *Megasphera elsdenii* can differ greatly in their ability to produce the *trans*-10, *cis*-12 CLA isomer and that CLA production is not a phylogenetically conserved trait. Counotte et al. (1981) observed that grain feeding promotes the growth of *Megasphera elsdenii*, which is consistent with the observations on diets inducing MFD. *Propionibacteria* isolated from the mouse cecum have been found to produce *trans*-10, *cis*-12 CLA when cultured in the presence of linoleic acid (Verhulst et al., 1987). Wallace et al. (2007) used *P. agnes* to produce *trans*-10, *cis*-12 CLA from linoleic acid in an *in vitro* rumen culture. Further, *Lactobacillus rhamnosus* was reported to produce *trans*-10, *cis*-12 CLA (Lee et al., 2006), whereas *Lactobacillus acidophilus* and *L. casei* synthesized only minor amounts of *trans*-10, *cis*-12 CLA during linoleic acid metabolism (Alonso et al., 2003).

A dramatic reduction in milk fat content was caused by post-ruminal infusion of *trans*-10, *cis*-12 CLA (Loor and Herbein, 1998; Chouinard et al., 1999a, 1999b, Lock et al., 2007). Baumgard et al. (2000) were the first to identify the role of *trans*-10, *cis*-12 CLA in MFD by utilizing post-ruminal infusions of pure CLA isomers (Figure 6). They observed that *cis*-9, *trans*-11 CLA had no effect on milk fat content, whereas *trans*-10, *cis*-12 CLA induced MFD. *Trans*-10, *cis*-12 was until recently the only fatty acid shown unequivocally to inhibit milk fat synthesis. Recently, two additional CLA isomers were isolated, inducing the inhibition of milk fat synthesis after abomasal infusion. Perfield et al. (2007) specified one as *trans*-9, *cis*-11 CLA, whereas Sæbø et al. (2005a) identified the other as *cis*-10, *trans*-12 CLA. However, the effect of *trans*-9, *cis*-11 C18:2 was less than that of *trans*-10, *cis*-12 CLA, whereas *cis*-10, *trans*-12 CLA seems to have similar effects as *trans*-10, *cis*-12 CLA. Other CLA isomers, including *trans*-8, *cis*-10 CLA, *cis*-9, *trans*-11 CLA and *cis*-11, *trans*-13 CLA, have been tested and no effects on milk fat concentrations were observed (Baumgard et al., 2000, 2002a; Loor and Herbein, 2003; Perfield et al., 2004a). Likewise, *trans*-10, *trans*-12 CLA has no effect on the rate of milk fat secretion (Perfield et al., 2004b; Sæbø et al., 2005a) as well as

the levels of conjugated dienes C18:3 *cis*-6, *trans*-10, *cis*-12 and *cis*-6, *trans*-8, *cis*-12 C18:3 (Sæbø et al., 2005b). Yet, small changes within the fatty acid structure can lead to striking results with respect to action and potency. For example, *trans*-10, *trans*-12 CLA and *trans*-9, *trans*-11 CLA does not reduce milk fat synthesis, but they are both potent inhibitors of SCD (Harvatine et al., 2009; Perfield et al., 2006).

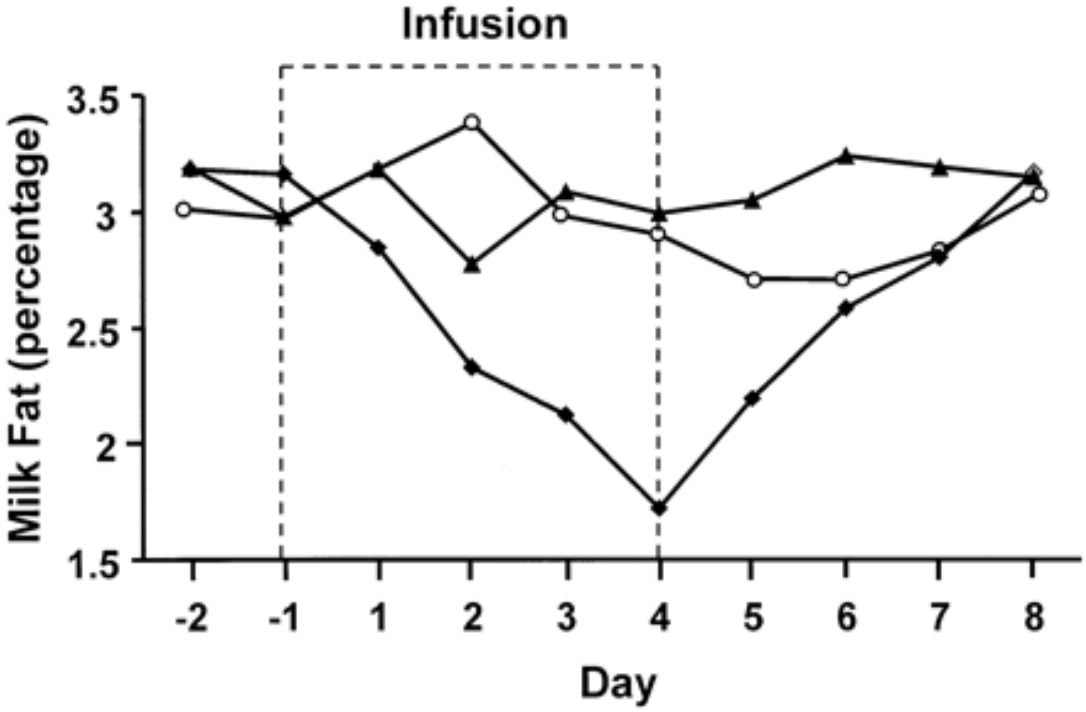

Figure 6. Development of milk fat content during abomasal infusion of conjugated linoleic acid (CLA) supplements.

Dotted lines imply a 4 day infusion period of treatments, which were control (▲), 9,11 CLA supplement (○; 10 g/day), and 10,12 CLA supplement (●; 10 g/day). Values represent means from 3 cows; the SE for milk fat percentage ranged from 0.003 to 0.300. From Baumgard et al. (2000)

Effect of trans-10, cis-12 CLA on milk fat synthesis (post-ruminally infused)

The milk fat depressing action of *trans*-10, *cis*-12 CLA was validated by several studies in which *trans*-10, *cis*-12 CLA was infused post-ruminally, covering a range from 1.25 to 14 g/day (Baumgard et al., 2000, 2001, 2002a, 2002b; Peterson et al., 2002; Loor and Herbein 2003; Perfield et al., 2004a; de Veth et al., 2004). Based on these data, the dynamics in CLA-induced MFD has been shown to occur in a dose-dependent manner. As shown in Figure 7, a maximum reduction of milk fat yield (50%) is reached at a dose of approximately 15 g of

trans-10, *cis*-12 CLA per day. The mechanisms involved in MFD are shown by the alterations in milk fatty acid composition.

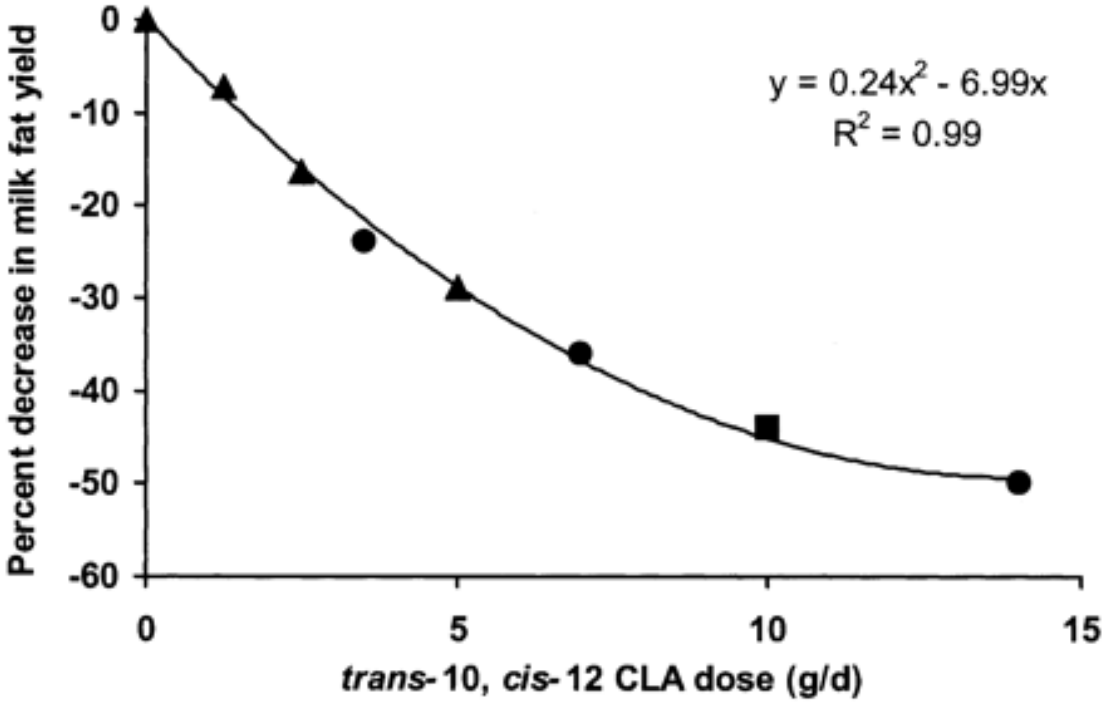

Figure 7. Dose dependency between the decrease in milk fat yield and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) infused abomasally into lactating dairy cows.

Triangle symbols represent the values from Peterson et al. (2002), circles represent values from Baumgard et al. (2001) and the square represents data from Baumgard et al. (2000). All values represent the mean (n = 4) on d 5 of infusion. From Peterson et al. (2002).

Effects of trans-10, cis-12 CLA on milk fat composition

Post-ruminally infused *trans*-10, *cis*-12 CLA alters the fatty acid composition of milk fat. The pattern of milk fatty acid composition provides information on the mechanisms by which milk fat synthesis is inhibited. (The section on milk fat synthesis (page 19) provides further information about the fatty acid composition of milk). In general, fatty acids up to carbon atom 14 are completely synthesized *de novo*, and fatty acids greater than or equal to C18 are solely derived from plasma lipoproteins (Bauman et al., 1970; Brumby and Welch, 1978; Moore and Christie, 1979). Fatty acids with 16 carbon atoms are derived from blood plasma TG and are synthesized within the gland. The percentage of *de novo* synthesized palmitate has been estimated at 60% (Palmquist et al., 1969), 63% (Bickerstaffe et al., 1974) 55% (Glascock and Welch, 1974) and 65% (Peeters et al., 1979). Odd (C15 and C17)

branched-chain fatty acids in milk fat are largely derived from bacteria leaving the rumen (Vlaeminck et al., 2006).

Peterson et al. (2002) infused 1.25, 2.5 and 5 g/d *trans*-10, *cis*-12 CLA into the abomasum of dairy cows, which induced a reduction in *de novo* synthesized and preformed fatty acids. Both effects contributed similarly (on a molar basis) to a curvilinear reduction in milk fat yield across all doses of *trans*-10, *cis*-12 CLA. The observations of Baumgard et al. (2001) were similar with an infusion of 3.5 g/d of *trans*-10, *cis*-12 CLA, but they found more pronounced effects on *de novo* synthesis at higher doses (7, 10, 14 g/d). In addition, Peterson et al. (2002) found no effects of *trans*-10, *cis*-12 CLA on the ratios of fatty acids representing product/substrate pairs for SCD. The lower doses (3.5 g/d) used by Baumgard et al. (2001) showed no effects either, whereas higher doses (7 and 14 g/d) altered the ratios in a manner indicating that this CLA isomer reduces SCD activity.

The desaturase index is represented by the substrate-to-product ratios of C14:0/C14:1, C18:0/C18:1 and *trans*-11 C18:1/*cis*-9, *trans*-11 C18:2 and serve as a proxy for SCD activity. With abomasal infusion of *trans*-10, *cis*-12 CLA, a reduction in mammary expression and/or activity of SCD occurred, whereas *cis*-9, *trans*-11 CLA had no effect on milk fat composition (Baumgard et al., 2000). The study of Baumgard et al. (2002b) showed that an infusion of 10 g *trans*-10, *cis*-12 CLA decreased the desaturase index. However, lower doses of *trans*-10, *cis*-12 CLA exhibited lower but significant inhibition of milk fat synthesis, without any changes in the SCD index (Baumgard et al. 2001; Peterson et al. 2002).

The effects of trans-10, cis-12 CLA on milk fat yield and content

The milk fat reducing potency of *trans*-10, *cis*-12 CLA is supported by the fact that there is a dose-response relationship related to the isomer content in milk fat (Figure 8). A curvilinear relationship exists between post-ruminally infused *trans*-10, *cis*-12 CLA and milk fat reduction. Infusing large amounts of a CLA mixture (150 g/d, with 31.7% *cis*-9, *trans*-11 C18:2 (= 47.6 g) and 30.4% *trans*-10, *cis*-12 C18:2 (= 45.6 g) after the rumen lowered the fat content and yield during a period of 11 days by about 25% and 57%, respectively (Bell and Kennelly, 2003). Different studies have indicated a declining marginal gain for *trans*-10, *cis*-12 CLA on depressing the milk fat yield and content (Loor and Herbein, 1998; Chouinard et al., 1999a; 1999b; Gervais et al., 1999; Figure 8). However, most of these studies included a very short period of *trans*-10, *cis*-12 CLA abomasal infusion (48 h, Loor and Herbein, 1998; 5 d, Chouinard et al., 1999a; 3 d, Chouinard et al., 1999b; 5 d, Baumgard et al., 2001; 5 d, Baumgard et al. 2002b; 11 d, Bell and Kennelly, 2003; 4 d, Mackle et al., 2003; 5 d, Gervais

et al., 2009). The question is if the nadir in MFD is reached by this time and at which point milk yield is affected by *trans*-10, *cis*-12 CLA abomasal infusion. Increases in milk yield are able to overrule the depression in milk fat yield. Taking a closer look at the studies of Loor and Herbein (1998), Baumgard et al. (2002b) and Mackle et al. (2003) showed that MFD was not at its nadir when infusion stopped. Possible effects on milk yield were not observed in these short periods of *trans*-10, *cis*-12 CLA abomasal infusion.

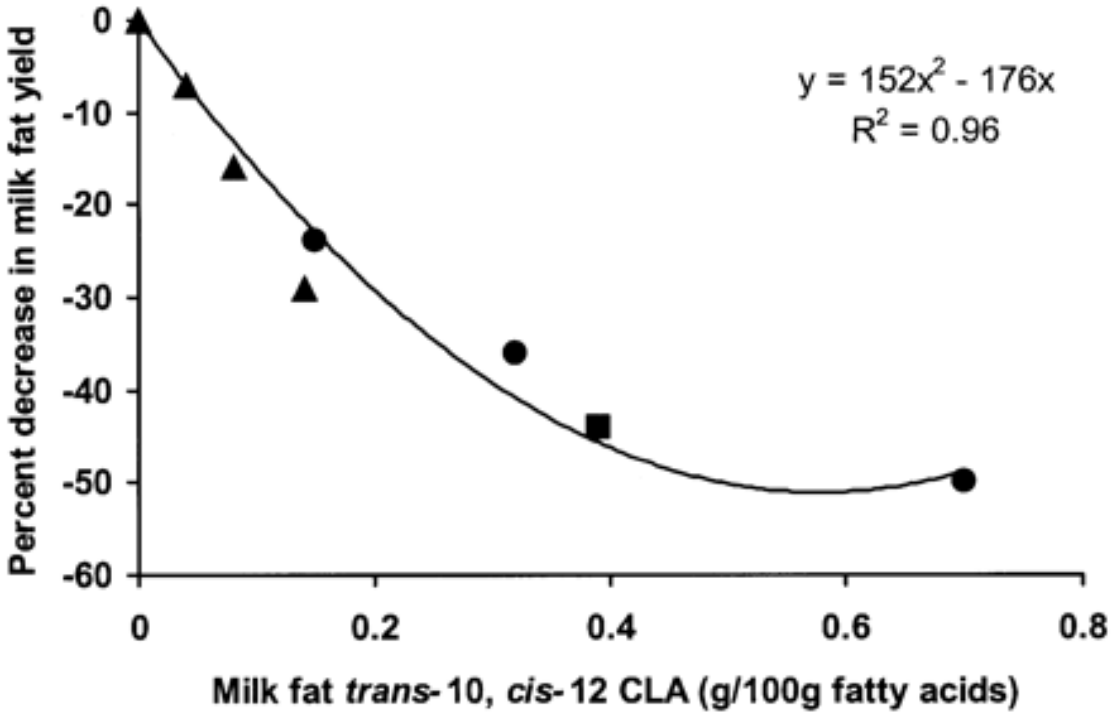

Figure 8. Relationship between the *trans*-10, *cis*-12 C18:2 (CLA) dose and the relative reduction in milk fat yield ($R^2 = 0.99$; $P < 0.001$).

Triangle symbols characterize values from Baumgard et al. (2001), diamond symbols represent values from Peterson et al. (2002) and square symbols represent values from Baumgard et al. (2000). All values represent the mean (n=4) of day 5 of infusion. From Peterson et al. (2003).

There seems to be a divergence in the reduction in milk fat yield depending on the method of *trans*-10, *cis*-12 CLA supplementation. Abomasal infusions seem to induce less severe MFD than if MFD is diet-induced (Figure 9). Peterson et al. (2003) suggested that the higher reductions in milk fat yield during diet-induced MFD were due to additional fatty acid intermediates inhibiting milk fat synthesis. A shift in biohydrogenation could result in the synthesis of the milk fat depressing isomers *trans*-9, *cis*-12 CLA and *cis*-10, *trans*-12 CLA (Perfield et al., 2007; Sæbø et al., 2005a). However, another explanation for the differences between studies could be the different duration of CLA supplementation, with a mean (n = 4) infusion time of 5 d. In contrast, the data collected by Peterson et al. (2003) used a mean (n =

3) feeding time of 19 d with milk fat depressing CLA isomers. In feeding studies, animals had a longer period of time to adjust to *trans*-10, *cis*-12 CLA, which might indicate that the nadir in infusion studies was not reached by the time samples were collected.

The study of Maxin et al. (2011) showed that the effects of rumen infused propionate and acetate, and propionate and *trans*-10, *cis*-12 CLA on milk fat secretion were additive. This explains that propionate could contribute to milk fat reductions unaccounted for by *trans*-10, *cis*-12 CLA during MFD induced by high concentrate diets. Since these diets increase ruminal production of propionate.

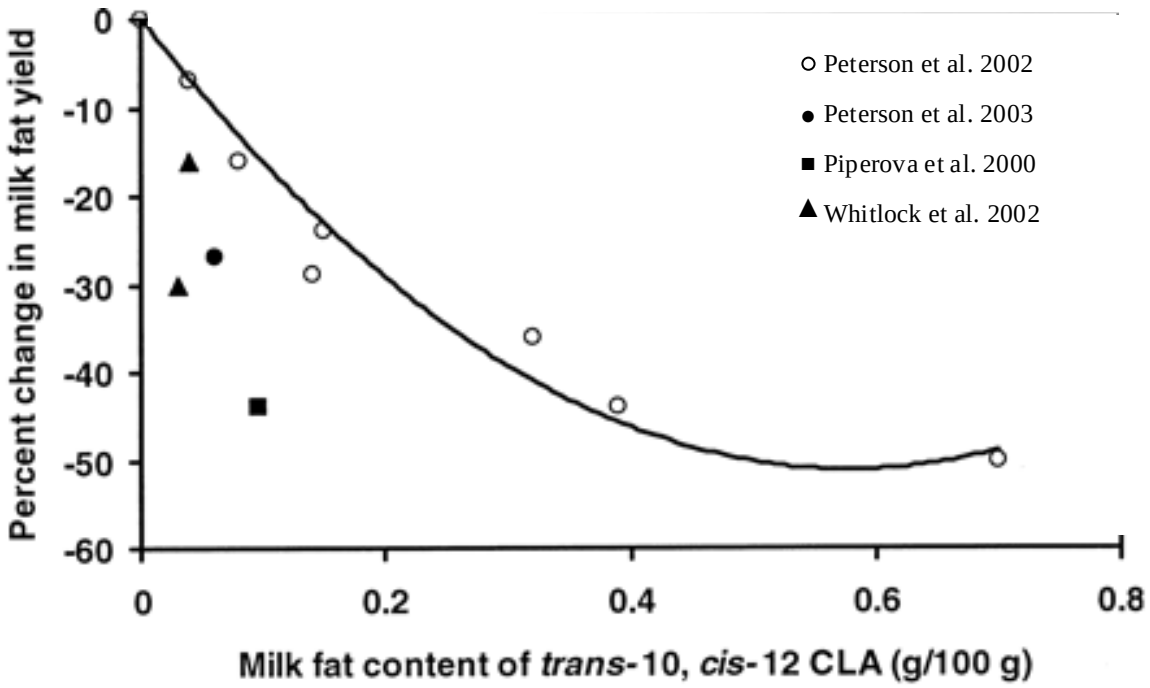

Figure 9. Relationship between *trans*-10, *cis*-12 CLA appearing in milk fat and the reduction in milk fat yield comparing the effects of diet-induced MFD with abomasally infused CLA.

Data indicated by ●, ■ and ▲ represent values of diet-induced MFD, while the dose response curve (○) represents values of abomasally infused *trans*-10, *cis*-12 CLA. From Peterson et al. (2003).

Post-ruminal infusion studies

An evaluation of the literature on supplementing *trans*-10, *cis*-12 CLA post-ruminally (Table 3) shows an eminent effect of this isomer on milk fat synthesis. Supplementation with a low amount, ~4 g *trans*-10, *cis*-12 CLA per day, provokes an effect on milk fat yield as well as on milk fat content.

Table 3. Literature overview showing effects of different amounts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) supplemented post-ruminally upon milk and milk components in dairy cows compared to the respective control (%).

Unit / Authors [1]	CLA, g	MFY[2]	MFC[2]	MPY[2]	MPC[2]	MY[2]
Baumgard et al., 2000	10	-44	-42	--[3]	--	--
Baumgard et al., 2001	3.5	-25	-24	--	--	--
	7	-33	-37	--	--	--
	14	-50	-46	--	--	--
Baumgard et al., 2002a	3.5	-25		--	--	--
	7	-33		--	--	--
	10	-28	-27	--	--	--
	14	-50	-42	--	--	--
Baumgard et al., 2002b	13.6	-48	-42	-16	--	-14
Bell and Kennelly, 2003	45.6	-43	-25	-13	+31	-38
Chouinard et al., 1999b	10.6	-52	-49	--	--	--
	21.1	-51	-51	--	--	--
	31.7	-62	-56	--	--	-15
Gervais et al., 2009	10	-38	-46	--	--	--
Mackle et al., 2003	5.9	-32	-36	--	--	--
	11.9	-36	-43	--	--	--
	23.8	-60	-62	--	--	+11
Perfield et al., 2004b	4	-35	-39	--	--	--
Viswanadha et al., 2003	2	-13	-15	--	--	+27
	4	-15	-21	--	--	+10
	6	-20	-30	--	--	+11

[1] All studies were conducted on cows in established or late lactation. Periods ranged from 80 days to 286 days post partum. Milk samples were collected within the period of 4 to 11 days after the start of the trial. Values represent the difference between the control and treatment group (%) of the least square means collected on the last day of the trial period.

[2] MFC= Milk fat content in %, MFY= Milk fat yield in kg, MPC= Milk protein content in %, MPY= Milk protein yield in kg, MY= Milk yield in kg.

[3] -- no significant differences noted.

A dose-response relationship for MFD was noticeable between studies. Milk fat content and yield were reduced by about 60% when high amounts of *trans*-10, *cis*-12 CLA were supplemented (Table 3). However, the degree of MFD was not consistent for similar amounts of *trans*-10, *cis*-12 CLA between studies.

Only two studies (Baumgard et al., 2002b; Bell and Kennelly, 2003) observed an effect on milk protein synthesis. The milk protein yield was reduced in both studies by around 15%, whereas milk protein content was affected (positively) in the study by Bell and Kennelly (2003). The amount of supplemented *trans*-10, *cis*-12 CLA (45.6 g/d) used in the study of Bell and Kennelly (2003) was on the high side. This had a significant effect in reducing the milk yield in treated cows. The effect of *trans*-10, *cis*-12 on milk yield was inconsistent.

Five out of the ten studies noted an effect of CLA on milk yield, and the highest supplementation rate of these studies resulted in a response. However, the effect on milk yield was inconsistent between studies. Three of them showed reductions and two studies registered increases in milk yield. With the exception of Viswanadha et al. (2003), only high inclusion rates of *trans*-10, *cis*-12 CLA (> 13.5 g/d) resulted in a milk yield response. The positive response of milk yield to CLA supplementation by Viswanadha et al. (2003) has to be carefully compared to the results of other studies, since the isomer was intravenously administered, whereas it was abomasally infused in the other studies.

Baumgard et al. (2002a) and Mackle et al. (2003) have suggested that *trans*-10, *cis*-12 CLA could be a tool to reduce the energy required for lactation. This is possible if CLA supplementation only resulted in a transformation of milk fat synthesis and all other milk variables stayed constant, or if increases in milk yield would energetically not exceed the energy conserved by MFD.

Feeding studies

The results shown in Table 3 were generated only from post-ruminal infusion studies, and did not consider the influence of this intestinal compartment upon the effects of *trans*-10, *cis*-12 CLA. The effect of supplementing a rumen-inert source of the milk fat depressing isomer to the diet of dairy cows was investigated by several studies. These had to be divided into two groups according to the stage of lactation, since differences were observed in the action of *trans*-10, *cis*-12 CLA on milk variables in cows in early lactation and those in established lactation.

Established lactation

The MFD effect of *trans*-10, *cis*-12 CLA was uniformly significant in all studies selected for the evaluation of the response in the yield and content of milk fat (Table 4). De Veth et al. (2006) did not collect data for the milk fat content. Similar amounts of the milk fat depressing CLA isomer did not necessarily lead to equal reductions in the milk fat content and yield as was found in the infusion studies. Possible reasons might be found in the varying duration of supplementation (7 to 140 d).

Table 4. Literature overview of diet-induced milk fat depression in established lactation dairy cows, showing effects upon milk yield and milk composition compared to the respective control (%).

Author \ Unit	N	DIM	Duration of suppl.	CLA	MFC	MFY	MPC	MPY	MY
De Veth et al., 2005	3	202±6	7	10	-28	-42	--[1]	--	--
			7	10	-35	-44	--	--	--
De Veth et al., 2006	48	112±5	16	12		-21		+2.8	+2.6
Gervais et al., 2005	240	171	42	0.7	-13	-11	--	--	--
			42	1.4	-22	-20	--	--	--
			42	2.8	-28	-28	--	--	--
Kay et al., 2007	12	204±7	10	9	-43	-44	+7	+6	--
Moore et al., 2005	13	97±17	21	3.8	-26	-30	--	--	--
Perfield et al., 2002	23	227	140	8.8	-23	-23	--	--	--
Perfield et al., 2004b	3	78±13	7	10	-27	-21	--	+6	--
			7	10	-28	-22	--	+8	--
Peterson et al., 2003	3	111±12	21	Diet	-25	-27	--	--	--

N = number, DIM = days in milk, CLA = *trans*-10, *cis*-12 CLA in g, Diet = Diet induced by ration, MFC = Milk fat content in %, MFY = Milk fat yield in kg, MPC = Milk protein content in %, MPY = Milk protein yield in kg, MY = Milk yield in kg.

[1] -- no significant differences noted

Three out of the eight studies observed significant positive effects on milk protein yield. These studies fed high amounts (≥9 g) of the milk fat depressing isomer. In the trial by De Veth et al. (2005), 10 g of *trans*-10, *cis*-12 CLA was not able to stimulate milk protein yield, whereas Kay et al. (2007) found a positive impact of feeding 9 g *trans*-10, *cis*-12 CLA on

milk protein content and yield. Perfield et al. (2004) observed increasing amounts of milk protein yield with comparable CLA amounts. The study that fed the highest amount (12 g) of *trans*-10, *cis*-12 CLA (De Veth et al., 2006) was the only one to observe significant increases in milk yield. These results indicated that higher amounts of *trans*-10, *cis*-12 CLA were able to affect milk fat synthesis as well as milk protein synthesis and milk yield.

Kay et al. (2007) and Moore et al. (2005) tested the hypothesis that supplementing *trans*-10, *cis*-12 CLA in the diet of dairy cows will improve energy balance. In both studies, the supplementation did not reach 10 g/d, and therefore the studies did not show any effects on milk protein synthesis and milk yield.

Early lactation

The milk fat depressing action of *trans*-10, *cis*-12 CLA was verified in several studies for dairy cows in early lactation, both for milk fat content and yield (Table 5). Castañeda-Gutiérrez et al. (2007) and Sigl et al. (2010) did not find any significant response in milk fat yield. Sigl et al. (2010) had problems with cows ingesting all the CLA and in both studies the CLA supplementation started after parturition, while all the other studies mentioned in Table 5 fed the milk fat depressing CLA isomer during the transition period.

The problems in evaluating a possible dose response on MFD were based on the fact that some studies surveyed the least square means from a specific lactation day, while others evaluated the data obtained during the entire trial period. Since most studies (Bernal-Santos et al., 2003; Moore et al., 2004; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2007; Odens et al., 2007; von Soosten et al., 2011) observed a delay in the CLA response of milk fat synthesis with the onset of lactation, the dose response in MFD is not consistent during this period. A dose response in MFD within trials was apparent, yet similar doses between trials showed differences.

No effect of *trans*-10, *cis*-12 CLA was observed on milk protein yield and content, and only the trials by Bernal-Santos et al. (2003) and Moallem et al. (2009) resulted in significant milk yields. The data in Table 5 indicated that supplementation with *trans*-10, *cis*-12 CLA during the transition period and early lactation might have some benefits on the animals' energy balance, since in most cases only milk fat synthesis was affected, but not milk yield.

Table 5. Literature overview of diet-induced milk fat depression in early lactation cows, effects on milk yield and milk composition compared to respective control (%).

Author \ Unit	N	Duration of supplementation	CLA	MFC	MFY	MPC	MPY	MY
Bernal-Santos et al., 2003	3	14 a.p.-140 p.p.		-13	-8	--[1]	--	+8
Castañeda-Gutiérrez et al., 2005	4	14 a.p.-63 p.p.	9	-10	-11	--	--	--
			18	-19	-21	--	--	--
Castañeda-Gutiérrez et al., 2007	4	20 p.p.- 57 p.p.	2.4	-6	--	--	--	--
			7.1	-14	--	--	--	--
Moallem et al., 2009	4	21 p.p.-100 p.p.	4.7	-12.5	-9	--	--	+4.
Moore et al., 2004	1	10 a.p.-21 p.p.	5	-13	-12	--	--	--
			10	-27	-22	--	--	--
			15	-32	-30	--	--	--
Odens et al., 2007	3	9 a.p.-40 p.p.	3.2	-18	-17	--	--	--
			9.7	-26	-23	--	--	--
Selberg *et al*., 2004	3	28 a.p.-49 p.p.	12	-14	-14	--	--	--
Sigl et al., 2010	1	14 a.p.-28 p.p.	10	--	--	--	--	--
Von Soosten et al., 2011	2	1 p.p.-105 p.p.	6	-5.5	-9.8	--	--	--

N = number, CLA = *trans*-10, *cis*-12 CLA, MFC = Milk fat content in %, MFY = Milk fat yield in kg, MPC = Milk protein content in %, MPY = Milk protein yield in kg, MY = Milk yield in kg, a.p. = ante partum, p.p. = post partum

[1] -- no significant differences noted

The effects of *trans*-10, *cis*-12 CLA on milk yield and components shown in Tables 3 to 5 demonstrated that the response of dairy cows, amongst others, was dependent on the method and duration of CLA supplementation and on the stage of lactation. Almost all studies evaluating the effects of *trans*-10, *cis*-12 CLA during early lactation fed about 10 g of this isomer. Furthermore, it was evident that rumen-inert products providing around 10 g of *trans*-10, *cis*-12 CLA had a significant effect in reducing milk fat synthesis even in established lactation. It can therefore be concluded that this amount is sufficient to induce MFD in lactating dairy cows. The suggestions of several authors (Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005; Moore et al., 2005; Kay et al., 2007) could motivate producers to induce MFD through the diets of dairy cows with the aim of modulating the energy required for milk synthesis. Saving energy is of specific interest in early lactation, since this is the period during which most cows are in negative energy balance. A specific and

quick reduction in milk fat synthesis with the onset of lactation would impair the enhancement in energy balance by lowering the total energy output. However, the inconsistent findings in milk yield and the milk protein response to *trans*-10, *cis*-12 CLA only allow vague predictions of actual energy savings. A further limitation in the calculation of energy savings during early lactation results from the fact that milk fat synthesis is first reduced a few weeks after the onset of lactation.

REFERENCES

AbuGhazaleh, A. A., D. J. Schingoethe, A. R. Hippen, and K. F. Kalscheur. 2003. Conjugated linoleic acid and vaccenic acid in rumen, plasma, and milk of cows fed fish oil and fats differing in saturation of 18 carbon fatty acids. J. Dairy Sci. 86:3648-3660.

Alonso, L., E. P. Cuesta, and S. E. Gilliland. 2003. Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. J. Dairy Sci. 86:1941-1946.

Amemiya-Kudo, M., H. Shimano, T. Yoshikawa, N. Yahagi, A. H. Hasty, H. Okazaki, Y. Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Sato, S. Kimura, S. Ishibashi, and N. Yamada. 2000. Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J. Biol. Chem. 275:31078-31085.

Annison, E. F. 1976. Energy utilisation in the body. In: Principles of Cattle Production, H. Swan, W. H. Broster, eds., pp. 169-199, London-Butterworth.

Arab, L. 2003. Biomarkers of fat and fatty acid intake. J. Nutr. 133:925S-932S.

Askew, E. W., R. S. Emery, and J. W. Thomas. 1971. Characteristics of fatty acid esterification by homogenates of bovine mammary tissue. Lipids 6:326-331.

Astrup, H. N., L. Vik-Mo, A. Ekern, and F. Bakke. 1976. Feeding protected and unprotected oils to dairy cows. J. Dairy Sci. 59:426-430.

Baldwin, R. L., N. E. Smith, J. Taylor, and M. Sharp. 1980. Manipulating metabolic parameters to improve growth rate and milk secretion. J. Anim. Sci. 51:1416-1428.

Baldwin, R. L. and N. E. Smith. 1971. Intermediary aspects and tissue interactions of ruminant fat metabolism. J. Dairy Sci. 54:583.

Baldwin, R. L., H. J. Lin, W. Chen, R. Cabrera, and M. Ronning. 1969. Enzyme and metabolite levels in mammary and abdominal adipose tissue of lactating dairy cows. J. Dairy Sci. 52:183-187.

Banks, J. M., J. L. Clapperton, D. D. Muir, and A. K. Girdler. 1986. The influence of diet and breed of cow on the efficiency of conversion of milk constituents to curd in cheese manufacture. J. Sci. Food Agric. 37:461-468.

Banks, W., J. L. Clapperton, A. K. Girdler, and W. Steele. 1984. Effect of inclusion of different forms of dietary fatty acid on the yield and composition of cow's milk. J. Dairy Res. 51:387-395.

Barber, M. C., R. A. Clegg, M. T. Travers and R. G. Vernon. 1997. Lipid metabolism in the lactating mammary gland. Biochem. Biophys. Acta 1347:101-126.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual Rev. Nutr. 23:203-227.

Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Prod. Sci. 70:15-29.

Bauman, D. E., L. H. Baumgard, B. A. Corl and J. M. Grinarii. 2000. Biosynthesis of conjugated linoleic acid in ruminants. Am. Soc. Animal Sci. 1-15.

Bauman, D. E., R. E. Brown, and C. L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow and cow. Arch. Biochem. Biophys. 140:237.

Baumgard. L. H., B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002a. Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. J. Ani. Sci. 80:1285-1293.

Baumgard, L. H., E. Mattiasvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002b. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769.

Baumgard. L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278:R179-R184.

Beam, T. M., T. C. Jenkins, P. J. Moate, R. A. Kohn and D. L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J. Dairy Sci. 83:2564-2573.

Bell, J. A. and J. J. Kennelly. 2003. Short communication: postruminal infusion of conjugated linoleic acids negatively impacts milk synthesis in Holstein cows. J. Dairy Sci. 86:1321-1324.

Ben Salem, H., R. Krzeminski, A. Ferlay, and M. Doreau. 1993. Effect of lipid supply on in vivo digestion in cows: comparison of hay and corn silage diets. Can. J. Ani. Sci. 73:547-557.

Bergström, B. 1964. Influence of bile salt, pH, and time on the action of pancreatic lipase; physiological implications. J. Lipid Res. 5:522-531.

Bernal-Santos, G., J. W. II Perfield, D. M. Barbano, D. E. Bauman, T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Bernard, L., C. Leroux, and Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. Adv. Exp. Med. Biol. 606:67-108.

Bickerstaffe, R., E. F. Annison, and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82:71-85.

Bickerstaffe, R. 1971. Uptake and metabolism of fat. In: Lactation. I. R. Falconer. ed., p. 317 Butterworth, London.

Bickerstaffe, R. and E. F. Annison. 1970. The desaturase activity of goat and sow mammary tissue. Comp. Biochem. Physiol. 35:635-665.

Breckenridge, W. C., and A. Kuskis. 1969. Structure of bovine milk fat triglycerides II. Long chain length. Lipids 4:197-204.

Brown, W. V. and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of a human very low density lipoprotein. Biochem. Biophys. Res. Comm. 46:375-382.

Brown, W. H., J. W. Stull, and G. H. Stott. 1962. Fatty acid composition of milk. I. Effect of roughage and dietary fat. J. Dairy Sci. 45:191-196.

Brumby, P. E. and V. A. Welch. 1978. Lipid precursors of milk fatty acids. Bienn Rev. Natl. Inst. Res. Dairying 39:39.

Brumby, P. E. 1971. Influence of plasma lipids on the activity of lipoprotein lipase. Biochem. J. 122:10-11.

Carroll, S. M., E. J. DePeters, S. J. Taylor, M. Rosenberg, J. Perez-Mnti, and V. A. Capps. 2006. Milk composition of Holstein, Jersey and Brown Swiss cows in response to increasing levels of dietary fat. Ani Feed Sci Tech. 131: 451-473.

Castañeda-Gutiérrez, E., B. C. Beefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253-4264.

Castañeda-Gutiérrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Chilliard, Y. and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Dev. 44:467-492.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids (Rev). Ann. Zootech. 49:181-205.

Chilliard, Y., G. Gagliostro, J. Fléchet, J. Lefaivre, and J. Sebasian. 1991. Duodenal rapeseed oil infusion in early and mid lactation cows. 5. Milk fatty acids and adipose tissue lipogenic activities. J. Dairy Sci. 74:1844-1854.

Chilliard, Y., C. Delouis, M. C. Smith, D. Sauvant, and P. Morand-Fehr. 1986a. Mammary metabolism in the goat during normal or hormonally-induced lactation. Reprod. Nutr. Dev. 26:607-615.

Chilliard, Y., J. Robelin, and B. Rémond. 1984. In vivo estimation of body lipid mobilization and reconstitution in dairy cattle. Can. J. Anim. Sci. 64:236-237.

Chouinard, P. Y., L. Corneau, W. R. Butler, Y. Chilliard, J. K. Drackley, and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84:680-690.

Chouinard, P. Y., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999a. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579-1584.

Chouinard, P. Y., L. Coreneau, A. Sæbø, and D. E. Bauman. 1999b. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737-2745.

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of delta (9)-desaturase in the production of cis-9, trans-11 CLA. J. Nutr. Biochem. 12:622-630.

Counotte, G. H. M., R. A. Prins, R. H. A. M. Janssen, and M. J. A. Debie. 1981. Role of Megasphaera elsdenii in the fermentation of lactate-2-C-13 in the rumen of dairy cattle. Applied and Environmental Microbiology 42:649-655.

Davis, C. L. and R. E. Brown. 1970. Low-fat milk syndrome. In Physiology of Digestion and Metabolism in the Ruminant, A. T. Phillipson, ed., pp. 545-565. Oriel Press, Newcastle upon Tyne.

Demeyer, D. 1973. Lipidstoffwechsel im Pansen. In “Biologie und Biochemie der mikrobiellen Verdauung". Publ. Giesecke and Hendricks. BLV Verlagsgesellschaft, München, Bern, Wien.

DePeters, E. J. and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J. Dairy Sci. 75:2043-2070.

De Veth, M. J., E. Castañeda-Gutiérrez, D. A. Dwyer, A. M. Pfeiffer, D. E. Putnam, and D. E. Bauman. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J. Dairy Sci. 89:4620-4631.

De Veth, M. J., S. K. Gulati, N. D. Luchini, and D. E. Bauman. 2005. Comparison of calcium salts and formaldehyde-protected conjugated linoleic Acid in inducing milk fat depression. J. Dairy Sci. 88:1685-1693.

De Veth, M. J, J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-37.

Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016-1027.

Dhiman, T. R., E. D. Helmink, D. J. McMahon, R. L. Fife, and M. W. Pariza. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412-219.

Doreau, M., D. I. Demeyer, and C. Van Nevel. 1997. Transformations and effects of unsaturated fatty acids in the rumen. Consequences on milk fat secretion. In Milk

Composition, Production and Biotechnology, R. A. S. Welch, D. J. W. Burns, S. R. Davis, A. I. Popay, and G. G. Prosser, eds., pp. 73-92. CAB International, Wallingford.

Doreau, M. and A. Ferlay. 1995. Effect of dietary lipids on nitrogen metabolism in the rumen: a review. Livestock Prod. Sci. 43:97-110.

Eastridge, M. L. 2006. Major advances in applied dairy cattle nutrition. J. Dairy Sci. 89:1311-1323.

Emery, R. S. 1988. Milk fat depression and the influence of diet on milk composition. Vet. Clin. North Am. Food Anim. Pract. 4:289-305.

Emery, R. S. 1978. Feeding for increased milk protein. J. Dairy Sci. 61:825-828.

Emery, R. S. 1973. Biosynthesis of milk fat. J. Dairy Sci. 56:1187-1195.

Emery, R. S., M. Schurman, and K. S. Sidhu. 1972. Specificity of mammary and adipose lipoprotein lipase for different apolipoproteins. Fed. Proc. 31:682 (abstract).

Enjalbert, F., M. C. Nicot, C. Boyourthe, and R. Moncoulon. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525-1532.

Fellner, V., F. D. Sauer, and J. K. Kramer. 1995. Steady-state rates of linoleic acid biohydrogenation by ruminal bacteria in continuous culture. J. Dairy Sci. 78:1815-1823.

Gagliostro, G., Y. Chilliard, and M. J. Davicco. 1991. Duodenal rapeseed oil infusion in early and mid lactation cows. 3. Plasma hormones and mammary apparent uptake of metabolites. J. Dairy Sci. 74:1893-1903.

Galbraith, H., T. B. Miller, A. M. Paton, and J. K. Thompson. 1971. Antibacterial activity of long-chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. J. Appl. Bacteriology 34:803-813.

Gervais, R., J. W. McFadden, A. J. Lengi, B. A. Corl, and P. Y. Chouinard. 2009. Effects of intravenous infusion of *trans*-10, *cis*-12 18:2 on mammary lipid metabolism in lactating dairy cows. J. Dairy Sci. 92:5167-5177

Gervais, R., R. Spratt, M. Líonard, and P. Y. Chouinard. 2005. Lactation response of cows to different levels of ruminally inert conjugated linoleic acids under commercial conditions. Can. J. Anim. Sci. 85:231-242.

Glascock, R. F. and V. A. Welch. 1974. Contribution of the fatty acids of three low density serum lipoproteins to bovine milk fat. J. Dairy Sci. 57:1364.

Griinari, J. M. and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Advances in Conjugated Linoleic Acid Research. Vol. 1., M. P. Yurawecz, M. M. Mossoba, M. W. Pariza, and G. J. Nelson, eds., pp. 180-200. AOCS Press, Champaign, Illinois.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. Trans-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

Grummer, R. R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74:3244-3257.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. In: The rumen microbial ecosystem, 2nd edition, P. N. Hobson and D. S. Stewart, eds., pp. 382-426. Chapman and Hall, London.

Harfoot, C. G., M. L. Crouchman, R. C. Noble, and J. H. Moore. 1974. Competition between food particles and rumen bacteria in the uptake of long-chain fatty acids and triglycerides. J. App. Bacteriology 37:633-641.

Harvatine, K. J., J. W. Perfield II, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-54.

Innis, S. M., R. Dyer, and C. M. Nelson. 1994. Evidence that palmitic acid is absorbed as sn-2 monoacylglycerol from human milk by breast-fed infants. Lipids 29:541-545.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863.

Jenny, B. F., C. E. Polan, and F. W. Thye. 1974. Effects of high grain feeding and stage of lactation on serum insulin, glucose and milk fat percentage in lactating cows. J. Nutr. 104:379-385.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997a. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104-2114.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of fat source on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2115-2126.

Kay J. K., R. R. Mackle, D. E. Bauman, N. A. Thomson, and L. H. Baumgard. 2007. Effects of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. J. Dairy Sci. 90:721-730.

Kay, J. K., T. R. Mackle, M. J. Auldist, N. A. Thomson, and D. E. Bauman. 2004. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Kelly G. S. 2001. Conjugated linoleic acid: a review. Altern. Med. Rev. 6:367-382.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, D. E. Van Abmbugh, and D. E. Bauman. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. Am. Soc. Nutr. Sci. 128:881-885.

Kemp, P. and D. J. Lander. 1984. Hydrogenation in vitro of alpha-linolenic acid to stearic-acid by mixed cultures of pure strains of rumen bacteria. J. Gen. Microbiol. 130:527-533.

Kennedy, K., M. S. Fewtrell, R. Morley, R. Abbott, P. T. Quinlan, J. C. K. Wells, J. G. Bindels, and A. Lucas. 1999. Double-blind, randomized trial of a synthetic triacylglycerol in formula-fed term infants: effects on stool biochemistry, stool characteristics and bone mineralization. Am. J. Clin. Nutr. 70:920-927.

Kepler, C. R. and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids. 3. Purification and properties of a linoleate delta12-cis, delta11-trans-isomerase from Butyrivibrio fibrisolvens. J. Biol. Chem. 242:5686-5692.

Kepler, C. R., W. P. Tucker, and S. B. Tove. 1970. Biohydrogenation of unsaturated fatty acids. 4. Substrate specificity and inhibition of linoleate delta12-cis, delta11-trans-isomerase from Butyrivibrio fibrisolvens. J. Biol. Chem. 245:3612-3620.

Kim, Y. J., R. H. Liu, J. L. Rychlik, and J. B. Russel. 2002. The enrichment of a ruminal bacterium (*Megasphaera elsdenii* YJ-4) that produces the *trans*-10, *cis*-12 isomer of conjugated linoleic acid. J. Appl. Microbiology, 92: 976-982.

Kim, Y. J., R. H. Liu, D. R. Bond, and J. B. Russell. 2000. Effect of linoleic acid concentration on conjugated linoleic acid produced by Butyrivibrio fibrisolvens A38. Appl. Environ. Microbiol. 66:5226- 5230.

Kinsella, J. E. 1972. Stearoyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglyceride synthesis. Lipids 7:349-355.

Knudsen, J. and I. Grunenet. 1982. Transacylation as a chain-termination mechanism in fatty acid synthesis by mammalian fatty acid synthetase. Biochem. J. 202:139-143.

Krukovsky, B. N. 1961. Vitamin A, carotenoid, iodine, and thiocyanogen values, and the refractive index of milk fat as influenced by feed, and by individual and breed differences. Agric. Fod. Chem. 9:326-329.

Landeskuratoriom der Erzeugerringe in der Rinderzucht in Bayern. 2008. Jahresberichte aus den Leistungsprüfungen: Milchleistungsprüfungen in Bayern. http://www.lkv.bayern.de/media/mlp_jahresbericht2008.pdf

Larsen, T. M., S. Toubro, and A. Astrup. 2003. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. J. Lipid Res. 44:2234-2241.

Latham, M. J., J. E. Storry, and M. E. Sharpe. 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Appl. Micorbiol. 24:871-877.

Lawson, R. E., A. R. Moss, and D. I. Gives. 2001. The role of dairy products in supplying conjugated linoleic acid to man's diet: a review. Nutr. Res. Reviews 14:153-172.

Lee, H. Y., J. H. Park S. H. Seok. M. W. Baek, D. J. Kim, K. E. Lee, K. S. Paek, Y. Lee, and J. H. Park. 2006. Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim. Biophys. Acta 1761:736-744.

Lock, A. L., C. Tyburczy, D. A. Dwyer, K. J. Harvatine, F. Destaillats, Z. Moulougui, L. Candy, and D. E. Bauman. 2007. Trans-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Lock, A. L. and P. C. Garnsworthy. 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. Animal Sci. 74:163-167.

Loor, J. J. and J. H. Herbein. 2003. Dietary canola or soybean oil with two levels of conjugated linoleic acids (CLA) alter profiles of 18:1 and 18:2 isomers in blood plasma and milk fat from dairy cows. Anim. Feed Sci. Tech. 103:63-83.

Loor, J. J. and J. H. Herbein. 1998. Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting *de novo* fatty acids synthesis. J. Nutr. 128:1422-1419.

Mackle, T. R., J. K. Kay, M. J. Auldist, A. K. H. McGibbon, B. A. Philpott, L. H. Baumgard, and D. E. Bauman. 2003. Effects of abomasal infusion of conjugated linoleic acid on milk fat concentration and yield from pasture-fed dairy cows. J. Dairy Sci. 86:644-652.

Maczulak, A. E., B. A. Dehority, and D. L. Palmquist. 1981. Effect of long-chain fatty acids on growth of rumen bacteria. App. Environm. Microbiol. 42:856-862.

Martin, S. A. and T. C. Jenkins. 2002. Factors affecting conjugated linoleic acid and trans-C18:1 fatty acid production by mixed ruminal bacteria. J. Anim. Sci. 80:3347-3352.

Maxin, G., F. Glasser, C. Hurtaud, J. L. Peyraud, and H. Rulquin. 2011. Combined effects of *trans*-10, *cis*-12 conjugated linoleic acid, propionate and acetate on milk fat yield and composition in dairy cows. J. Dairy Sci. 94:2051-2059.

McClymont, G. L. and S. Vallance. 1962. Depression of blood glycerides and milk-fat synthesis by glucose infusion. Proc. Nutr. Soc. 21:R41.

Molkentin, J. and D. Precht. 1995. Development of a precise capillary GC method for rapid triglyceride analysis of milk fats. Fett Wissenschaft Technologie-Fat Science Technology 97:43-49.

McDonald, T. M. and J. E. Kinsella. 1973. Stearoyl-CoA desaturase of bovine mammary microsomes. Arch. Biochem. Biophys. 156:223-231.

Mele, M., R. Dal Zotto, M. Cassandro, G. Conte, A. Serra, A. Buccioni, G. Bittante, and P. Secchiari. 2009. Genetic parameters for conjugated linoleic acid, selected milk fatty acids and milk fatty acid unsaturation of Italian Holstein-Friesian cows. J. Dairy Sci. 92:392-400.

Moallem, U., H. Lehrer, M. Zachut, L. Livshitz, and S. Yacoby. 2010. Production performance and pattern of milk fat depression of high-yielding dairy cows supplemented with encapsulated conjugated linoleic acid. Animal. 4:641-652

Moate, P. J., W. Chalupa, R. C. Boston, and I. J. Lean. 2007. Milk fatty acids. I. Variation in the concentration of individual fatty acids in bovine milk. J. Dairy Sci. 90:4730-4739.

Moore, C.E., J. K. Kay, R. J. Collier, M. J. Vanbaale, and L. H. Baumgard. 2005. Effect of supplemental conjugated linoleic acids on heat-stressed Brown Swiss and Holstein cows. J. Dairy Sci. 88:1732-1740.

Moore, C. E., H. C. Haflinger, O. B. Mendivil, S. R. Saders, D. E. Bauman, and L. H Baumgard. 2004. Increasing amounts of conjugated linoleic acid progressively reduces milk fat synthesis immediately postpartum. J. Dairy Sci. 87:1886-1895.

Moore, J. H. and W. W. Christie. 1981. Lipid metabolism in the mammary gland of ruminant animals, in: Christie W. W. (Ed.) Lipid metabolism in ruminant animals. Pergamon Press, pp. 227-277.

Moore, J. H. and W. W. Christie. 1979. Lipid metabolism in the mammary gland of ruminant animals. Prog. Lipid Res. 17:347-395.

Mosley, E. E., B. Shafii Dagger, P. J. Moate, and M. A. McGurire. 2006. Cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Nakamura, M. T. and T. Y. Nara. 2004. Structure, function, and dietary regulation of Δ6, Δ5 and Δ9 desaturase. Annu. Rev. Nutr. 24:345-376.

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. J. Dairy Sci. 90:293-305.

Page, A. M., C. A. Sturdivant, D. K. Lunt, and S. B. Smith. 1997. Dietary whole cottonseed depresses lipogenesis but has no effect on stearoyl coenzyme desaturase activity in bovine subcutaneous adipose tissue. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 118:79-84.

Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feeding and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771.

Palmquist, D. L. and F. L. Schanbacher. 1991. Dietary fat composition influences fatty acid composition of milk fat globule membrane in lactating cows. Lipids 26:718-722.

Palmquist, D. L., T. C. Jenkins, and A. E. Joyner. 1986. Effect of dietary fat and calcium source on insoluble soap formation in the rumen. J. Dairy Sci. 69:1020-1025.

Palmquist, D. L. and T. C. Jenkins. 1980. Fat in lactation rations: a review. J. Dairy Sci. 63:1-14.

Palmquist, D. L. and W. Mattos. 1978. Turnover of lipoproteins and transfer to milk fat of dietary 1-carbon-14 linoleic acid in lactating cows. J. Dairy Sci. 61:561-565.

Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)-β-hydroxybutyrate. J. Dairy Sci. 52:633-638.

Peeters, G., A. Houvenaghel, E. Roets, A. M. Massart-Leen, R. Verbeke, G. Dhondt, and F. Verschooten. 1979. Electromagnetic blood flow recording and balance of nutrients in the udder of lactating cows. J. Anim. Sci. 48:1143-1153.

Perfield II, J. W., A. L. Lock, J. M. Griinari, A. Sæbø, P. Delmonte, D. A. Dwyer, and D. E. Bauman. 2007. Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Perfield II, J. W., P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2006. Trans-10, trans-12 conjugated linoleic acid does not affect milk fat yield but reduces delta(9)-desaturase index in dairy cows. J. Dairy Sci. 89:2559-2566.

Perfield II, J. W., P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2004b. Trans-10, trans-12 conjugated linoleic acid (CLA) reduces the Δ^9-desaturase index without affecting milk fat yield in lactating cows. J. Dairy Sci. 87 (Suppl.1):297 (Abstract).

Perfield II, J. W., A. Sæbø, and D. E. Bauman. 2004c. Use of conjugated linoleic acid (CLA) enrichments to examine the effects of trans-8, cis-10 CLA and cis-11, trans-13 CLA on milk fat synthesis. J. Dairy Sci. 87:1196-1202.

Perfield II, J. W., G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased trans-10, cis-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Short communication: milk fat response to low doses of trans-10, cis-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85:1764-1766.

Sæbø, A, P-C. Sæbø, J. M. Griinari, and K. J. Shingfield. 2005a. Effect of abomasal infusions of geometric isomers of 10,12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids 40:823-832.

Sæbø, A., J. W. Perfield II, P. Delmonte, M. P. Yurawecz, P. Lawrence, J. T. Brenna, and D. E. Bauman. 2005b. Milk fat synthesis is unaffected by abomasal infusion of the conjugated diene 18:3 isomers cis-6, trans-10, cis-12 and cis-6, trans-8, cis-12. Lipids 40:89-95.

Sawal, R. K. and C. K. Kurar. 1998. Milk yield and its fat content as affected by dietary factors: a review. AJAS 11:217-233.

Selberg, K. T., A. C. Lowe, C. R. Staples, N. D. Luchini, and L. Badinga. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J. Dairy Sci. 87:158-168.

Shirley, J. E., R. S. Emery, E. M. Convery, and W. D. Oxender. 1973. Enzymatic changes in bovine adipose and mammary tissue with initiation of lactation. J. Dairy Sci. 56:569-574.

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer and, M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scan. 52:16

Singh, S. and J. C. Hawke. 1979. The in vitro lipolysis and biohydrogenation of monogalactosyldiglyceride by whole rumen contents and its fractions. J. Sci. Food Agric. 30:603-612.

St. John, L. C., K. K. Lunt, and S. B. Smith. 1991. Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J. Anim. Sci. 69:1064-1073.

Storry, J. E. 1972. Milk fat: its synthesis and composition in relation to the nutrition of the dairy cow. J. Soc. Dairy Technol. 25:40-46.

Stull J. W. and W. H. Brown. 1964. Fatty acid composition of milk. II. Some differences in common dairy breeds. J. Dairy Sci. 47:1412.

Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801-2814.

Sutton, J. D., I. C. Hart, S. Morant, V. Schuller, and A. D. Simmonds. 1988. Feeding frequency for lactation cows: diurnal patterns of hormones and metabolites in peripheral blood in relation to milk-fat concentration. Br. J. Nutr. 132:940-945.

Timmen, H. and S. Patton. 1988. Milk fat globules: fatty acid composition, size and *in vivo* regulation of fat liquidity. Lipids 23:685-689.

Verhulst, A., G. Janssen, G. Parmentier, and H. Eyssen. 1987. Isomerisation of polyunsaturated long chain fatty acids by Propionibacteria. Syst. Appl. Microbiol. 9:12-15.

Viswanadha, S., J. G. Giesy, T. W. Hanson, and M. A. McGuire. 2003. Dose response of milk fat to intravenous administration of the trans-10, cis-12 isomer of conjugated linoleic acid. J. Dairy Sci. 86:3229-3236.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: a review. Animal Feed Sci. Techn. 131:389-417.

Von Soosten, D., U. Meyer, E. M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J. Dairy Sci. 94:2859-2879.

Waghorn, G. C. and R. L. Baldwin. 1984. Model of metabolite flux within mammary gland of the lactating cow. J. Dairy Sci. 67:531-544.

Wallace, R. J., N. McKain, K. J. Shingfield, and E. Devillard. 2007. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. J. Lipid Res. 48:2247-2254.

Ward, P. F. V., T. W. Scott, and M. R. C. Dawson. 1964. Hydrogenation of unsaturated fatty acids in ovine digestive tract. Biochem. J. 92:60-68.

West, C. E., R. Bickerstaffe, E. F. Annison, and J. L. Linzell. 1972. Studies on the mode of uptake of blood triglycerides by the mammary gland of the lactating goat. Biochem. J. 126:477-490.

White, S. L., J. A. Bertarnd, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301.

White, T. W., R. B. Grainger, F. H. Baker, and J. W. Stroud. 1958. Effect of supplemental fat on digestion and the ruminal calcium requirement of sheep. J Anim. Sci. 17:797-803.

Wiking, L., J. Stagsted, L. Bjørck, and J. H. Nielsen. 2004. Milk fat globule size is affected by fat production in dairy cows. Int. Dairy J. 14:909-913.

Wiking, L., L. Bjørck, and J. H. Nielsen. 2003. Influence of feed composition on stability of fat globules during pumping of raw milk. Int. Dairy J. 13:797-803.

Wonsil, B. J., J. H. Herbein, and B. A. Watkins. 1994. Dietary and ruminally derived *trans*-18:1fatty acids alter bovine milk lipids. J. Nutr. 124:556-565.

Yang, A., T. W. Larsen, S. B. Smith, and R. K. Tume. 1999. Delta-9 desaturase activity in bovine subcutaneous adipose tissue of different fatty acid composition. Lipids 34:971-978.

Zinn, R. A. 1989. Influence of level and source of dietary fat on its comparative feeding value in finishing diets of feedlot steers: metabolism. J. Anim. Sci. 67:1038-1049.

3 THE SCOPE OF THE STUDIES

The first of the following studies was conducted to evaluate the effects of supplementing a source containing 10 g each of rumen-inert *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA to early lactation dairy cows. Many studies have reported an influence of *trans*-10, *cis*-12 CLA on milk fat synthesis during different stages of lactation. However, no studies have investigated supplementation with the milk fat depressing CLA isomer starting at the onset of lactation for different periods (80 d and 120 d), or further evaluated the effects of supplementation not only during the feeding period but also beyond the supplementation period for nearly an entire lactation (240 d). Data were collected to estimate the influence of the mixed CLA supplement on the progression of milk variables and energy balance during and beyond supplementation. These data should give answers beyond the depression of milk fat synthesis, meaning the development of milk energy output and energy balance throughout the trial period. Because primi- and multiparous cows are metabolically different at the beginning of lactation, the first study included both lactation groups to evaluate how supplementation with CLA impacts milk variables and the energy balance of both groups.

The second study was performed in order to evaluate the effect of a mixed CLA source containing 10 g of *cis*-9, *trans*-11 CLA and 10 g of *trans*-10, *cis*-12 CLA on the performance of cows fed the product during the transition period and during early lactation. Animals were fed 80 d into lactation with the CLA product; milk variables and energy balance were followed until lactation day 100. Data of these studies were obtained to be compared to literature data and evaluate different feeding strategies of CLA product in early lactation dairy cows.

Because MFD was inconsistent when supplementing similar amounts of *trans*-10, *cis*-12 CLA, and since these differences might have resulted from the diet, cows in both studies were fed the same ration. Most studies conducted on MFD by supplementing *trans*-10, *cis*-12 CLA have been performed in North America with rations typical for that region. The generation of data evaluating MFD in early lactation cows fed with rations typical for grass-based dairy regions could provide information on how the energy balance and milk variables are affected by these diets when feeding *trans*-10, *cis*-12 CLA.

4 STUDY ONE

How does a variable duration of supplementation of a mixed CLA source during early lactation influence milk traits and performance variables in dairy cows across an entire lactation period?

ABSTRACT

The objective of this study was to evaluate the lactation effects of a rumen-inert conjugated linoleic acid (CLA) product containing equal amounts of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers. The CLA product was fed during early lactation (6 ± 3.3 DIM (SD)) to German Holstein cows (n = 74). The observation period of lactation variables was 240 d. Cows were assigned to one of three treatments at trial start; either fed a) without (control) or with a source of rumen-protected CLA beginning at the start of the trial until b) 80 d (CLA80) or c) 120 d (CLA120) of the trial. The CLA product was pelleted into a concentrate and fed by automatic concentrate feeders. Forage was fed once daily as a basal ration based on grass (45%) and corn silage (32%). Dry matter intake, milk yield and body weight were registered on a daily basis, whereas milk components were measured every second week. Milk acetone was measured on trial d 1, 7, 14, 28 and 56, whereas milk fat analysis was performed three times (control) or four times (CLA80 and CLA120) throughout the trial. Serum free fatty acids and β-hydroxybutyrate were analyzed on trial d 7, 14 and 28. Analysis of variance showed parity effects between the treatment groups. The maximum decrease in the milk fat content resulting from CLA supplement was 14.5% and 20% for CLA80 and CLA120, respectively, in multiparous cows. The effect of the CLA product on the milk fat content was delayed at the onset of lactation and after discontinuing CLA supplementation. The milk fat content reverted back to control levels in late lactation after the cessation of CLA supplementation. A trend for increased milk yields across the 240 d observation period occurred in the multiparous CLA treatment groups with higher persistence in milk yields after discontinuing CLA supplementation. No effects upon milk fat, protein or lactose yield or their contents were found, and the energy-corrected milk yield, energy balance, health and fertility variables were unchanged for multiparous cows during the 240 d of the trial. Milk fat content was inconsistently depressed during CLA supplementation with significant increases after the cessation of CLA product delivery in primiparous cows. During CLA treatment, a trend for increased milk yield was observed without any carry-over effects after terminating CLA supplementation. No effects on other variables were observed. These data imply that the CLA product provokes different effects in the secretion pathways of the mammary gland in primi- and multiparous cows, without favoring the energy balance during early lactation.

Keywords: conjugated linoleic acid, milk fat depression, energy balance, lactation performance

INTRODUCTION

Diet-induced milk fat depression (**MFD**) has been observed in dairy cows for many decades. Van Soest (1963) and Davis and Brown (1970) reviewed the role of substrate supply limitation. Bauman and Griinari (2001) were the first to propose the biohydrogenation theory of MFD, based on the premise that certain dietary conditions alter rumen biohydrogenation pathways, shifting the synthesis of particular conjugated linoleic acids (**CLA**). Martin and Jenkins (2002) showed that alterations in biohydrogenation result from high intake of soluble carbohydrates and polyunsaturated fatty acids (**PUFA**), low ruminal pH and high rumen passage rates. Several studies (Baumgard et al., 2000; Corl et al., 2000; Sæbø and Bauman, 2000) demonstrated that the *trans*-10, *cis*-12 CLA isomer is a potent inhibitor of milk fat synthesis. Following this observation, many trials have been conducted investigating the effect of *trans*-10, *cis*-12 CLA on milk component synthesis. Baumgard et al. (2000) and Peterson et al. (2002) showed a dose-dependent relationship on milk fat yield in abomasally infused dairy cows with no effects on milk yield or other milk components. Similar effects were observed for cows in established to late lactation fed with a rumen-inert *trans*-10, *cis*-12 CLA isomer (Perfield et al., 2002; De Veth et al., 2005; Moore et al., 2005), exhibiting an effect on milk fat content and yield shortly after the initiation of treatment.

Trans-10, *cis*-12 CLA fed to cows during the transition period (Bernal-Santos et al., 2003; Selberg et al., 2004; Odens et al., 2007) and early lactation (Castañeda-Gutiérrez et al., 2007) induced MFD, but not until several weeks after parturition. At this point, MFD fully developed and trends for elevated milk yield (Bernal-Santos et al. 2003) and beneficial effects on energy balance (**EB**; Liermann et al., 2008) were observed. Recent studies evaluating the effect of *trans*-10, *cis*-12 CLA in lactating dairy cows considered only the effects during supplementation period. Pappritz et al. (2011) considered the effects of CLA supplementation during early lactation and during a depletion period after CLA termination, on lactation performance and energy status. While Pappritz et al. (2011) evaluated the effect of different *trans*-10, *cis*-12 CLA concentrations, the present study investigated the effects of two different supplementation periods (80 and 120 d) during a 240 d lactation period in primiparous and multiparous cows.

MATERIALS AND METHODS

Animals, treatments and design

Early lactation German Holstein cows from the Research Centre “Haus Riswick”, Landwitschaftskammer North Rhine Westphalia, Germany were blocked according to parturition day, parity and 305 d mature-equivalent milk production. They were allotted to one of three treatments in a completely randomized block design: 1) **control** (without CLA supplementation), 2) **CLA80**, 10 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA fed during the first 80 d and 3) **CLA120** 10 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA fed during the first 120 d of the trial. Treatments started at 6 ± 3.3 DIM (SD), and the lactation performance and energy status of dairy cows were observed for 240 d. All procedures involving dairy cows were conducted according to the regulations of the German law on animal welfare (Anonymous, 2010).

All animals were housed together in a free-stall barn with *ad libitum* access to a basal ration that was fed once daily into weighing troughs located in front of self-locking gates. Each visit was monitored by a transponder located on a neck collar. Two concentrates, differing only in fatty acid composition, were supplemented individually by concentrate feeders. The basal ration provided nutrients for 25 kg of energy-corrected milk (**ECM**) yield. The forage ratio in the basal ration was 58% grass silage to 42% corn silage on a DM basis with temporary tolerated deviations of 10 percentage units for both components. Table 6 provides information in the components and chemical composition of the basal ration.

Table 6. Components and chemical composition of the basal ration.[1]

Component composition, % of DM	
Grass silage (40% DM; 6.0 MJ NEL/kg DM)	44.6
Corn silage (35% DM, 6.8 MJ NEL/kg DM)	31.9
Sugar beet pulp, pressed, ensiled (22% DM, 7.5 MJ NEL/kg DM)	4.6
Concentrate (18/3)	7.2
Protein supplement	10.5
Straw	0.9
Mineral mixture	0.3
Chemical composition, % of DM	
Crude protein	14.9
Utilizable crude protein	14.7
RNB	0.5
Crude lipid	3.2
NDF	37.1
ADF	22.5
NEL, MJ/ kg DM	6.61

[1]Values represent averages of samples obtained from the individual stocks prior to feeding or at delivery.

DM: Dry matter, RNB: Ruminal nitrogen balance, NEL: Net energy lactation

Concentrates fed additionally with the basal mixed ration were formulated to be isocaloric and isonitrogenous, differing only in the lipid source (Table 7). The lipid source in the concentrate delivered to control cows was given as a rumen-inert fat source of palmitic acid Ca salts (Bergafat, Berg und Schmidt GmbH, Hamburg, Germany), and in CLA fed cows, this was delivered as Lutrell (BASF SE, Ludwigshafen, Germany) containing 10% each of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. All cows were fed with 3 kg of the concentrate respective to their trial group from the beginning of the trial. If this amount did not meet individual nutrient requirements calculated by the recommendations of GfE (2001), all animals were fed with additional control group concentrate. The maximum overall concentrate supplementation was 10 kg.

Table 7. Components and chemical composition of concentrates fed to the control (Control) and treatment (CLA) groups.[1]

	Control	CLA
Component composition, % of DM		
Wheat	26	26
Corn grains	14	14
Soybean meal (protected)	11.5	11.5
Soybean meal	12.8	12.8
Rapeseed meal	8.9	8.9
Molasses pulp	18.8	18
Bergafat (Berg und Schmidt GmbH, Hamburg)	2.5	
Lutrell (BASF SE, Ludwigshafen)		3.3
Rest	5.5	5.5
Chemical analysis, % of DM		
Crude protein	23.2	23.4
Utilizable crude protein	17.5	17.5
RNB	3	3
NDF	19.1	18.8
ADF	9.5	9.3
Crude lipid	4.2	4.2
NEL, MJ/ kg DM	8.1	8

[1] Values are averages of samples collected at the delivery of each batch (n = 3)

DM = Dry matter, RNB = Ruminal nitrogen balance, NEL = Net energy lactation

Sampling

Chemical composition. Samples of forages were collected weekly from the surface of each silage stock and stored at -20°C. A cumulative sample for each stock was added up for analyses. DM was estimated from samples collected from the basal mixed ration. They were gathered five days a week and analyzed immediately. Concentrate samples were taken from each batch at delivery and stored at -20°C until analysis for the chemical composition and energy value. Collective samples of three batches of the control concentrate and protein

supplement were analyzed. Batches of the CLA concentrate were always analyzed as single samples. A second aliquot of CLA concentrates was stored at -20°C before analysis for the content of CLA isomers. The first three samples were stored for two months before analysis; all subsequent samples were analyzed within a week after delivery.

Milk. All cows were milked twice daily in a 14 parlor carrousel (Westfalia Surge GmbH, Bönen, Germany) and milk production was recorded electronically. Every second week, milk performance monitoring was performed on a pooled sample, collected as a cross-section from the evening and morning milking periods. The sample was stored at 4°C with a preservative (bronopol) until analysis. On trial d 1, 7, 14, 28 and 56, milk samples were collected for subsequent acetone analysis. They were stored in an airtight bottle at 4°C in the presence of a conservation agent (bronopol) until analysis. The fatty acid pattern was analyzed from milk samples collected from a limited number of cows. Control (n = 8) samples were taken on trial d 1, 84 and 124, while in the CLA80 group (n = 7), samples were taken on trial d 1, 78, 84 and 124. Samples for the CLA120 group (n = 6) were taken on trial d 1, 84, 118 and 124. Samples were pooled from the morning and evening milking periods and stored in a freezer at -28°C with no antidegradant added.

Blood. Samples were taken on trial days 7, 14 and 28 via coccygeal venipuncture and collected into tubes to harvest serum. The tubes were centrifuged immediately after sample collection at 5000 rpm for 15 min at room temperature. The supernatant was stored in Eppendorf tubes at -28°C until analysis at the Veterinary University of Hanover/Germany for BHBA and FFA. Blood was collected from seven cows randomly assigned from the control groups and from 14 cows randomly assigned from both CLA treated groups without differentiation between CLA80 and CLA120, as these treatments were identical during the early lactation stage.

Body condition. All cows were weighed twice a day by electronically recording BW. Back fat thickness (**BFT**) was measured monthly on each cow starting after parturition with an ultrasonic device (Personal Ultrasound-400; Proxima medizinische Geräte GmbH, Berlin, Germany) with a 5 MHz linear sonic head. The sonic head was placed on alcohol-wetted skin. The point of measurement was in the conduit between the dorsal part of the *Tuber ischiadicum* and the upper part of the *Tuber coxae* at the end of the *Crista sacrales* and the beginning of the coccyx, following the method of Staufenbiel (1992). All measurements were performed by the same person.

Analysis

Chemical composition. Analysis of the chemical composition was done by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt NRW (Münster, Germany). All components of the basal ration were analyzed individually.

All methods for analyzing forage components are described in the book of methods published by Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (**VDLUFA**), volume III, from Naumann et al. (2004). The DM of forages was estimated by oven-drying at 103°C for 4 hours (VDLUFA method 3.1). Ash was determined by ashing in a muffle furnace at 550°C overnight (16 h) (VDLUFA method 8.1). NDF (VDLUFA method 6.5.1) and ADF (VDLUFA method 6.5.2) were determined by boiling the sample for 1 h with neutral or acid detergent, respectively. Crude lipid (**CL**) was analyzed by treating the sample with benzene (VDLUFA method 5.1.1/A), while CP was analyzed by the Kjeldahl method (VDLUFA method 4.1.1). The concentrates were analyzed by the same institute that analyzed the forages. DM (VDLUFA method 3.1) was estimated by oven-drying at 103°C for 4 h. Ash (VDLUFA method 8.1) was determined by ashing in a muffle furnace at 550°C overnight (16h). CP was analyzed by the Kjeldahl method (VDLUFA method 4.1.1). These methods have been described in detail by Naumann et al. (2004). The CL content was analyzed after acid hydrolysis ("Amtsblatt" of EG L 257/23-25 1998/ procedure B).

The analytics for CLA isomers were done by BASF SE (Ludwigshafen, Germany) to determine the total CLA content and the content of CLA-ME isomers by HPLC according to the method AM/00887/01e. Both, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were evaluated. In addition, the sum of *cis, trans* CLA-ME isomers, *trans, trans* CLA-ME isomers and *cis, cis* CLA-ME isomers were evaluated according to the following principle.

CLA-Ca^{++} salt was initially reacted with acetic acid to form CLA. CLA and/or CLA-ME were extracted with cyclohexane and quantified by HPLC (wavelength: 233nm, C-18 column) against an external standard. The total CLA content was obtained by adding up the individual CLA and CLA-ME contents. To identify the CLA-ME isomers, part of the extracting solutions were evaporated to dryness under a stream of nitrogen and the CLA obtained was esterified with hydrogen chloride in methanol to yield CLA-ME; this was subsequently extracted with cyclohexane. The isomers of CLA-ME were separated by HPLC on a ChromSpher 5 lipid column and then subjected to UV detection at a wavelength of 233 nm.

Based on the area percentages of the individual CLA-ME isomers in the total CLA-ME area, their contents were established by multiplying the value by the total CLA content.

Milk. Milk lactose, protein and fat content, as well as the somatic cell count and urea content were analyzed by Landeskontrollverband NRW (Krefeld, Germany) using a MILCOSCAN (Foss Electric, Hillerød, Denmark). Acetone was measured by infrared analysis (Landeskontrollverband Rhineland-Palatinate) using a MILCOSCAN (Foss Electric, Hillerød, Denmark). The milk fatty acid pattern was analyzed by gas chromatography by MUVA Kempten (Bavaria, Germany) using the method MUVA MET412. Samples were prepared by mixing the milk with a blend of sodium sulfate and sea sand. The fat was extracted from this powder using a blend of petrol and acetone (2:1). Thereafter, the solvent was evaporated at 40°C (Rotavapor) and the sample was dried at 102°C. Next, 0.1 g of fat was solubilized in 4.9 ml of t-butyl-methyl-ether and 0.5 ml of this mixture was injected into a GC-sampler after it was blended with trimethylsulfoniumhydroxide and heated in a closed sampler for 15 min at 100°C. The sampler content was used for the gas chromatographic analysis, which was done by the Agilent 6890 gas chromatograph with an autosampler. A CP7420 FAME (Fa Varian) column (100 m x 0.25 mm x 0.25 µm) was used with helium as the carrier gas. A 1 µl aliquot of the sample was injected in a split ratio of 45:1 at a temperature of 250°C. Devices were steered by the Chromelon system (Fa Dionex). Calibration was done using a mixture of fatty acid methyl esters which were singly derived from pure substances. CLA (*cis*-9, *trans*-11) was a part of the calibration standard.

Blood. The BHBA content of serum samples was analyzed by an enzymatic procedure based on the oxidation of 3-D-hydroxybutyrate to acetoacetate. 75 µl of the sample were reacted with 500 ml of a buffer enzyme solution containing 100 mmol/l Tris buffer (pH 8.5), 2 mmol/l EDTA, 20 mmol/l oxalic acid, 2.5 mmol/l NAD^+ and 0.12 U/ml 3-D-hydroxybutyrate dehydrogenase. Serum was incubated for 60 seconds at 37°C and then analyzed at a wavelength of 340 nm in a 1 cm light path cuvette against a reagent blank.

The serum NEFA content was measured using the ACS-ACOD method, an *in vitro* test to determine the quantity of free FA in serum. 50 µl of the sample were incubated with 1000 µl of color reagent A at 37°C for exactly 10 min, followed by a 10 min incubation with color reagent B at 37°C. Samples were analyzed at a wavelength of 550 nm in a 1 cm cuvette.

Statistics and calculations

The energy content of forages was calculated as MJ NEL (NEL (MJ) = 0.6[1 + 0.004 (q-57)] * ME (MJ); GfE, 2001).The utilizable crude protein at the duodenum (uCP = [187.7 – (115.4 (UDP/CP))] DOS + 1.03 UDP) and the ruminal nitrogen balance (RNB = [(CP – uCP)/6.25] were determined by formulae provided by GfE (2001). In the concentrate, the nitrogen-free extracts were calculated according to the Weende analysis (DM - ash - CP - CF - CL). The ECM was calculated by a GfE (2001) recommended formula: Milk yield * [(milk fat content * 0.38) + (milk protein content * 0.21) + 1.05] / 3.28.

The normal distribution of the data on milk components, DMI, EB, BFT, BW, blood and milk metabolites and fertility variables was tested by the Kolmogorov-Smirnov test before analysis of variance. Here, the MIXED procedure in SAS (2001) was used for a completely randomized design with repeated measures. The model included the effects of treatment, parity and season, and the interactions between parity and treatment; the random variable was the cow.

$$Y = \mu + G_i + \beta_j + \gamma_k + e_{ijklm}$$

Y = value of the observation

μ = total mean

G_i = treatment ($i = 1, 2, 3$; 1 = control, 2 = CLA80, 3 = CLA120)

β_j = parity ($j = 1, 2$; 1 = primiparous, 2 = multiparous)

γ_k = season ($k = 1 – 18$ months of the trial)

e_{ijklm} = residual error

Significance was declared at $P < 0.05$ and trends at $P \leq 0.10$. Least square means (**LSM**) and standard error (**SE**) are reported throughout.

Data on the fatty acid distribution in milk fat were analyzed by the MIXED procedure in SAS (2001). The model included the effects of treatment and sampling day, with the cow as the random factor.

$$Y = \mu + G_i + \beta_j + e_{ij}$$

Y = value of the observation

μ = total mean

G_i = group (i = 1, 2, 3; 1 = control, 2 = CLA 80, 3 = CLA 120)

β_j = sampling day (k = 1, 78, 84,188, 124)

e_{ij} = residual error

RESULTS

Daily intake of CLA isomers

The daily CLA intake during the treatment period in animals treated with the CLA product is provided in Table 8, and represents the intake of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA per d and animal, respectively, calculated by considering the total recovery of CLA isomers from the feed concentrate.

Table 8. Average daily intake of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA in grams during the treatment period for primi- and multiparous cows.

	CLA80	CLA120
Multiparous cows	3.29	3.22
Primiparous cows	3.31	3.36

CLA80 = CLA supplement fed for 80 d, starting after parturition

CLA120 = CLA supplement fed for 120 d, starting after parturition

Production variables

Parity effects ($P < 0.05$) were observed for milk yield, milk component yield, ECM yield, DMI and BW (Table 9). No parity effects were detected for milk fat, milk protein content or EB. In multiparous cows over the entire supplementation period, milk yield was affected ($P = 0.09$) by supplementation in both treatment groups, while the milk fat content was reduced ($P = 0.07$) solely in CLA120. No differences over the entire supplementation period were observed in milk fat yield, milk protein or lactose yield and content in multiparous cows. Neither were ECM, EB, DMI, BW or BFT of multiparous cows affected. Reductions in milk protein content across the entire supplementation period were observed for primiparous cows in CLA80 (Table 9) with respect to control ($P = 0.05$) and to CLA120 ($P = 0.02$). No treatment effects across the 240 d trial period were registered for the remaining milk components of primiparous cows. DMI, BW, BFT and EB were not affected by treatment across the entire trial period in primiparous cows (Table 9). Treatment parity interactions were not detectable.

During the CLA treatment period, the milk fat content, but not the milk fat yield, decreased progressively with CLA supplementation in multiparous cows (Table 9). About a month (4 to 5 weeks) after the trial started, differences in both treatment groups became significant compared to control. The nadir of milk fat content was reached during trial wk 11 (3.13%) and wk 15 (3.05 %) for CLA80 and CLA120, respectively, with maximum reductions of 14.5% (CLA80) and 20% (CLA120). After terminating CLA supplementation, the milk fat content in both groups treated with the CLA supplement progressively returned to the control level. Regeneration from low milk fat levels occurred within two and four weeks for CLA80 and CLA120, respectively (Figure 10). Progress in multiparous cow milk yield (Figure 11) revealed a trend for increases after lactation was established. Yields persisted throughout lactation as a tendency for elevated milk yields, even if the CLA treatment was discontinued (Figure 11).

Table 9. Least square means for performance measurements of cows fed with or without conjugated linoleic acids (CLA) across a trial period of 240 d starting on the sixth (2.5) d of lactation.

	Multiparous cows				Primiparous cows						
	Treatment[1]			SEM	Treatment[1]			SEM	P-value		
Variable	Control	CLA80	CLA120		Control	CLA80	CLA120		TRT	Parity	TRT*Parity
Milk yield, kg/d	32.9_{b}	35.7_{a}	35.7_{a}	2.64	28.1	26.0	28.4	1.61-2.23	0.41	<0.01	0.41
Milk fat											
%	4.04_{a}	3.86_{ab}	3.81_{b}	0.115	3.84	3.63	3.73	0.114-0.163	0.34	0.77	0.41
kg/d	1.27	1.32		0.085	1.03	0.90	1.05	0.060-0.091	0.77	<0.01	0.71
Milk protein											
%	3.31	3.30	3.22	0.091	3.37_{A}	3.19_{B}	3.38_{A}	0.068-0.089	0.30	0.12	0.65
kg/d	1.06	1.14	1.11	0.061	0.93	0.83	0.94	0.053-0.067	0.59	<0.01	0.71
Milk lactose											
%	4.67	4.66	4.63	0.036	4.79	4.71	4.78	0.048-0.052	0.61	0.03	0.35
kg/d	1.56	1.69	1.66	0.128	1.34	1.26	1.37	0.077-0.104	0.37	<0.01	0.97
ECM[2] yield, kg/d	32.0	34.0	33.3	2.174	26.9	24.1	27.2	1.57-2.15	0.59	<0.01	0.82

	Multiparous cows				Primiparous cows						
	Treatment[1]			SEM	Treatment[1]			SEM	P-value		
Variable	Control	CLA80	CLA120		Control	CLA80	CLA120		TRT	Parity	TRT*Parity
Energy variables											
DMI, kg/d	20.1	20.6	19.9	0.54	16.6	15.4	16.5	0.72-0.95	0.88	<0.01	0.41
BW,[3] kg	678	680	678	12.0	620	604	606	17.2-24.4	0.89	<0.01	0.85
BFT,[4] mm	10.0	8.9	7.6	0.71	11.0	11.2	9.1	1.32-1.85	0.10	0.06	0.98
EB,[5] MJ NEL/d	-2.5	-9.1	-6.1	5.67	-1.9	-7.9	-4.2	2.61-3.69	0.45	0.87	0.96

$_{a,b}$ Values within rows and parities differ at $P < 0.1$

$_{A,B}$ Values within rows and parities differ at $P < 0.05$

[1] Cows received 1) control (Ca salts of palmitic acid), 2) CLA80 (3.29 g (multiparous) and 3.31 g (primiparous) of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11, respectively, in the first 80 days plus Ca salts of palmitic acid) and 3) CLA120 (3.22 g (multiparous) and 3.36 g (primiparous) *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 respectively in the first 120 days plus Ca salts of palmitic acid)

[2] Energy corrected milk (ECM) yield was calculated based on GfE (2001); ECM = Milk yield (kg/d) * (((0.38* (fat %) + 0.21 * (protein %)) + 1.05) / 3.28)

[3] Body weight (BW) was measured daily

[4] Measurement of back fat thickness (BFT) was performed once a month for all cows in the trial on the same date

[5] Energy balance (EB) was calculated by subtracting the energy requirement for lactation and maintenance from energy intake

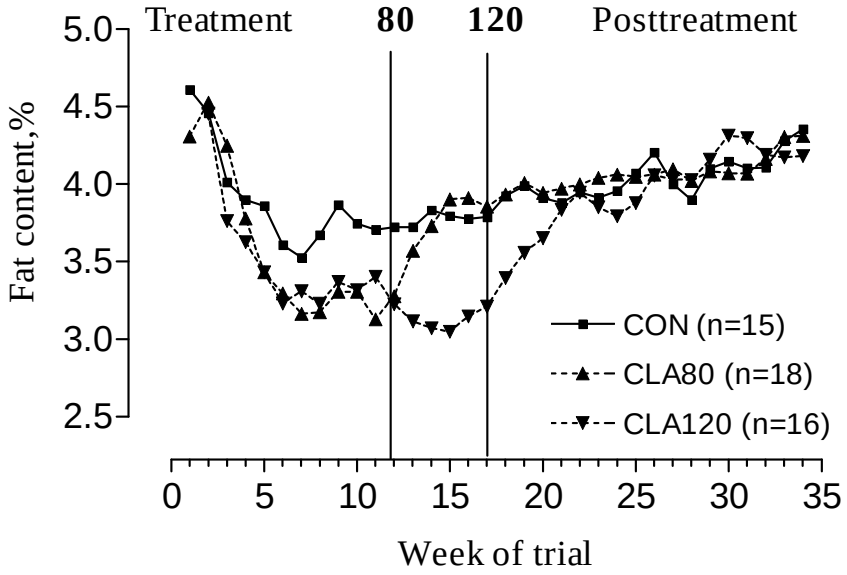

Figure 10. Milk fat content of multiparous cows during the treatment and post-treatment periods from cows fed 1) Ca palmitate (CON), 2) 3.31 g/d *trans*-10, *cis*-12 CLA for 80 days and 3) 3.36 g/d *trans*-10, *cis*-12 CLA for 120 days (CLA120).

The values shown are treatment by week LSM; the SEM for milk fat content averaged 0.11%. Over the entire trial period, the P-value for the treatment effect was 0.34 and 0.41 for the treatment by week interaction. The P-value for the treatment effect for CLA80 at wk 11 of supplementation was 0.51 and was 0.20 for CLA120 at wk 17 of supplementation.

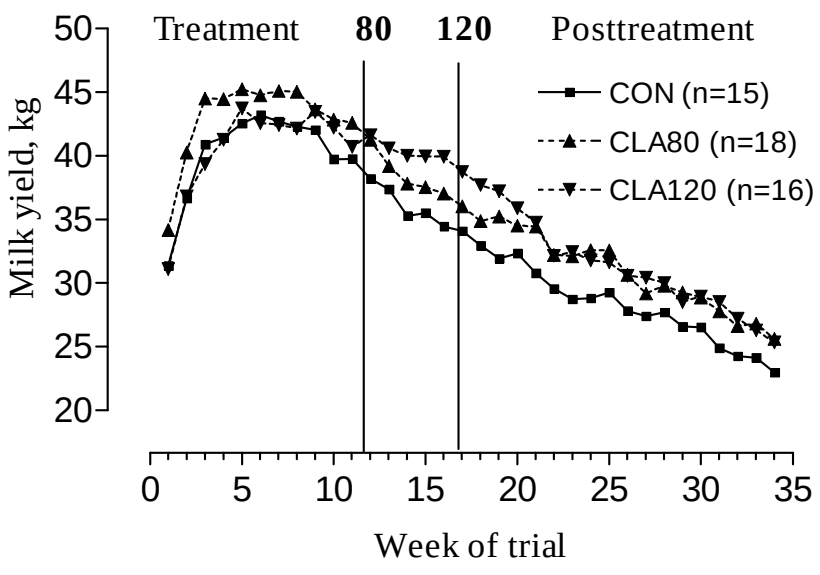

Figure 11. Milk production by multiparous cows during the treatment and post-treatment periods from cows fed 1) Ca palmitate (CON), 2) 3.29 g/d *trans*-10, *cis*-12 CLA for 80 days and 3) 3.22 g/d *trans*-10, *cis*-12 CLA for 120 days (CLA120).

The values shown are treatment by week LSM; the SEM for milk yield averaged 2.64 kg. Over the entire trial period, the P-value for the treatment effect was 0.41 and 0.41 for the treatment by week interaction. The P-value for the treatment effect for CLA80 at wk 11 of supplementation was 0.05 and was 0.01 for CLA120 at wk 17 of supplementation.

The changes in milk protein content showed a trend for discontinuous reductions during CLA treatment in CLA120. Differences were reversed by numerical elevations after the cessation of CLA supplementation in CLA120. No differences in milk protein and milk lactose yields were observed between the multiparous treatment groups. The changes in milk lactose content were similar to the changes in milk yield in all three multiparous treatment groups.

The milk fat content in CLA treated primiparous cows progressively decreased and revealed reduced contents ($p < 0.05$) in the CLA treated groups by trial wk 6 compared to control (Figure 12). The development of CLA-induced MFD was different between both CLA treated primiparous groups and was more pronounced in CLA80 as in CLA120. Increases in milk fat content occurred in CLA80 before stopping CLA supplementation. Following the termination of CLA supplementation, milk fat content in CLA80 was maintained throughout the rest of the trial at a similar level as control (Figure 12). MFD in primiparous CLA120 was not as pronounced as in CLA80 and quickly returned to below control levels before the termination of CLA supplementation. Following trial wk 20, significant increases in milk fat content occurred, which were maintained at a significantly elevated level compared with control throughout the rest of trial (Figure 12). The changes in the protein content in milk from primiparous cows (Figure 13) was similar between control and CLA120 throughout the entire trial, whereas that of CLA80 showed lower values than the two other treatment groups following wk 5. The milk yield in primiparous cows was similar during the first 7 weeks of the trial (Figure 14). By trial wk 8, milk yields of CLA80 and CLA120 were elevated by 3.3 kg and 3.2 kg, respectively, compared with control. After the cessation of CLA administration, the differences between control and CLA80 disappeared within two weeks, and milk yields in CLA80 were numerically below control (Figure 14). The milk yield in primiparous CLA120 cows decreased from high levels one week prior to the termination of CLA to levels similar to control; these levels were maintained throughout the remaining trial period (Figure 14).

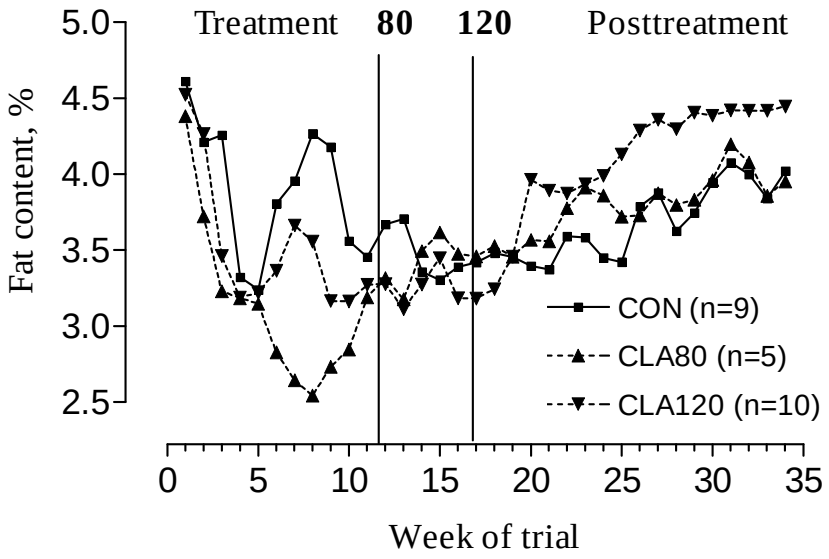

Figure 12. Milk fat content of primiparous cows during the treatment and post-treatment periods from cows fed 1) Ca palmitate (CON), 2) 3.31 g/d *trans*-10, *cis*-12 CLA for 80 days and 3) 3.36 g/d *trans*-10, *cis*-12 CLA for 120 days (CLA120).

The values shown are treatment by week LSM; the SEM for milk fat content averaged from 0.114 to 0.163%. Over the entire trial period, the P-value for the treatment effect was 0.34 and 0.41 for the treatment by week interaction. The P-value for the treatment effect for CLA80 at wk 11 of supplementation was 0.51 and was 0.20 for CLA120 at wk 17 of supplementation.

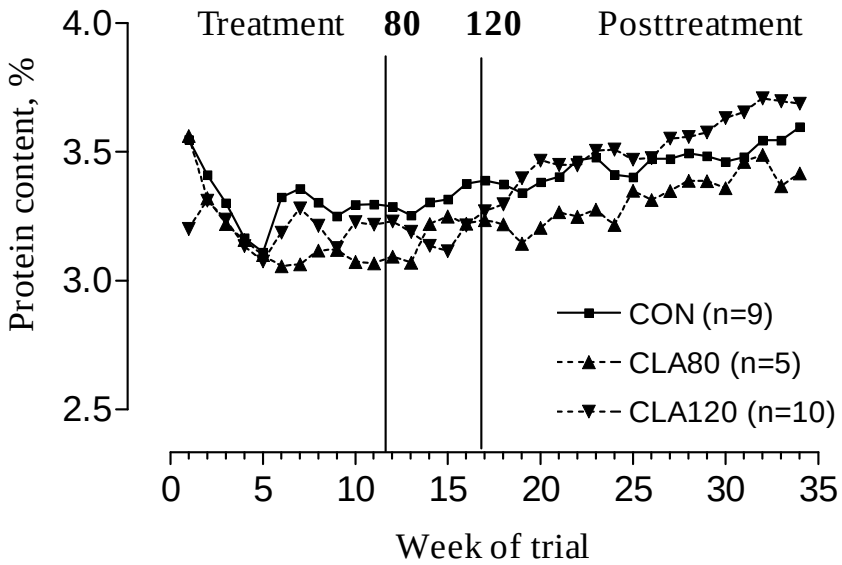

Figure 13. Milk protein content of primiparous cows during the treatment and post-treatment periods from cows fed 1) Ca palmitate (CON), 2) 3.31 g/d *trans*-10, *cis*-12 CLA for 80 days and 3) 3.36 g/d *trans*-10, *cis*-12 CLA for 120 days (CLA120).

The values shown are treatment by week LSM; the SEM for milk protein content averaged from 0.068 to 0.089%. Over the entire trial period, the P-value for the treatment effect was 0.30 and 0.65 for the treatment by week interaction. The P-value for the treatment effect for CLA80 at wk 11 of supplementation was 0.11 and was 0.12 for CLA120 at wk 17 of supplementation.

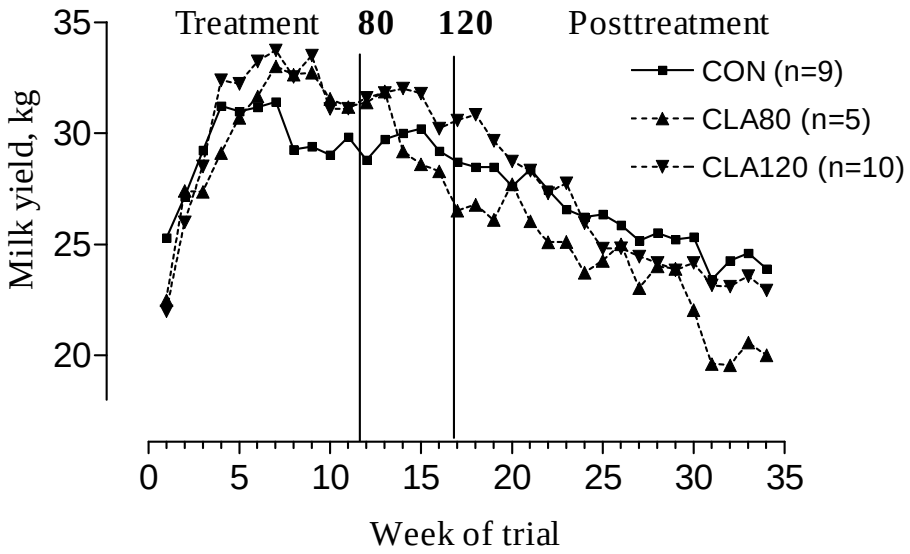

Figure 14. Milk yield of primiparous cows during the treatment and post-treatment periods from cows fed 1) Ca palmitate (CON), 2) 3.31 g/d *trans*-10, *cis*-12 CLA for 80 days and 3) 3.36 g/d *trans*-10, *cis*-12 CLA for 120 days (CLA120).

The values shown are treatment by week LSM; the SEM for milk yield averaged from 1.61 to 2.23 kg. Over the entire trial period, the P-value for the treatment effect was 0.41 and 0.41 for the treatment by week interaction. The P-value for the treatment effect for CLA80 at wk 11 of supplementation was 0.74 and was 0.47 for CLA120 at wk 17 of supplementation.

The results of fatty acid analysis revealed no treatment differences in milk fatty acid composition on the sampling day, with the exception of the milk fat content of *trans*-10, *cis*-12 CLA. This isomer was significantly ($P < 0.01$) elevated in CLA80 and CLA120 on trial d 78 compared with control. On trial d 118 ($P < 0.01$) and 124 ($P = 0.05$), *trans*-10, *cis*-12 CLA was significantly elevated in CLA120 compared with control and CLA80. An effect of lactation week on milk fatty acid composition was found. On trial d 7, milk fat had a significant lower content of short chain fatty acids, C16:0 fatty acid and *cis*-9, *trans*-11 CLA than on trial d 78, 84, 118 and 124. On d 78, the content of SCFA, C16:0 and *cis*-9, *trans*-11 CLA was significant lower than on trial d 118 and 124. The long chain fatty acids, PUFA and MUFA content decreased significantly ($P < 0.05$) in milk fat from trial d 7 to trial d 78. From trial d 78, a significant decline ($P < 0.05$) in LCFA, PUFA and MUFA occurred in milk fat for all treatment groups.

In early lactation, serum concentrations of BHBA and NEFA revealed no differences between the CLA treated and control groups (Table 10), comparing both the results of sampling day and the results across the sampling period. In multiparous cows, the concentration of 1mmol BHBA in serum indicates a ketotic state of metabolism. The control values were just above this range and the values in CLA treated cows were just below this range. The BHBA serum concentration in primiparous cows in all treatment groups was constantly below the state of ketosis, which is indicated in primiparous cows at a serum concentration of 0.6 mmol/l NEFA. The serum values during early lactation did not show any significant fat mobilization (>600 µmol/l) in the multiparous trial groups. The range for pathophysiological fat mobilization in primiparous cows was >300 µmol/l, and both treatment groups exceeded this range on trial d 7, whereas no pathogenic mobilization nor significant treatment effects were observed on trial d 14 and 28 in the primiparous treatment groups.

The milk acetone content did not differ according to treatment or parity (Table 10) within the first 56 days of the trial. On a sampling day basis, these values were not different from each other and did not indicate subclinical acidosis, with the exception of the multiparous control on trial d 1. Acetone levels in this group indicated subclinical acidosis on this day, whereas the CLA group stayed significantly below the cutoff value.

Table 10. Least square means of serum metabolites and milk acetone in early lactation multi- and primiparous cows fed with (CLA) or without (CON) conjugated linoleic acid.

	Treatment[1]								
	Multiparous			Primiparous			P-value		
Variable	CON	CLA	SEM	CON	CLA	SEM	TRT	PAR	TRT* PAR
BHBA[2], mmol/l	1.08	0.66	0.273-0.384	0.46	0.40	0.172-0.274	0.40	0.13	0.53
NEFA[2], µmol/l	505	442	53.8-83.5	331	391	83.7-131.5	0.99	0.23	0.51
Milk acetone[3], mol/l	0.11	0.09	0.019-0.026	0.07	0.05	0.026-0.035	0.55	0.11	0.95

[1] Cows received 1) Ca salts of palmitate, 2) 3.26 g/d *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA and 3) 3.34 g/d *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA

[2] Least squares means representing values collected on trial days 7, 14 and 28

[3] Least squares means representing values collected on trial days 7, 14, 28 and 56

BHBA = beta-hydroxybutyrate; NEFA = Non-esterified fatty acids; TRT = Treatment; PAR= Parity

CLA treatment did not affect the services per conception (**SPC**), but a significant parity effect was noticeable (Table 11) for this variable. The same result could be observed for the fertility variable days open (**DO**). However, no parity or treatment effects could be observed for the variable days to first service (**DFS**; Table 11).

Table 11. Least square means of fertility variables in cows fed conjugated linoleic acid for 80 days (CLA80) or 120 days (CLA120) or a control diet (CON).

	Treatment[1]								
	Multiparous			Primiparous			P-value		
Variable	CON	CLA80	CLA120	CON	CLA80	CLA120	T	PA	T*PA
SPC[2]	2.1	2.6	2.3	1.6	2.0	1.3	0.44	0.04	0.84
DO[3], day	128.2	154.9	128.6	116.6	92.6	84.0	0.57	0.01	0.41
DFS[4], day	75.1	81.4	77.0	80.6	66.4	67.3	0.75	0.33	0.41

[1] Cows received 1) control (Ca salts of palmitic acid), 2) CLA80 (3.29 g (multiparous) and 3.31 g (primiparous) *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11, respectively, in the first 80 days plus Ca salts of palmitic acid) and 3) CLA120 (3.22 g (multiparous) and 3.36 g (primiparous) *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11, respectively, in the first 120 days plus Ca salts of palmitic acid)

[2] Values represent LSM of services per conception

[3] Values represent LSM of days open

[4] Values represent LSM of days to first service

T = Treatment, PA = Parity

DISCUSSION

Lactogenesis and subsequent lactation are characterized by shifting metabolic challenges in dairy cows. Many studies (Perfield et al., 2002, 2004; Bernal-Santos et al., 2003; Moore et al., 2004, 2005; Selberg et al. 2004; Castañeda-Gutiérrez et al., 2005, 2007; Odens et al., 2007) have evaluated the influence of CLA products containing *trans*-10, *cis*-12 CLA in early lactation cows, but the present study represents the first to feed CLA products starting soon after parturition until established lactation considering two different supplementation periods; moreover, the treatment effects were evaluated beyond the supplementation period throughout nearly an entire lactation period. A similar study carried out by Pappritz et al. (2011) investigated the effects of two different *trans*-10, *cis*-12 CLA supplement concentrations fed to dairy cows during early lactation and monitored their effects during the depletion period.

The aim of the present study was to feed a daily amount of 10 g each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA to each animal. However, the mean recovery rate of total CLA isomers in the pelleted concentrate was only 39% with a standard deviation of 0.20, indicating considerable differences in CLA concentrations between single concentrate deliveries. Yang et al. (2000), Lee et al. (2003) and Moon et al. (2008) have stated that CLA is extremely unstable in air and decomposes rapidly. The high temperature and pressure during the pelleting process segregates the methylester bonds of CLA as well, explaining the low recovery rates. The pelleting process was optimized during the trial by reducing the pressure and temperature, resulting in a maximum total CLA recovery rate of 58%. Presumably, losses of protected CLA molecules have to be taken into account if the product is fed as a pelleted concentrate. Top dressing the ration with the CLA product has less impact on the product's rumen stability, but it is harder to ensure the accuracy of intake recordings.

The low amount of *trans*-10, *cis*-12 intake, approximately 3.2 g/d for each trial group, has to be considered if discussing the effects of dietary CLA supplementation on lactation variables. The present intakes of *trans*-10, *cis*-12 CLA are somewhat comparable to treatments in studies by Moore et al. (2004), Castañeda-Gutiérrez et al. (2007) and Odens et al. (2007). The trial design of Castañeda-Gutiérrez et al. (2007) was the most similar of the three to the present study, because others supplemented the CLA product during the transition period. The most comparable trial design was achieved by Pappritz et al. (2011) as mentioned earlier, they fed 6 and 12 g *trans*-10, cis-12 CLA per d. Significant effects of the CLA product on reducing *de novo* synthesized fatty acids in milk fat were observed in transition trials

(Bernal-Santos et al., 2003; Odens et al., 2007) but not in trials that initiated CLA supplementation (Castañeda-Gutiérrez et al., 2007; von Soosten et al., 2011; Pappritz et al., 2011; present study) during early lactation. It might be concluded that the period of lactation during which CLA supplementation commences has an effect on the mammary response to CLA-induced nutrient repartitioning. As a result, MFD was more rapid and severe in studies in which CLA supplementation was started during the transition period (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007). However, the mechanisms responsible for these reactions are not quite clear, because the uptake of *trans*-10, *cis*-12 CLA into mammary tissue is consistent in all cases throughout the treatment period in the literature (Bernal-Santos et al., 2003). Odens et al. (2007) showed that by tripling the amount of CLA fed to transition cows, MFD was achieved within five days after parturition. MFD induced by CLA supplementation had no effects on the plasma concentrations of metabolites, including glucose, NEFA and BHBA, or on metabolic hormones, including insulin, IGF-I and leptin during short- (< 1 wk) and long-term (up to 20 wk) treatment (Baumgard et al., 2000, 2002; Perfield et al., 2002; Castañeda-Gutiérrez et al., 2005; de Veth et al., 2006; von Soosten et al., 2011; Pappritz et al., 2011; Schlegel et al., 2012). However, Bernal-Santos et al. (2003) speculated that the delay in the response of mammary synthesis during early lactation is due to missing mRNA for key enzymes responsible for lipid synthesis, or that essential cellular signaling systems are attenuated in such a way that *trans*-10, *cis*-12 CLA cannot provoke a coordinated reduction in the gene expression of key lipogenic enzymes. Baumann et al. (2011) explained in their review, that a coordinated regulation of enzymes for lipid synthesis was provoked by *trans*-10, *cis*-12 CLA and diet induced MFD. This happened by a decreased expression of lipid synthesis enzymes which means an mRNA abundance for FAS, ACC, lipoprotein lipase, SCD, FA-CoA- ligase, glycerol-phosphate-acyl-transferase, and acyl-glycerol-phosphate-acyl-transferase.

The dissimilarities in the time of milk fat content recovery after terminating CLA supplementation between both CLA treated groups in multiparous cows could be due to differences in energy status at the termination point. The changes in EB in multiparous cow observed in the present study were in accordance with the findings of Coffey et al. (2002, 2004). They determined that EB generally returns to positive values between lactation d 70 and 90 depending on parity, with increasing time for increasing parity. During positive EB, lipogenesis is greater than lipolysis and fatty acids are incorporated into adipose tissue. This is also true for CLA isomers (Wang et al., 2008), supporting the suggestion that *trans*-10, *cis*-12 CLA is released for a longer period under positive EB. Furthermore, adipose tissue turnover

depends on the animal's energy status (Chilliard et al., 2003), i.e. slower fatty acid release is found in more established lactation. In our case this means that CLA120 cows incorporated *trans*-10, *cis*-12 CLA into the adipose tissue from about lactation day 80, when they recovered to positive EB, until the cessation of the CLA product. Due to the natural adipose tissue turnover, which slows down with the progression of lactation, *trans*-10, *cis*-12 CLA is released again, and becomes available for mammary uptake. Fatty acid analysis in milk fat asserts these explanations, because the content of *trans*-10, *cis*-12 CLA was still significantly elevated 4 days after terminating supplementation in the CLA120 group compared to control. In contrast, no differences in milk *trans*-10, *cis*-12 CLA content were registered 4 days after the cessation of CLA product delivery between CLA80 and control. The monetary interest in CLA-induced MFD can be optimized by prolonging CLA supplementation until animals recover from negative EB. This will only be the case if low milk fat content is desired by the dairy.

CLA-induced MFD correlates negatively with the severity of EB, regardless of parity. The regression line in Figure 15 clearly indicates a negative relationship between milk fat content and EB in primi- and in multiparous cows fed the CLA product. This supports the assumption that, under conditions of high adipose tissue mobilization, NEFA and CLA isomers compete for receptors, reducing CLA-induced MFD in the case that NEFA is present in great amounts. High amounts of *trans*-10, *cis*-12 CLA are then required to reduce the milk fat content.

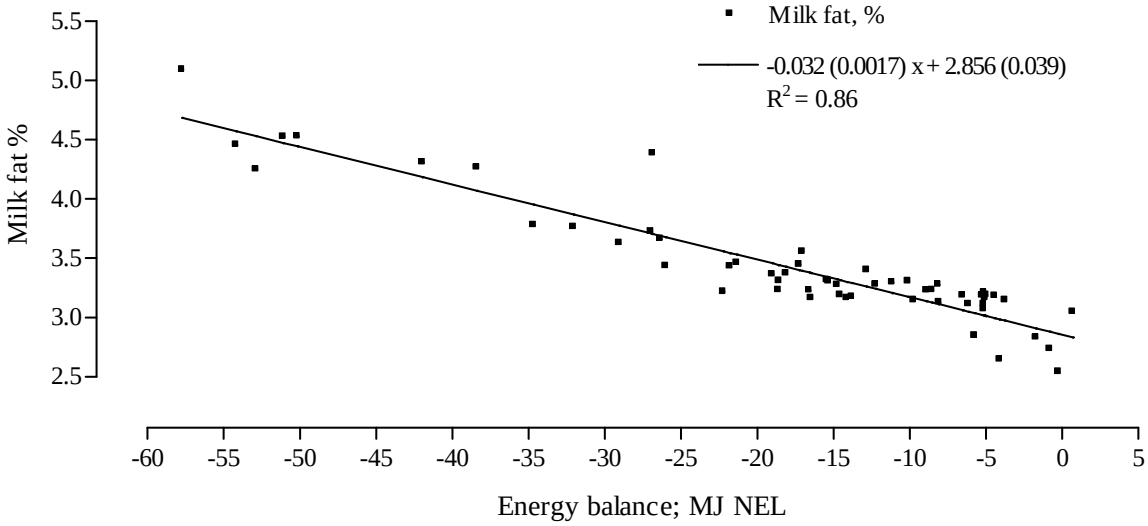

Figure 15. Relationship between energy balance and milk fat content during the supplementation period of the CLA product to early lactation primi- and multiparous cows; CLA supplementation was initiated post-partum.

Nutrients spared from CLA-induced MFD were repartitioned to other sites of mammary synthesis. Both CLA treated multiparous groups revealed a trend for increased milk yields after lactation was established. This trend was persistent throughout the entire trial period in both CLA fed groups, suggesting that, during early lactation, stimulated milk lactose secretion is not reversed by terminating CLA supplementation. However, Pappritz et al. (2011) reported no prolonged effects of SLA supplementation on natural milk yield after cessation of CLA product for each treatment group. It is not clear why the progression of natural milk yield differs between the two studies.

The evaluation of the ideal duration of CLA supplementation for primiparous cows was more difficult, because these animals seemed to react differently to CLA-induced MFD than multiparous cows. Different changes were observed in the milk fat content 1) in comparison to multiparous cows and 2) within primiparous treatment groups. The indifferent findings in terms of changes in the milk fat content between the two CLA treated primiparous groups was attributed to the low number (n = 5) of animals in CLA80. The results of the primiparous CLA80 group are, for this reason, generally not considered for discussion in this study. Because MFD in the primiparous CLA120 group was abolished prior to the termination of CLA supplementation, and as the cessation of CLA product delivery was followed by a significant elevation in milk fat content, it is suggested that mammary synthesis in primiparous cows is regulated differently than in multiparous cows. Whates et al. (2007) suggested that nutrient repartitioning in primiparous cows between body tissue and milk yield is less steered than in multiparous cows, because the metabolic challenge in early lactation is greater due to body growth. The considered primiparous cows calved at an average age of 27 months; Coffey et al. (2006) analyzed the growth trajectories of dairy heifers from birth until the end of their third lactation, although growth rates slowed once the animal reached about 450 days of lactation. The demands of the mammary gland during the initiation of lactation are superimposed on the requirements for growth. Higher insulin and IGF-1 levels, which have positive growth-promoting effects with the additional effects of stimulating protein synthesis and inhibiting protein degradation (Etherton, 1982), were found by Whates et al. (2007) in young lactating animals regardless of their number of lactation periods. However, it might be hypothesized that mammary metabolism in primiparous cows adapts to CLA-induced nutrient repartitioning if CLA is fed during early lactation. Their metabolism is confronted with lactogenesis for the first time, and might therefore be susceptible to alterations. Reist et al. (2003) observed that multiparous cows successfully adapted to higher metabolic and nutritional challenges, which might indicate that primiparous cows could react

similarly. Further studies evaluating the effects of CLA products on mammary metabolism in primiparous cows must be conducted in order to gain information on the mechanisms influencing mammary adaptations to CLA-induced nutrient repartitioning.

In the study of von Soosten et al. (2011) the progression of milk fat content in dairy heifers fed *trans*-10, *cis*-12 CLA from 21 d prior to parturition to 105 d in milk was comparable to expectation for multiparous cows. It would be interesting to know why these cows reacted differently from the primiparous cows in the present study. Could the differences be contributed to the CLA application during transition period, or that they were fed 12 g instead of approximately 3 g in the present study? Another interesting question is the role of growth, because these animals were younger. Their average parturition age was 23 ± 0.2 month.

Differences in the development of BFT throughout the trial period were not observed between parities. BFT could have explained possible differences occurring due to adipose tissue-related hormonal control (leptin) of lipid metabolism. Although leptin is an endocrine product of adipose tissue (Zang et al., 1994), it is also expressed in several other tissues including, apparently, the mammary gland (Chilliard et al., 2000). Mammary adipocytes and fat are present during lactation (Janke et al. 2002; Komatsu et al. 2003), and leptin secreted from mammary fat has a clear role in the regulation of milk synthesis in the bovine mammary gland (Feuermann et al., 2006). Leptin and prolactin interact to alter milk synthesis in the bovine mammary gland (Feuermann et al., 2004), and their plasma levels are significantly increased during the fourth and sixth months of lactation (Accorsi et al., 2005). Feuermann et al. (2004) noted upregulated leptin mRNA expression in lactating cows compared with heifer mammary glands. It should be examined if there are parity effects on hormonal milk secretion control, which could explain the differences between parities in CLA-induced MFD.

The CLA product did not seem to influence the health variables of animals under the high metabolic stress of early lactation, regardless of parity. This was validated by equivalent concentrations of milk acetone, serum BHBA and NEFA between the control and CLA treated groups. As a result, the CLA product is not assumed to have benefits for the health of dairy cows during early lactation. This is underlined by the fact that CLA treatment did not alter the EB. These results are supported by the findings of Castañeda-Gutiérrez et al. (2007). Sigl et al. (2010) and Pappritz et al. (2011) observed a more negative EB in CLA treated cows during early lactation and no effect during established lactation. A decrease in DMI was the trigger for the differences in EB. However, Liermann et al. (2008) and Hutchinson et al.

(2011) found a positive effect of a CLA supplementation on EB in early lactation cows. It remains to be specified how the genetic ability for milk production is related to nutrient repartitioning of *trans*-10, *cis*-12 CLA since published studies have investigated CLA effects in different dairy cow breeds.

High metabolic turnover induces infertility, which is a metabolic response rather than a pathological failing (Knight et al. 1999). It seems as if CLA isomers provided in doses less than 10 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA have a beneficial effect on reproduction (Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005). Fertility is measured by earlier postpartum ovulation and increased pregnancy rates. The present CLA treated primiparous cows followed, as a tendency, the results of other studies (Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005). Multiparous cows, however, reacted differently. No tendency or numerical trend could be noted in decreasing the days open. Interestingly, primiparous cows conceived earlier, although they remained in negative EB for a longer period than multiparous cows. This is in contrast to the findings of Knight et al. (1999), who stated that high metabolic turnover induces infertility. Hutchinson et al. (2011), did not differentiate between primi- and multi-parous cows, but reported no effect of CLA treatment on fertility variables with the exception of a trend of lesser services per conception.

CONCLUSION

Postpartum supplementation of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA led to progressively reduced milk fat contents in multiparous cows. The nadir was reached several weeks after treatment was started. A long recovery period of milk fat content was found after terminating CLA product delivery when animals returned back to positive EB. This was due to *trans*-10, *cis*-12 CLA release that had previously been incorporated into adipose tissue. Differences in the nutrient repartitioning pathways were found in animals in first parity and at higher parities. In multiparous cows, nutrients tend to be repartitioned, leading to elevated milk yields, starting in early lactation and persisting throughout the entire lactation period regardless of the point of CLA product cessation. In primiparous cows, a tendency for increased milk yield occurred during MFD as well, but these differences diminished after stopping CLA supplementation. In the post-treatment period, the milk fat content was significantly increased in first parity animals, leading to the suggestion that CLA provokes different metabolic reactions in different parities.

REFERENCES

Accorsi, P. A., N. Govoni, R. Gaiani, C. Pezzi, E. Seren, and C. Tamanini. 2005. Leptin, GH, PRL, insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Reprod. Domest. Anim. 40:217-223.

Anonymous, 2010. Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), das zuletzt durch Artikel 20 des Gesetzes vom 9. Dezember 2010 (BGBl. I S. 1934) geändert worden ist Fünfter Abschnitt, Tierversuche §7-§9a.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nurtigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman, D. E. and J. M., Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Prod. Sci. 70:15-29.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278:R179-R184.

Bernal-Santos, G., J. W. Perfield II, D. M. Barbano, D. E. Bauman, T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Castañeda-Gutiérrez, E., B. C. Beefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253-4264.

Castañeda-Gutiérrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Chilliard, Y., A. Ferlay, J. Rouel, and B. Lamberet. 2003. A Review of Nutritional and Physiological Factors Affecting Goat Milk Lipid Synthesis and Lipolysis. J. Dairy Sci. 86:1751-1770.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids (Rev). Ann. Zootech. 49:181-205.

Coffey, M. P., J. Hicey, and S. Brotherstone. 2006. Genetic aspects of growth of Holstein-Frisian dairy cows from birth to maturity. J. Dairy Sci. 89:322-329.

Coffey, M. P., G. Doreau, J. D. Oldham, W. G. Hill, and S. Brotherstone. 2004. Genotype and diet effects on energy balance in the first three lactations of dairy cows. J. Dairy Sci. 87:4318-4326.

Coffey, M. P., G. Simm, and S. Brotherstone. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. J. Dairy Sci. 85:2669-2678.

Corl, B. A., D. A. Baumgard, D. E. Bauman, and J. M. Griinari. 2000. Role of Δ9-desaturase in the synthesis of the anticarcinogenic isomer of conjugated linoleic acid and other milk fatty acids. Proceedings of the Cornell nutrition conference. pp 203-212, Cornell University, Rochester, NY.

Davis, C. L. and R. E. Brown. 1970. Low-fat milk syndrome. In Physiology of Digestion and Metabolism in the Ruminant, A. T. Phillipson, ed., pp. 545-565. Oriel Press: Newcastle upon Tyne.

De Veth, M. J., E. Castañeda-Gutiérrez, D. A. Dwyer, A. M. Pfeiffer, D. E. Putnam, D. E. Bauman. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J. Dairy Sci. 89:4620-4631.

De Veth, M. J., S. K. Gulati, N. D. Luchini, and D. E. Bauman. 2005. Comparison of calcium salts and formaldehyde-protected conjugated linoleic acid in inducing milk fat depression. J. Dairy Sci. 88:1685-1693.

Etherton, T. D. 1982. The role of insulin-receptor interactions in regulation of nutrient utilization by skeletal muscle and adipose tissue: a review. J. Anim. Sci. 54:38-67.

Feuermann, Y., S. J. Mabjeesh, L. Niv-Spector, D. Levin, and A. Shamay. 2006. Prolactin affects leptin action in the bovine mammary gland via the mammary fat pad. J. Endocrinol. 191:407-413.

Feuermann, Y., S. J. Mabjeesh, and A. Shamay. 2004. Leptin affects prolactin action on milk protein and fat synthesis in the bovine mammary gland. J. Dairy Sci. 87:2841-2946.

GfE. 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. DLG-Verlag: Frankfurt am Main, Germany.

Hutchinson, I., M. J. de Veth, C. Stanton, R. J. Dewhurst, P. Lonergan, A. C. O. Evans, and S. T. Butler. 2011. Effects of lipid-encapsulated conjugated linoleic acid supplementation on milk production, bioenergetic status and indicators of reproductive performance in lactating dairy cows. J. Dairy Res. 78:308-317.

Janke, J., S. Engeli, K. Gorzelniak, F. C. Luft, and A. M. Sharma. 2002. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes. Res. 10:1-5.

Knight, C. H., D. E. Beever, and A. Sørensen. 1999. Metabolic loads to be expected from different genotypes under different systems. Br. Soc. Anim. Sci. Occasional Publication no. 24:27-35. Pencuik, Midlothian: British Society of Animal Science.

Komatsu, T., F. Itho, S. Mikawa, and K. Hodate. 2003. Gene expression of resistin in adipose tissue and mammary gland of lactating and non-lactating cows. J. Endocrinol. 178:R1-5.

Lee, J., S-M. Lee, I-H. Kim, J-H. Jeong, C. Rhee, and K-W. Lee. 2003. Oxidative instability of CLA concentrate and its avoidance with antioxidants. J. Am. Oil Chem. Soc. 80:807-810.

Liermann, T., A.-M. Pfeiffer, and F. J. Schwarz. 2008. Effects and post-effects on performance and metabolic parameters of early lactation dairy cows to dietary rumen-protected CLA and rumen protected fat. Proc. Soc. Nutr. Physiol. 17:30.

Martin, S. A. and T. C. Jenkins. 2002. Factors affecting conjugated linoleic acid and trans-C18:1 fatty acid production by mixed ruminal bacteria. J. Anim. Sci. 80:3347-3352.

Moon, H. S., H. G. Lee, C. S. Chung, Y. J. Choi, and C. S. Cho. 2008. Physico-chemical modifications of conjugated linoleic acid for ruminal protection and oxidative stability. Nutr. Metab. (Lond). 1:5-16.

Moore, C. E., J. K. Kay, R. J. Collier, M. J. Vanbaale, and L. H. Baumgard. 2005. Effect of supplemental conjugated linoleic acids on heat-stressed Brown Swiss and Holstein cows. J. Dairy Sci. 88:1732-1740.

Moore, C. E., H. C. Haflinger, O. B. Mendivil, S. R. Saders, D. E. Bauman, and L. H Baumgard. 2004. Increasing amounts of conjugated linoleic acid progressively reduces milk fat synthesis immediately postpartum. J. Dairy Sci. 87:1886-1895.

Nauman, C., R. Bassler, R. Seiblod, and C. Barth. 2004. Verband Deutscher Landwirtschaflicher Untersuchungs- und Forschungsanstalten; Methodenbuch, Band III/1, Die chemischen Untersuchungen von Futtermitteln. Dritte Auflage mit 5. Ergänzungslieferung, VDLUFA-Verlag-Darmstadt.

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. J. Dairy Sci. 90:293-305.

Pappritz, J., U. Meyer, R. Kramer, E.-M. Weber, G. Jahreis, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effects of long-term supplementation of dairy cow diets with rumen-protected conjugated linoleic acids (CLA) on performance, metabolic parameters and fatty acid profile in milk fat. Arch. Anim. Nutr. 65:89-107.

Perfield II, J. W., P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2004. Trans-10, trans-12 conjugated linoleic acid (CLA) reduces the Δ^9-desaturase index without affecting milk fat yield in lactating cows. J. Dairy Sci. 87 (Suppl.1):297 (Abstract).

Perfield II, J. W., G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Short communication: milk fat response to low doses of trans-10, cis-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85:1764-1766.

Reist, M., D. Erdin, D. von Euw, K. Tschuemperlin, H. Leuenberger, C. Delavaud, Y. Chilliard, H. M. Hammon, N. Kuenzi, and J. W. Blum. 2003. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin. J. Dairy Sci. 86:1690-1706.

SAS/STAT. 2001. Changes and Enhancements, Release 8.2. SAS Inst., Cary, NC.

Schlegel, G., R. Ringseis, W. Windisch, F. J. Schwarz, and K. Eder. 2012. Effects of a rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows. J. Dairy Sci. 95:3905-3918.

Selberg, K. T., A. C. Lowe, C. R. Staples, N. D. Luchini, and L. Badinga. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J. Dairy Sci. 87:158-168.

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer, and M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scan. 52:16.

Staufenbiel, R. 1992. Energie- und Fettstoffwechsel des Rindes- Untersuchungskonzept und Messung der Rückenfettdicke. Mh. Vet.-Med. 47:467-474.

Van Soest, P. J. 1963.Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. Dairy Sci. 46:204-216.

Von Soosten, D., U. Meyer, E.M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J. Dairy Sci. 94:2859-2879.

Wang, Y., J. Lu, M. R. Ruth, S. D. Goruk, M. J. Reaney, D. R. Glimm, D. F. Vine, C. J. Field, and S. D. Proctor. 2008. Trans-11 vaccenic acid dietary supplementation induces hypolipidemic effect in JCR:LA-cp rats. J. Nutr. 138:2117-2122

Wathes, D. C., Z. Cheng, N. Bourne, V. J. Taylor, M. P. Coffey, and S. Brotherstone. 2007. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the peripaturient period. Domest. Anim. Endocrinol. 33:203-25.

Yang, L., L. K. Leung, Y. Huang, and Z. Y. Chen. 2000. Oxidative stability of conjugated linoleic acid isomers. J. Agric. Food Chem. 48:3072-3076.

Zang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432.

5 STUDY TWO

Influences of typical Central European grass-based rations on milk fat depression in dairy cows fed a source of mixed conjugated linoleic acids during the transition period

ABSTRACT

Multiparous German Holstein cows were treated either without (CON) or with a rumen-inert CLA product (TCLA), providing 4.96 g/d each of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA throughout the periparturient period. Treatment started 18 d prior to parturition and continued until lactation d 80. Progress of performance variables and energy status were obtained from the beginning of the trial until lactation d 100. During the transition period, animals were fed a total mixed ration based on grass and corn silage (42:58) providing 140 g CP/kg DM and 6.55 MJ NEL/kg DM. During lactation, a basal ration with a ratio of 66:34 grass to corn silage providing 150 g CP /kg DM and 6.5 MJ NEL /kg DM was fed. The basal ration was supplemented with a concentrate providing 232 g/kg DM CP and 8.1 MJ MEL/kg DM depending on the demands based on energy corrected milk yields. The CLA product was pressed into a concentrate that was isonitrogenous and isocaloric to the concentrate fed to control animals.

The objective of the present study was to evaluate if the milk fat depressing ability of a CLA product could be reproduced by feeding rations common in Central European regions, because the majority of studies researching the mechanisms of CLA effects have been conducted in America. Animals fed with the CLA product showed a significant reduction in the milk fat content three weeks after parturition. Trends were observed for reduced milk protein content in early lactation and increased milk yield until lactation d 100 with CLA treatment. No effect on yields in milk fat, protein and lactose were observed because elevations in milk yield balanced the depression in milk fat and protein content, resulting in equal yields of energy corrected milk. CLA supplementation did not benefit energy balance and no differences in dry matter intake and body weight were recorded. These results are similar to those recorded by studies conducted under similar trial designs in the United States of America.

Keywords: conjugated linoleic acid, transition period, diet composition, energy balance

INTRODUCTION

Conjugated linoleic acids (**CLA**) are geometric and positional isomers of linoleic acid containing conjugated double bonds. *Cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA are the two most frequently studied isomers with respect to dairy cow nutrition. It has been shown that *trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis during late lactation (Baumgard et al., 2000, 2001, 2002; Viswanadha et al., 2003) in a dose-dependent manner (Baumgard et al., 2001; Viswanadha et al., 2003). The isomer acts by inhibiting *de novo* milk fat synthesis and the uptake of fatty acids into the mammary gland (Piperova et al., 2000; Baumgard et al., 2001; Peterson et al., 2002; Harvatine et al., 2006; Perfield et al., 2006) and further by interacting with the gene receptors encoding for key enzymes in milk fat synthesis (Piperova et al., 2000; Baumgard et al., 2002; Harvatine et al., 2008).

The effects of rumen-protected mixed CLA isomers, containing *trans*-10, *cis*-12 CLA, were determined in various studies concerning lactation variables in dairy cows. Many studies have shown the milk fat depressing ability of *trans*-10, *cis*-12 CLA (Bernal-Santos et al., 2003; Selberg et al., 2004; De Veth et al., 2005; Kay et al., 2007; Odens et al., 2007), but the effects on milk yield were variable between studies. Bernal-Santos et al. (2003) speculated that energy balance (**EB**) could be positively influenced by feeding mixed CLA isomers during the transition period and early lactation because of spared energy from reduced milk fat secretion. These results were supported by Liermann et al. (2008), Hutchinson et al. (2011) and Schlegel et al. (2012), who found significant benefits on EB when CLA was fed during early lactation. However, other studies did not support these findings (Moore et al., 2004; Castañeda-Gutiérrez et al., 2005, 2007, von Soosten et al. 2011), and even registered negative effects during early lactation (Pappritz et al., 2011).

The majority of studies evaluating CLA-induced milk fat depression (**MFD**) have been conducted in America. These rations differ in composition to those fed in Central Europe. The objective of the present study was to determine if the results from those feed trials with mixed CLA products could be repeated. The focus of this study was on changes in lactation variables, EB and fertility.

MATERIALS AND METHODS

Animals, treatments and design

Seventeen multiparous German Holstein cows were grouped according to the criteria of expected parturition day, prior 305 d mature-equivalent milk production and parity into a block design of two treatments: 1) fed without the CLA product (**CON**) and 2) fed with 100 g/d of the CLA product (Lutrell; BASF SE, Ludwigshafen, Germany) starting at 18.5 d (±1.4 SD) prior to parturition until lactation d 80 (**TCLA**). The CLA supplement provided, considering the recovery rate in the concentrate pellet, 4.96 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA. All animals started the transition period at 18 d antepartum (**a.p.**), and data on animal performance were collected until 100 d postpartum (**p.p.**). The trial started in January 2007 and continued until July 2007. Fertility variables were recorded as they accrued. All procedures involving dairy cows were conducted according to the regulations of the German law on animal welfare (Anonymous, 2010).

Transition Period. Animals were housed respective to the treatment group in straw bedded free stalls. They calved in these stalls and stayed there for the first two days of lactation. Transition cows were fed a total mixed ration (TMR) for *ad libitum* intake without the possibility of measuring daily feed intake for single animals. The ration was fed every third day and, in order to decelerate fermentation, the TMR was preserved with potassium sorbate (80 g/1000 kg FM TMR). The composition of TMR was nearly identical between both treatment groups, except that 3 kg of a concentrate containing 100 g Lutrell (BASF SE, Ludwigshafen, Germany) was blended into the TMR of the TCLA group. The concentrate fed to the CON group was isoenergetic and isonitrogenic, containing 2.5% of a rumen-inert palmitic fatty acid source (Bergafat, Berg und Schmidt GmbH, Hamburg, Germany). Detailed information on the composition of concentrates and TMR is provided in Table 12 and Table 13, respectively.

Table 12. Components and chemical composition of concentrates fed to control (CON) and the group fed with conjugated linoleic acid (TCLA)[1]

Concentrate composition	CON	TCLA
Component, % of DM		
Wheat	26	26
Corn grains	14	14
Soybean meal (protected)	11.5	11.5
Soybean meal	12.8	12.8
Rapeseed	8.9	
Molasses pulp	18.8	18
Protected fat, (Bergafat, Berg und Schmidt, Hamburg, Germany)	2.5	
Lutrell (BASF SE, Ludwigshafen, Germany)		3.3
Rest	5.5	5.5
Chemical analysis, % of DM		
Crude protein	23.2	23.4
Utilizable crude protein at the duodenum	17.5	17.5
RNB	3	3
NDF	17.1	16.9
ADF	8.9	8.7
Crude lipid	4.2	4.2
NEL, MJ/ kg DM	8.1	8

[1] Values are averages of samples collected at each delivery

DM = Dry matter, RNB = Ruminal nitrogen balance, NEL = Net energy lactation

Table 13. Components and chemical composition of the total mixed ration (TMR) fed during the transition period.[1]

Diet composition	TMR
Component, %	
Grass silage	33.8
Corn silage	35.8
Concentrate (Bergafat/Lutrell)	20.8
Straw	7.9
Glycerin	1.3
Mineral mixture	0.4
Chemical analysis, % of DM	
Crude protein	14.0
Utilizable crude protein at the duodenum	14.1
RNB	0
Crude lipid	3.4
NDF	39.5
ADF	23.1
NEL, MJ/ kg DM	6.55

[1]Values represent averages of samples obtained from the individual stocks prior to feeding or at delivery.

DM = Dry matter, CLA = Conjugated linoleic acid, RNB = Ruminal nitrogen balance, NEL = Net energy lactation

The forage ratio of TMR was 49% grass silage to 51% maize silage on the base of DML

Lactation period. Two days after parturition, cows were grouped into a free barn system including 48 single beds covered with rubber mats littered with shavings twice a day. The beds were arranged on both sides of a crevice alley automatically scraped by a chain-drawn slider. A basal ration, providing nutrients for 25 kg of ECM yield and formulated by utilizing the recommendations of GfE (2001), was fed into 24 weighing troughs located in front of self-locking doors. The ratio of grass to corn silage was 66% to 34%. The basal ration provided 6.5 MJ NEL/kg DM and 152 g CP/kg DM (Table 14). All cows had access to all troughs and fresh matter (**FM**) intake was monitored by a transponder located on a neck collar. Five times a week, the DM of the basal ration was determined as a basis for DMI

calculations. Cows of the TCLA group were fed 3 kg of the concentrate containing 3.3% CLA product (Lutrell, BASF SE, Ludwigshafen, Germany; Table 12) until 80 d in lactation, providing 4.96 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA; this was fed before parturition as well. Additional nutrient needs based on energy corrected milk yield (**ECM**) were met by an isoenergetic and isonitrogenic concentrate (Table 12) containing 2.5% of a rumen-inert palmitic acid fat source (Bergafat, Berg und Schmidt GmbH, Hamburg, Germany).

Table 14. Component and chemical composition of upgraded mixed ratio fed from the start of lactation.[1]

Diet composition	Basal ration
Component %	
Grass silage	47.8
Corn silage	24.8
Sugar beet pulp, pressed, ensiled (22% DM, 7.5 MJ NEL/kg DM)	6.5
Protein supplement	18.6
Straw	2.0
Mineral mixture	0.3
Chemical analysis % of DM	
Crude protein	15.2
Utilizable crude protein in the duodenum	14.5
RNB	1.1
Crude lipid	3.3
NDF	41.7
ADF	24.6
NEL, MJ/ kg DM	6.50

[1]Values represent averages of samples obtained from the individual stocks prior to feeding or at delivery

DM = Dry matter, RNB = Ruminal nitrogen balance, NEL = Net energy lactation

The maximum concentrate intake per day was 10 kg/cow. Cows of the CON group were fed an isoenergetic and isonitrogenic concentrate starting with 3 kg after parturition and

increasing continuously throughout lactation to a maximum intake of 10 kg. Animals of the TCLA group were fed identically to the CON group after stopping the CLA supplementation. The daily concentrate intake was computer registered. Milking was performed twice a day, at 5:30 am and 3:30 pm, in a Westfalia Surge (Bönen, Germany) carousel with 14 parlors. Since only 17 animals were included in the trial, the cow house was filled with non-experimental cows, treated as the CON group, in order to diminish systematic errors.

Sampling and analysis

Feeds. Analysis of the chemical composition and energy value of feedstuffs was done by Landwirtschaftliche Untersuchungs- und Forschungsanstalt NRW; Münster/Germany. All components of the basal ration were analyzed individually. Samples of forages were collected weekly from the surface of each silage stock and stored at -20°C. A cumulative sample was added up for each stock, which was analyzed further.

All methods for analysis of forage components are described in the book of methods by Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (**VDLUFA**) volume III (Naumann et al., 2004). The DM of forages was estimated by oven-drying at 103°C for 4 h (VDLUFA method 3.1). Ash was determined by ashing in a muffle furnace at 550°C overnight (16 h) (VDLUFA method 8.1). NDF (VDLUFA method 6.5.1) and ADF (VDLUFA method 6.5.2) were determined by boiling the sample for 1 h with neutral or acetic detergent, respectively. Crude lipid (**CL**) was analyzed by treating the sample with benzene (VDLUFA method 5.1.1/A), whereas CP was analyzed by the Kjeldahl method (VDLUFA method 4.1.1).

Samples of fed concentrates were taken during delivery and stored at -20°C until analysis for the chemical composition and energy value. Samples of the concentrates containing CLA were analyzed individually, while three individual samples of the concentrate containing the palmitic fat source were pooled before analysis. The concentrates were analyzed by LUFA NRW, Münster/Germany. DM (VDLUFA method 3.1) was estimated by oven-drying at 103°C for 4 h. Ash (VDLUFA method 8.1) was determined by ashing in a muffle furnace at 550°C overnight (16 h). CF was determined by maceration with a boiling sulfuric acid and potassium hydroxide solution (method 6.1.1) and CP was analyzed by the Kjeldahl method (method 4.1.1); these methods are described in the method book of the VDLUFA, volume III (Naumann et al., 2004). The CL content was analyzed after acid hydrolysis (“Amtsblatt” of EG L 257/23-25 1998/ procedure B).

Milk. Every second week, milk performance monitoring of each cow was carried out. For this reason, a pooled milk sample was collected individually during the evening and morning milking periods. The analysis of milk lactose, protein and fat content, as well as the somatic cell count and urea content was performed at the Landeskontrollverein (**LKV**) NRW, Krefeld, Germany using a MILCOSCAN (Foss Electric, Hillerød, Denmark). Additional milk samples were collected on lactation day 1, 7, 14, 28 and 56 during the morning milking period, giving an average across this milking period. These samples were hereafter stored in an airtight bottle at 4°C in the presence of a conservation agent (Bronopol) until analysis for the content of fat, true protein, lactose, urea and SCC by infrared analysis (LKV Rhineland-Palatinate) using a MILCOSCAN (Foss Electric, Hillerød, Denmark). The milk acetone content was further analyzed using the same utilities.

Blood. From each cow, blood samples were taken before and after parturition. Samples taken a.p. were collected as cows entered the transition period and three days prior to parturition. More samples were gathered on parturition and on d 7, 14, 28 and 56 p.p. Blood samples were taken via coccygeal venipuncture and collected into tubes to harvest serum. The tubes were centrifuged immediately after sample collection at 5000 rpm for 15 min at room temperature. The supernatant was stored in Eppendorf tubes at -28°C until analysis. Contents of FFA, BHBA and glucose were analyzed at the Veterinary University in Hannover/Germany. The BHBA content of serum samples was analyzed by an enzymatic procedure, based on the oxidation of 3-D-hydorxybutyrate to acetoacetate. 75 µl of the sample were reacted with 500 ml of a buffer enzyme solution containing 100 mmol/l Tris buffer (pH 8.5), 2 mmol/l EDTA, 20 mmol/l oxalic acid, 2.5 mmol/l NAD^+ and 0.12 U/ml 3-D-hydoxybutyrate dehydrogenase. Serum was incubated for 60 seconds at 37°C and then analyzed at a wavelength of 340nm in a 1 cm light path cuvette against a reagent blank. The serum NEFA content was measured using the ACS-ACOD method, an *in vitro* test to determine the quantity of free FA in serum. 50 µl of the sample were incubated with 1000 µl of color reagent A at 37°C for exactly 10 min, followed by a 10 min incubation with color reagent B at 37°C. Samples were analyzed at a wavelength of 550 nm in a 1 cm cuvette. Serum glucose was analyzed by the phoshorylation of glucose with ATP to glucose-6-phosphate which was then transformed by NADP to gluconate-6-P. The synthesized $NADPH_2$ was then measured. 10 µl of serum were incubated with 1000 µl of reactive solution at 20 to 25°C and analyzed at a wavelength of 334 nm in a 1 cm cuvette.

Dale E. Bauman at Cornell University (Ithaca, NY, USA) analyzed serum samples for IGF-1 by determining the circulating levels of IGF-1 by RIA (Butler et al., 2004). The first

step was an extraction with ethanol:acetone:acetic acid (60:30:10) to remove all binding proteins, as described by Enright et al. (1989). Complete removal of all binding proteins was verified by Western blot. The primary antibody (anti-hIGF-1 (rabbit) #AFP 4892898) was sourced from Dr. A. F. Parlow (National Hormone and Peptide Program) and the hormone for iodination and standards (lot#AAG-CO1) were obtained from GROPEP, Adelaide, Australia.

Body condition. BW data were recorded automatically as cows were weighed twice daily after milking. The back fat thickness (**BFT**) of each cow in the trial was measured using a PERSONAL ULTRASOUND-400 ultrasonic device (Proxima medizinische Geräte GmbH, Berlin, Germany) with a 5 MHz linear sonic head. The sonic head was placed on alcohol-wetted skin. The point of measurement was in the conduit between the dorsal part of the *Tuber ischiadicum* and the upper part of the *Tuber coxae* at the end of the *Crista sacrales* and the beginning of the coccyx, following the method of Staufenbiel (1992). The measurement was performed once a month without regard for to parturition day by the same person throughout the entire trial. The BFT measurements started two months prior to the calculated parturition day.

CLA isomers. Individual samples of all delivered CLA concentrate batches were taken and stored at -20°C before being sent out for analysis of CLA isomers by HPLC at BASF SE (Ludwigshafen/Germany). They were analyzed within a week of sample collection in order to prevent storage losses of CLA methylesters (**CLA-ME**). The analytics determined the total CLA content and content of CLA-ME isomers by HPLC using BASF SE method AM/00887/01e. Both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were evaluated. In addition, the sum of *cis, trans* CLA-ME isomers, *trans, trans* CLA-ME isomers and *cis, cis* CLA-ME isomers were evaluated according to the following principle.

CLA-Ca^{++} salt was initially reacted with acetic acid to form CLA. CLA and/or CLA-ME were extracted with cyclohexane and quantified by HPLC (wavelength: 233nm, C-18 column) against an external standard. The total CLA content was obtained by adding up the individual CLA and CLA-ME contents. To identify the CLA-ME isomers, part of the extracting solutions were evaporated to dryness under a stream of nitrogen and the CLA obtained was esterified with hydrogen chloride in methanol to yield CLA-ME; this was subsequently extracted with cyclohexane. The isomers of CLA-ME were separated by HPLC on a ChromSpher 5 lipid column and then subjected to UV detection at a wavelength of 233 nm. Based on the area percentages of the individual CLA-ME isomers in the total CLA-ME area, their contents were established by multiplying the value by the total CLA content.

Statistics and calculations

Feeds. The energy content of forages was calculated in MJ NEL (NEL (MJ) = 0.6[1 + 0.004 (q-57)] * ME (MJ); GfE, 2001); the available protein (uCP = [187.7 – (115.4 (UDP/CP))] DOS + 1.03 UDP) and the ruminal nitrogen balance (RNB = [(CP – uCP)/6.25] were determined by formulas provided by GfE (2001). The energy content of concentrates was calculated as follows: NEL (MJ) = 0.6 [1 + 0.04 (q – 57)] * ME (MJ) and nitrogen-free extracts were calculated by Weende analysis (DM - ash - CP - CF – CL; GfE, 2001).

Energy balance. The EB was calculated as the difference between energy intake through feed intake on a DM basis and the energy requirement for milk production and maintenance. Energy requirements for maintenance were calculated according to Kirchgessner (1997), who stated that the metabolic rate of grown mammals is approximately 297 KJ NEL per kg of metabolic live weight. Using this calculation, the required energy for maintenance is between the suggestions of ARC (1980) and Chwaligbog (2000). The energy output of milk was calculated by multiplying the ECM yield by 3.28 MJ NEL/kg ECM (GfE, 2001). The energy requirement for fetal development and growth was not included.

Energy corrected milk yield. The following formula (GfE, 2001) was used to calculate ECM: (Milk yield * ((milk fat content * 0.38) + (milk protein content * 0.21) + 1.05) / 3.28).

Statistics. The data obtained for DMI, EB, BFT, BW, blood and milk metabolites and fertility variables were tested for normal distribution by the Kolmogorov-Smirnov test before analysis of variance was conducted. The MIXED procedure in SAS (2001) was used for a completely randomized design with repeated measures. The model included the effects of treatment, parity and season, as well as interactions between parity and treatment with the random variable as the cow. Significance was declared at $P < 0.05$ and trends at $P < 0.10$. Least square means (**LSM**) and standard error (**SE**) are reported throughout.

RESULTS

Production variables

The dietary CLA intake was calculated to be 4.96 g/d each of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA, considering the recovery rate in the concentrate. No effect of treatment was noted on DMI, ECM yield, BW, BFT or EB (Table 15) throughout the first 100 d of lactation

including a 20 d post-treatment period. Yields of milk fat, protein and lactose as well as lactose content were not statistically altered during the same period. Milk fat content, as expected, was significantly reduced ($P < 0.001$) by 15% during the observation period and milk protein content showed a tendency for reduction ($P = 0.09$; 3.7%). Furthermore, a trend ($P = 0.08$) for elevated milk yield was observed in the CLA treated group, which corresponds to 4.2 kg/d. During the treatment period, the reduction in milk fat content was 16.3%, and a maximum reduction of 20% was reached during lactation wk 4.

The development of milk fat content (Figure 16) during the first weeks in lactation was identical between the treatment groups. By wk 4, the treatments showed different changes. The milk fat content in TCLA decreased by approximately 3%, whereas the milk fat content increased in CON. In the post-treatment period, the differences between treatments were annulled three weeks after ending the CLA supplementation. Hereafter, the fat content in both treatment groups changed equally (Figure 16). Milk yield in TCLA changed by lactation wk 6, as a tendency, to a level higher than in CON (Figure 17). After terminating CLA supplementation, yields approached those of CON again. Nevertheless, numerical elevations were recognized throughout the entire observation period for TCLA (Figure 17).

Alterations in milk protein content during the first three weeks in lactation showed no differences between treatments (Figure 18). However, the protein content in TCLA showed a trend for lower levels during the CLA treatment period than CON. In lactation wk 11, the milk protein content of TCLA was reduced by 0.18 percentage points compared to CON. These differences were diminished in the post-treatment period, but levels in TCLA did not reach those of CON within the observation period. This change explains the trend of overall protein content reduction in TCLA across the observation period (Table 15). The EB (Figure 19) developed similarly in both treatment groups during early lactation. Animals of both treatment groups returned inconsistently back to positive values during lactation wk 10. Changes in DMI, BW, BFT and ECM yield showed no differences between treatments, thus explaining the lack of an effect on EB during the observation period.

Table 15. Least square means (LSM) and standard error (SE) of performance variables for the first 100 days of lactation in control (CON) and in the group fed 4.96 g/d conjugated linoleic acids (TCLA).

	Treatments			P-value	
	CON	TCLA*	SEM	TRT.	TRT*WO
Lactation variables					
Milk, kg	38.1	42.3	1.61-1.70	0.08	<0.001
Milk fat					
%	3.84	3.27	0.125	<0.001	<0.001
kg	1.44	1.36	0.093	0.53	<0.001
Milk protein					
%	3.26	3.14	0.058	0.09	0.003
Kg	1.23	1.32	0.043	0.20	<0.001
Milk lactose					
%	4.81	4.74	0.041	0.28	<0.001
Kg	1.79	1.96	0.088	0.14	0.01
ECM, kg	36.8	37.7	1.86-1.91	0.73	<0.001
Energy variables					
DM intake, kg	20.2	20.9	0.89-0.93	0.62	0.27
EB, MJ NEL	-13.4	-12.8	4.31-4.50	0.86	<0.001
BFT, mm	8.3	9.0	0.92	0.63	
BW, kg	649	660	17.7-18.9	0.53	0.002

* Conjugated linoleic acid was fed during the transition period (-18 days) and the first 80 days of lactation in relation to parturition.

ECM = energy corrected milk yield; EB = energy balance; DM = Dry matter; BFT = Back fat thickness; BW = Body weight

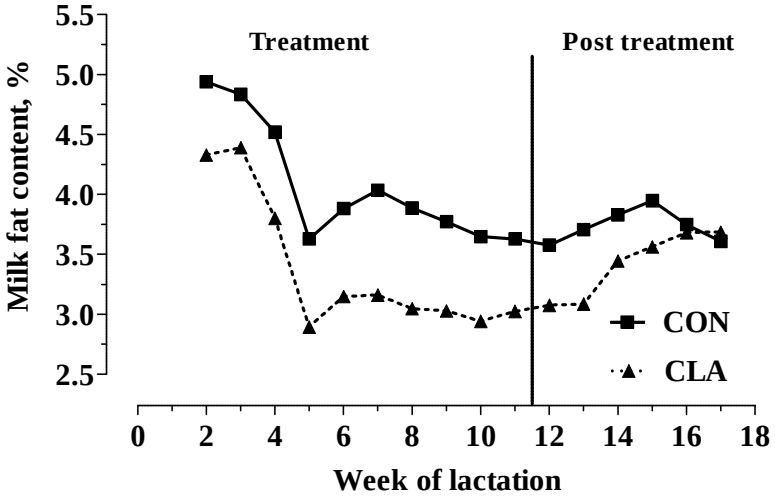

Figure 16. Development of milk fat content during the treatment and post-treatment periods in cows fed without (CON) and with 4.96 g/d *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA starting 18.5 d prior to parturition.

Values shown are treatment by week LSM; the SEM for milk fat content averaged 0.12%. Over the entire trial period, the P-value for the treatment effect was <0.001 and <0.001 for the treatment by week interaction.

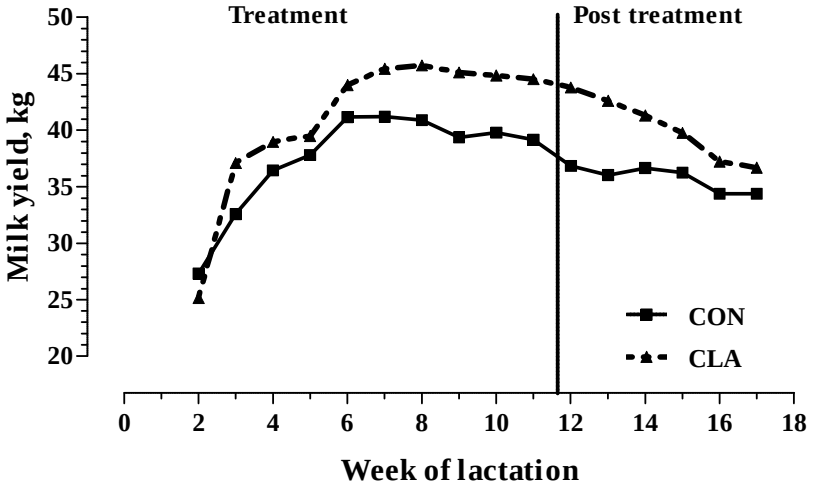

Figure 17. Development of milk yield during the treatment and post-treatment periods in cows fed without (CON) and with 4.96 g/d *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA starting 18.5 d prior to parturition.

Values shown are treatment by week LSM; the SEM for milk yield averaged from 1.61 to 1.70 kg. Over the entire trial period, the P-value for the treatment effect was 0.08 and <0.001 for the treatment by week interaction.

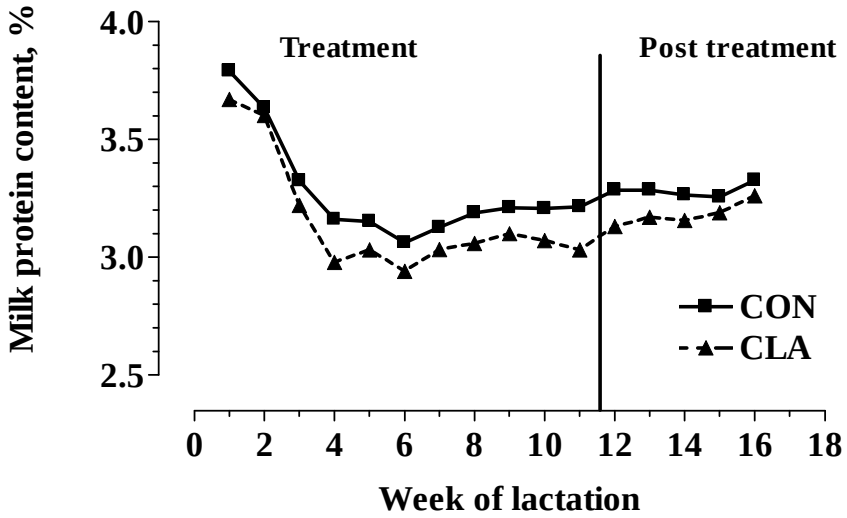

Figure 18. Development of milk protein content during the treatment and post-treatment periods in cows fed without (CON) and with 4.96 g/d *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA starting 18.5 d prior to parturition.

Values shown are treatment by week LSM; the SEM for milk fat content averaged 0.05%. Over the entire trial period, the P-value for the treatment effect was 0.09 and 0.003 for the treatment by week interaction.

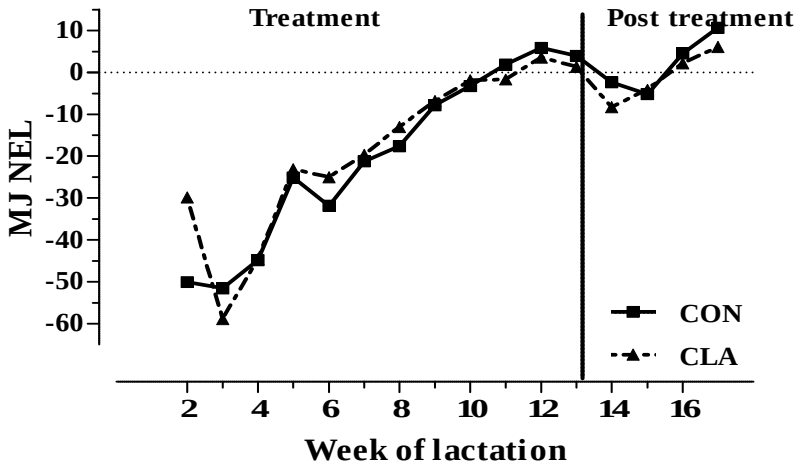

Figure 19. Development of energy balance during the treatment and post-treatment periods in cows fed without (CON) and with 4.96 g/d *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA starting 18.5 d prior to parturition.

Values shown are treatment by week LSM; the SEM for milk fat content averaged from 4.3 to 4.5 MJ NEL. Over the entire trial period, the P-value for the treatment effect was <0.86 and <0.001 for the treatment by week interaction.

Health and fertility variables

Changes in serum and milk variables mirrored the health status of dairy cows during the transition period and in early lactation (Table 16). The serum variable BHBA and the milk acetone content map ketotic states. Both variables showed no significant differences between treatments during early lactation. However, numerical increases in the BHBA serum concentration for TCLA were registered on lactation day 1. Both treatment groups reached the range indicating a ketotic state (1 mmol/l) on lactation days 7 and 14. Milk acetone contents did not indicate any subclinical states of ketosis, which is indicated at a concentration of 0.2 mmol/l. Fatty acid mobilization was significantly elevated ($P = 0.004$) in TCLA on parturition day and thereby surpassed the range (600 µmol/l) indicating pathophysiological fat mobilization (Table 16).

The glucose concentration in serum showed numerically higher values in CON than in TCLA throughout the observation period. Differences in the glucose concentration between treatments were significant on parturition day and on d 14 p.p. Reductions in the serum glucose concentration occurred in TCLA earlier p.p. than in CON, and fell below the optimum range (3.0 to 3.3 mmol/l during lactation) on lactation d 14. Differences between treatments were significant ($P = 0.05$) for the period including the transition phase and the first 56 d in lactation. The changes in the IFG-I concentration in serum did not show any differences between treatments and levels were typical for the studied period of lactation.

Table 16. Development of serum and milk variables during the transition period in cows fed with (TCLA) or without (CON) 4.96 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA.

	Day relative to parturition								|p|
Variable	-18	-3	1	7	14	28	56	SEM	TRT
BHBA[1], mmol/l									
CON	0.918	0.673	0.753	1.044	1.023	0.795	0.689	0.0741	0.62
TCLA	0.788	0.723	0.961	0.969	1.163	0.882	0.762	0.0665	
FFA[1], µmol/l									
CON	102.1	246.4	500.8[b]	474.9	601.5	197.3	172.1	41.22	0.97
TCLA	102.8	155.1	834.2[a]	382.6	443.9	165.5	195.4	38.82	
GLUCOSE[1], mmol/l									
CON	3.65	3.91	4.03[a]	3.15	3.27[a]	3.41	3.17	0.097	0.05
TCLA	3.53	3.62	3.35[b]	3.11	2.84[b]	3.22	3.08	0.091	
IGF-I[1], ng/ml									
CON	152.3	116.7	63.0	51.6	65.5	80.3	85.5	8.50	0.92
TCLA	147.6	124.1	41.8	57.7	66.1	81.7	94.6	8.02	
MILK ACETONE[2], mmol/l									
CON	-	-	0.011	0.120	0.040	0.035	0.016	0.0191	0.86
TCLA	-	-	0.011	0.035	0.111	0.017	0.006	0.0187	

[1] Variable measured in serum

[2] Variable measured in milk

a,b Different letters indicate significant ($P < 0.05$) treatment effects on a specific observation day for the measured variable.

Signs of heat were registered and insemination data were evaluated throughout the trial. No significant differences were observed in this data with respect to services per conception, days to first service and days open as shown in Table 17.

Table 17. Least square means (LSM) and standard error (SE) for observational variables of fertility in cows fed without (CON) and with 4.96 g/d each of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA during the transition period and first 80 days of lactation (TCLA).

	CON		TCLA		
Variable	LSM	SE	LSM	SE	P-value
SPC[1]	2.75	0.46	2.22	0.43	0.41
DFS[2], day	133.3	18.9	107.3	17.8	0.33
DO[3], day	83.3	11.5	64.2	10.8	0.25

[1] Values represent LSM of services per conception

[2] Values represent LSM of days to first service

[3] Values represent LSM of days open

DISCUSSION

As in the present study, several other studies (Bernal-Santos et al., 2003; Moore et al., 2004; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; Sigl et al., 2010; von Soosten et al., 2011; Schlegel et al., 2012) fed mixed CLA products including *trans*-10, *cis*-12 CLA during the transition period and early lactation. Between studies, differences in the duration of the treatment period, the amount of *trans*-10, *cis*-12 CLA fed and the composition of rations fed occurred. However, all studies started between 28 and 9 d a.p. and were conducted until 21 and 140 d p.p. The fed amount (4.96 g/d) of *trans*-10, *cis*-12 CLA in the present study was at the bottom of the range, which varied between studies from 3.8 to 18 g/d and showed dose-dependent MFD effects. The low rate of total CLA recovery from the concentrate was the main reason why such a low amount of *trans*-10, *cis*-12 CLA was fed in the present study. Yang et al. (2000), Lee et al. (2003) and Moon et al. (2008) have suggested that CLA as a whole is extremely unstable in air and decomposes rapidly. The high temperature and pressure during the pelleting process further reduced the amount of CLA methylesters analyzed in concentrate, reducing the initially calculated daily intake of 10 g/d *trans*-10, *cis*-12 CLA to less than half.

General differences in the type of ration fed were observed between the present study and the aforementioned studies. The ration in the present study was based on grass and corn silage, upgraded with additional concentrate and protein sources (soybean meal and rapeseed

meal). These are diets common for the grass-based dairy cow regions in Central Europe. The rations fed in most of the aforementioned studies were based on components typical for crops grown in regions in the USA. These diets included alfalfa hay, corn silage, legume silage, corn (meal, steam flaked), citrus pulp, soybean meal and cotton seed. Because alfalfa hay is highly digestible and corn silage in the USA is commonly fed in higher amounts than in Europe, the rumen environment and nutrient degradation in the digestive tract are likely to be different between studies. As CLA supplements mainly are fed as Ca salts, which dissociate in environments with a low pH, the type of ration could have an effect on CLA-induced MFD if the rumen stability of the product is not given.

In the study of Moore et al. (2004), 5 g/d *trans*-10, *cis*-12 CLA was equivalent with a 13% reduction of milk fat content from wk 1 to 3 of lactation. Schlegel et al. (2012) showed that 3.8 g/d *trans*-10, *cis*-12 CLA induces during week 5 of lactation a 14% reduction of milk fat content, indicating that lower amounts of *trans*-10, *cis*-12 influenced mammary synthesis. Reductions in milk fat yield amounted to 9.7% in the present study and 12% in Moore et al. (2004) during the first three weeks of the trial. Schlegel et al. (2012) reported a 12.5% reduction of milk fat yield during week 5 of lactation. This indicates similar effects of CLA supplementation on milk fat synthesis regardless of the diet during the start of lactation. With progressing lactation however, milk yields elevated in TCLA and reductions in milk fat yield were nullified. The alterations in milk yield due to CLA supplement observed in the present study are not supported by the studies of Moore et al. (2004), Selberg et al. (2004), Castañeda-Gutiérrez et al. (2005) ; Odens et al. (2007) and von Soosten et al. (2011). Schlegel et al. (2012) observed a positive alteration in milk yield in week 14 of lactation. The duration of treatment with the CLA product in early lactation cows seemed to have an influence on alterations in natural milk yield. Bernal-Santos et al. (2003) observed trends for elevated milk yields as well. Animals in that study were fed with the CLA product throughout the transition period until they reached balanced energy states. Bernal-Santos et al. (2003) observed a 3 kg/d increase in natural milk yield during the first 20 wk of lactation and a decrease in milk fat percentage of 12% (similar to the present study). Milk fat yield was only reduced by about 7.5%, which is similar to the 9% registered during the treatment period in the present study. It can be speculated that ingested nutrients might influence elevations in milk yield. The energy density of the rations seems to be without influence on nutrient repartitioning toward milk yield, because Odens et al. (2007) fed a ration only containing 5.6 MJ NEL/kg DM a.p. and 5.8 MJ NEL/kg DM p.p., whereas the diets fed by Selberg et al. (2004) had an energy content of 6.45 MJ NEL/kg DM and 6.9 MJ NEL/kg DM p.p.; neither study indicated a trend for

increased milk yields. However, they stopped CLA supplementation before lactation d 50, a stage at which animals still are in negative EB (Coffey et al. 2002, 2004; Banos et al. 2005), suggesting that nutrient repartitioning toward elevated milk yields was not implemented yet. The mechanisms responsible for inducing nutrient repartitioning have yet to be identified.

Variations occurred in the CP content in rations fed during the transition period in the literature studies (12.1 to 18.8%) and in the present study (14.1%). During lactation, the CP content in the rations amounted to 17.5% in present study, and 16.4 to 19% in the literature. Differences in nitrogen supply could have influenced the availability of precursors for microbial protein and mammary protein synthesis and may have influenced 1) nutrient digestion and degradation and 2) milk component synthesis. De Veth et al. (2006) altered dietary protein but found no effect on milk yield and milk fat protein content and yield in CLA fed dairy cows. On the other hand, Kay et al. (2007) provided information that CLA supplements may be an alternative for enhancing protein synthesis and improving the milk protein to fat ratio and calculated net EB in cows grazing in pasture. In the present study, TCLA showed a trend for reduced milk protein content, but milk protein yield was equal between treatments, due to elevated milk yields fluidizing the milk protein content.

EB seems to play an important role in mechanisms of CLA-induced nutrient repartitioning. During the first four weeks of lactation, adipose tissue mobilization overrules the MFD action of CLA, resulting in a lag in the response. These effects are due, according to Harvatine et al. (2008), to a reduced sensitivity of gene receptors encoding for key enzymes during this stage of lactation. The closer EB approaches a balanced state, the less these receptors for milk fat synthesis in the mammary gland are blocked. Because increases in milk yield were apparent following wk 5, this led to the suggestion that when the metabolism overcomes severe stress due to negative EB and tissue mobilization, nutrients are repartitioned into milk yield, resulting in equal EB between treatments. Liermann et al. (2008) reported the beneficial effects of CLA-induced MFD on EB in early lactation cows. However, animals in that trial yielded less milk while ingesting the same amount of DM, suggesting that cows were under less metabolic stress. Then the energy spared from MFD could have been retained in adipose tissue and improved the EB. Schlegel et al. (2012) reported positive effects on EB starting in wk 5 of lactation. Reasons can be found in a trend to higher DMI, while increases in milk yield first became significant by week 14 of lactation. Harvatine et al. (2009) reported that CLA doses that decreased milk fat yield by 38% and milk fat content by 34% led to an increased expression of lipid synthesis regulating enzymes in adipose tissue. Von Soosten et al. (2011) found no effects of CLA on the weights of liver, omental,

mesenteric, or s.c. adipose depots. But they suggested a CLA induced deceleration of mobilization of the retroperioneal adipose depot during the first 42 DIM. Hepatic expression of gene involved in dairy cows lipid metabolism are not influenced by a mixed CLA product (Schlegel et al., 2012). Further studies are needed to evaluate the mechanisms of CLA-induced nutrient repartitioning and to identify the factors that influence different pathways of nutrient redirection.

Reproductive performance has been highly linked to the extent and timing of the EB nadir (Lucy et al., 1992; Beam and Butler et al., 1999). Because no differences in the development of EB were observed, no effects on fertility were expected. This theory is supported by the equal levels of serum IGF-I concentration between treatments in the present study. However, Castañeda-Gutiérrez et al. (2007) detected greater plasma IGF-I concentrations by top dressing 7.1 g/d of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA to cows between lactation d 20 and d 57, but they did not observe differences in EB and plasma NEFA. Taylor et al. (2004) reported that cows were more likely to conceive with greater IGF-I plasma concentrations during the first 12 wk p.p. than with lower levels. The review of Bauman et al. (2011) reported that IGF-I was not influenced by CLA. Comparing the biological observations of animals between treatments in the present study, these data revealed interesting numerical differences between treatments. CLA fed cows had fewer days to first service than CON. The same was observed for the days open, resulting in numerically fewer services per conception needed in the TCLA group. Previous studies by Bernal-Santos et al. (2003) and Castañeda-Gutiérrez et al. (2005) support these findings. An explanation might be found in a report by Harvatine et al. (2009), who noted that dairy cattle adipose tissue is stimulated for lipid synthesis by *trans*-10, *cis*-12 CLA supplements. Because adipose tissue is an important site for the hormonal control of fertility variables, interactions between adipose tissue, fertility variables and *trans*-10, *cis*-12 CLA have to be clarified in the future.

The metabolic health of dairy cows in early lactation is a subject of great interest regarding overall lactation productivity. However, early lactation dairy cows react very sensitively to variations in nutrition and metabolic stress. Serum concentration of BHBA and FFA are indicators of the status of metabolism. The FFA concentration in serum is a reliable index of the magnitude of adipose tissue mobilization (Baumann et al., 1988). In the present study, no differences were observed in adipose tissue mobilization and ketone bodies in serum prior to parturition. This is consistent with earlier study by Bauman et al. (2011). On parturition day, however, adipose tissue mobilization increased significantly in CLA fed animals, supporting the findings of Selberg et al. (2004), thus suggesting that the CLA

product might influence lipolysis with the onset of lactation. During the following weeks, the serum FFA level in TCLA stayed below that of CON, indicating reduced adipose tissue mobilization. The BFT of TCLA showed numerically greater fat layers, which is supported by the findings of Harvatine et al. (2009) who showed that adipose tissue is stimulated to deposit fat in lactating dairy cows if they are fed *trans*-10, *cis*-12 CLA. Studies observing the health of dairy cows in detail during early lactation are needed to provide insight into the alterations of fat metabolism under the influence of *trans*-10, *cis*-12 CLA around the parturition period, and may lead to more profound conclusions about the health status of dairy cows.

Significant reductions in the serum glucose concentration after parturition were observed in CLA treated cows up to lactation d 56. This could indicate higher tissue nutrient uptake or a higher nutrient turnover. However, these findings are in contrast to the findings of Moore et al. (2004); Castañeda-Gutiérrez et al. (2005) and Selberg et al. (2005). Increased glucose concentrations were observed by Odens et al. (2007). Since this variable is very sensitive to the nutritional status of animals, time differences at sample collection between studies could have had an influence.

CONCLUSION

No differences in the mode of CLA action were found between the present study and the literature data from American studies. However, several questions arose regarding the influence of EB on the severity of CLA-induced MFD and the resulting metabolic changes. Numerical changes in improved fertility and BFT may be an indicator that nutrient repartitioning in CLA supplemented rations had other effects beyond improved milk yield. It remains to be examined under which conditions nutrients are repartitioned to which site of metabolism.

REFERENCES

Anonymous, 2010. Tierschutzgesetz in der Fassung der Bekanntmachung vom 18. Mai 2006 (BGBl. I S. 1206, 1313), das zuletzt durch Artikel 20 des Gesetzes vom 9. Dezember 2010 (BGBl. I S. 1934) geändert worden ist Fünfter Abschnitt, Tierversuche §7-§9a.

ARC. 1980. The Nutrient Requirements of Ruminant Livestock. pp. 351. Commonwealth Agriculture Bureau, London.

Banos, G., M. P. Coffey, and S. Brotherstone. 2005. Modeling daily energy balance of dairy cows I the first three lactations. J. Dairy Sci. 88:2226-2237.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, Rumen-Derived Bioactive Fatty Acids, and the Regulation of Milk Fat Synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman, D. E., C. J. Peel, W. D. Steinhour, P. J. Reynolds., H. F. Tyrrell, A. C. G. Brown, and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: Influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031-1040.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769.

Baumgard. L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278:R179-R184.

Beam, S. W. and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertility 54:411-424.

Bernal-Santos, G., J. W. Perfield II, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Butler, S. T., S. H. Pelton, and W. R. Butler. 2004. Insulin increases 17 beta-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction 127:537-545.

Castañeda-Gutiérrez, E., B. C. Beefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253-4264.

Castañeda-Gutiérrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Chwaligbog, A. 2000. Husdyrernaering, Bestemmelse af naeringsvaerdi og naeringsbehov. 5th ed., DSR Forlag, Copenhagen, pp.123.

Coffey, M. P., G. Simm, J. D. Oldham, W. G. Hill and S. Brotherstone. 2004. Genotype and diet effects on energy balance in the first three lactations of dairy cows. J. Dairy Sci. 87:4318-4326.

Coffey, M. P., G. Simm, and S. Brotherstone. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. J. Dairy Sci. 85:2669-2678.

De Veth, M. J., E. Castañeda-Gutiérrez, D. A. Dwyer, A. M. Pfeiffer, D. E. Putnam, and D. E. Bauman. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J. Dairy Sci. 89:4620-4631.

De Veth, M. J., S. K. Gulati, N. D. Luchini, and D. E. Bauman. 2005. Comparison of calcium salts and formaldehyde-protected conjugated linoleic acid in inducing milk fat depression. J. Dairy Sci. 88:1685-1693.

Enright, W. J., L. T. Chapin, W. M. Moseley, S. A. Zinn, M. B. Kamdar, L. F. Krabill, and H. A. Tucker. 1989. Effects of infusions of various doses of bovine growth hormone-releasing factor on blood hormones and metabolites in lactating Holstein cows. J. Endo. 122:671-679.

GfE. 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie, Frankfurt am Main, Germany. DLG-Verlag.

Harvatine, K. J., J. W. Perfield II, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-54.

Harvatine, K. J., Y. R. Boisclair, and D. E. Bauman. 2008. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Harvatine, K. J. and D. E. Bauman. 2006. SREBP-1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136: 2468-2474.

Hutchinson, I., M. J. de Veth, C. Stanton, R. J. Dewhurst, P. Lonergan, A. C. O. Evans, and S. T. Butler. 2011. Effects of lipid-encapsulated conjugated linoleic acid supplementation on milk production, bioenergetic status and indicators of reproductive performance in lactating dairy cows. J. Dairy Res. 78:308-317.

Kay J. K., R. R. Mackle, D. E. Bauman, N. A. Thomson, and L. H. Baumgard. 2007. Effects of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. J. Dairy Sci. 90:721-730.

Kirchgessner, M. 1997. Tierernährung, Leitfaden für Studium, Beratung und Praxis.10th ed., VerlagsUnionAgrar, Frankfurt/Main, Germany. pp. 126.

Lee, J., S-M. Lee, I-H. Kim, J-H. Jeong, C. Rhee, and K-W. Lee. 2003. Oxidative instability of CLA concentrate and its avoidance with antioxidants. J. Am. Oil Chem. Soc. 80:807-810.

Liermann, T., A.-M. Pfeiffer, and F. J. Schwarz. 2008. Effects and post-effects on performance and metabolic parameters of early lactation dairy cows to dietary rumen-protected CLA and rumen protected fat. Proc. Soc. Nutr. Physiol. 17:30.

Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry matter, milk production and energy balance on rime of postpartum ovulation and fertility in dairy cows. Anim. Prod. 54:323-331.

Moon, H. S., H. G. Lee, C. S. Chung, Y. J. Choi, and C. S. Cho. 2008. Physico-chemical modifications of conjugated linoleic acid for ruminal protection and oxidative stability. Nutr. Metab. (Lond). 1:5-16.

Moore, C. E., H. C. Haflinger, O. B. Mendivil, S. R. Saders, D. E. Bauman, and L. H Baumgard. 2004. Increasing amounts of conjugated linoleic acid progressively reduces milk fat synthesis immediately postpartum. J. Dairy Sci. 87:1886-1895.

Nauman, C., R. Bassler, R. Seiblod, and C. Barth. 2004. Verband Deutscher Landwirtschaflicher Untersuchungs- und Forschungsanstalten; Methodenbuch, Band III/1, Die chemischen Untersuchungen von Futtermitteln. Dritte Auflage mit 5. Ergänzungslieferung, VDLUFA-Verlag-Darmstadt.

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. J. Dairy Sci. 90:293-305.

Pappritz, J., U. Meyer, R. Kramer, E.-M. Weber, G. Jahreis, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effects of long-term supplementation of dairy cow diets with rumen-protected conjugated linoleic acids (CLA) on performance, metabolic parameters and fatty acid profile in milk fat. Archives of Animal Nutr. 65:89-107.

Perfield II J. W., P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2006. Trans-10, trans-12 conjugated linoleic acid does not affect milk fat yield but reduces delta9-desaturase index in dairy cows. J. Dairy. Sci. 89:2559-2566.

Peterson, D. G., L. H. Baumgard, and D. E. Bauman. 2002. Short communication: milk fat response to low doses of trans-10, cis-12 conjugated linoleic acid (CLA). J. Dairy Sci. 85:1764-1766.

Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Ku Fritsche, 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.

SAS/STAT. 2001. Changes and Enhancements, Release 8.2. SAS Inst., Cary, NC.

Schlegel, G., R. Ringseis, W. Windisch, F. J. Schwarz, and K. Eder. 2012. Effects of a rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows. J. Dairy Sci. 95:3905-3918.

Selberg, K. T., C. R. Staples, N. D. Lucini, and L. Badinga. 2005. Dietary trans octadeceoic acids upregulate the liver gene encoding peroxisome proliferators-activated receptor-alpha in transition dairy cows. J. Dairy Res. 72:107-114.

Selberg, K. T., A. C. Lowe, C. R. Staples, N. D. Luchini, and L. Badinga. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J. Dairy Sci. 87:158-168.

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer and, M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scan. 52:16.

Staufenbiel, R. 1992. Energie- und Fettstoffwechsel des Rindes- Untersuchungskonzept und Messung der Rückenfettdicke. Mh. Vet.-Med. 47:467-474.

Taylor, V. J., Z. Cheng, P. G. Pushpakumara, D. E. Beever, and D. C. Whates. 2004. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Vet. Rec. 155:583-588.

Viswanadha, S., J.G. Giesy, T.W. Hanson, M.A. McGuire. 2003. Dose response of milk fat to intravenous administration of the trans-10, cis-12 isomer of conjugated linoleic acid. J. Dairy Sci. 86:3229-3236.

Von Soosten, D., U. Meyer, E.M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J. Dairy Sci. 94:2859-2879.

Yang, L., L. K. Leung, Y. Huang, and Z. Y. Chen. 2000. Oxidative stability of conjugated linoleic acid isomers. J. Agric. Food Chem. 48:3072-3076.

6 GENERAL CONCLUSIONS

The studies in the present thesis were conducted at the research centre “Haus Riswick” in North Rhine Westfalia, Germany in order to elucidate how dietary supplements containing the two conjugated linoleic acid (**CLA**) isomers *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, the latter of which is a milk fat depressing (**MFD**) agent (Baumgard et al., 2001, 2000; Baumann et al., 2000, 2001, 2003), act in rations typical for Central European diets. The rations fed in the two experiments described in this thesis were comparable with each other. Literature data evaluating the effect of CLA supplements on milk yield and milk components have mostly been performed by American authors feeding diets typical of that part of the world (Bernal-Santos et al., 2003; Moore et al., 2004, Castañeda-Gutiérrez et al. 2005, 2007; De Veth et al. 2005). Studies evaluating the effect of *trans*-10, *cis*-12 CLA as an MFD agent were mainly conducted in dairy cows for a limited period, either in late lactation (Perfield et al., 2002, 2004; Peterson et al., 2003; De Veth et al., 2005, 2006; Gervais et al., 2005; Moore et al., 2005; Kay et al., 2007) or during the transition period and early lactation (Bernal-Santos et al. 2003; Moore et al., 2004; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Odens et al., 2007; von Soosten et al., 2011; Schlegel et al., 2012). Castañeda-Gutiérrez et al., (2007) and Pappritz et al., (2011) monitored the progress of mammary effects by starting CLA supplementation after parturition. One of the present studies was the first to investigate the influence of varying CLA product supplementation periods in early lactation upon the progress of milk yield and components during the treatment and post-treatment periods throughout the preceding lactation with the aim of providing information on the ideal supplementation period and for practical applications. The other study compared the nutritional effects of typical grass-based rations found in Central Europe with the results from North American studies.

Multiparous cows fed the CLA product during the transition period showed significant MFD ($p < 0.05$) sooner (3 wk after parturition) than multiparous cows fed the CLA product starting after the initiation of lactation (wk 4 to 5 after the start of the trial). The lag in the response during early lactation was not due to *trans*-10, *cis*-12 CLA not reaching the mammary gland, as shown by the results of milk fatty acid analysis and confirmed by the results of Bernal-Santos et al. (2003). Cows fed the CLA product during the transition period showed a trend for decreased milk fat content and yield during very early lactation. This was not apparent in cows fed the CLA product starting at parturition. Moore et al. (2004) showed that the insensitivity of the mammary gland during early lactation can be overcome by increasing the amount of *trans*-10, *cis*-12 CLA supplemented during the transition period and early lactation. Greater amounts are assumed to be more effective, since it is hypothesized

that CLA isomers compete with NEFA for epithelial uptake. Furthermore, CLA is believed to alter essential cellular signaling systems during early lactation, whereby *trans*-10, *cis*-12 CLA is unable to reduce the expression of mRNA encoding for key enzymes involved in mammary fat synthesis. Moore et al. (2004) observed that differences in MFD were apparent from the first day in milk (**DIM**) and became significant different by 5 DIM, if 15 g/d *trans*-10, *cis*-12 CLA was supplemented. Sigl et al., (2010), who fed 3.75 g *trans*-10, *cis*-12 CLA two weeks prior to parturition and 10 g *trans*-10, *cis*-12 CLA four weeks after parturition, observed no effects on milk fat secretion at all, indicating that 3.75 g *trans*-10, *cis*-12 CLA was too low to inflict an early effect. The early reductions of Moore et al. (2004) could not be confirmed by Selberg et al. (2004) and Castañeda-Gutiérrez et al. (2005). They observed significant reductions in the milk fat content first after the fourth week or during the third week of lactation, respectively (supplementing 12 g/d and 18 g/d *trans*-10, *cis*-12 CLA, respectively, during the transition period and early lactation). Similar amounts of the CLA product fed in both experiments induced comparable maximum MFD within the multiparous CLA treated groups. However, if the CLA product was fed during the transition period, the nadir was reached around lactation wk 6, while it took cows fed the CLA product starting at parturition 13 weeks to reach the nadir. Animals treated for 80 days with the CLA product starting at parturition did not reach the minimum nadir. This supports the hypothesis that reduced sensitivity of CLA receptors in the mammary gland during lactogenesis can be overcome by supplementing CLA products during the transition period.

The withdrawal of the CLA product during established lactation results in a lag in the return of milk fat contents to values seen in the multiparous control. Cows fed CLA for 80 days starting after parturition showed a lag in recovery of one week, while animals fed for 120 d after parturition or during the transition period and the first 80 days of lactation showed prolonged (2 weeks longer) reduced milk fat content. In the case of the 120 d feeding interval, the prolonged period of recovery was due to the energy balance (**EB**) returning back to positive values before stopping CLA supplementation; consequently, milk fat depressing isomers were incorporated in greater amounts into adipose tissue and circulated after the cessation of CLA product delivery due to lipid turnover for a longer period of time in plasma, also impacting the mammary gland. In the experiment in which the CLA product was fed during the transition period, EB in the treatment groups was balanced sooner than the group of multiparous cows fed the CLA product starting at lactation for 120 d. Especially the *trans*-10, *cis*-12 isomer is concentrated in the subcutaneous fat tissue (Mir et al., 2004) in ruminants. Clement et al. (2002) showed in mice that the disappearance of CLA in the neutral lipid

fraction of the mammary tissue after discontinuing CLA reached basal values 4 weeks later. This indicates that isomers are removed from fat depots set up prior to discontinuing CLA supplementation.

The ability of CLA products containing the *trans*-10, *cis*-12 isomer in inducing MFD is well-described and was confirmed by the present studies. Beyond the validation of the effect on MFD, the present studies were conducted in order to investigate the possible effects of the CLA product on EB and animal health during early lactation. The data in the literature provide evidence that CLA-induced MFD could positively influence the EB due to decreased energy output with milk (Bernal-Santos et al., 2003; Moore et al., 2005; Odens et al., 2007; Liermann et al., 2008; Schlegel et al., 2012). However, the present studies provided no evidence for a beneficial effect of CLA-induced MFD on EB in early lactation cows, similar to the findings of Moore et al. (2004) and von Soosten et al., (2011). Pappritz et al., (2011) observed a more severe negative EB in CLA fed cows during early lactation due to decreases in dry matter intake (**DMI**). No differences were observed in terms of the time of initiation for CLA supplementation on EB. Since EB is the difference between energy intake and energy expenditure for maintenance and performance, a depression in milk fat content can be overcome by increased energy requirements due to alterations in the synthesis of other milk components. Both present studies revealed (in multiparous cows) no difference between animals treated with or without the CLA product in terms of the yield of milk fat, milk protein and milk lactose (Table 18), resulting in equal amounts of energy corrected milk (**ECM**) yield. Since DMI was equal within the respective trials for the treatment groups, no differences in EB could be expected. Castañeda-Gutiérrez et al. (2007) found similar results in cows started on CLA treatment during early lactation. The question that remains to be answered is why some dairy cows supplemented with MFD CLA products show a repartitioning of nutrients leading to elevated milk yields, and why others do not.

The beneficial effects of CLA-induced MFD on EB in early lactation cows depend on the enhancement of animal productivity, since metabolic disorders such as fatty liver disease and ketosis often accompany a negative EB (Grummer, 1993). The EB results were confirmed by the non-existent differences in the milk acetone concentration and beta-hydroxybutyrate (**BHBA**) concentration in serum, indicating no influence of CLA product supplementation on ketosis during different early lactation periods which is consistent with the report of Bauman et al., (2011). However, fat mobilization in cows fed CLA during the transition period was significantly increased ($p<0.05$) on parturition day (plasma non-esterified fatty acids; **NEFA**), but these animals showed decreased fat mobilization shortly before parturition and between

lactation d 7 and 14. The latter results may be explained by the findings of Harvatine et al. (2009) that lipogenesis in lactating dairy cows is increased due to short-term *trans*-10, *cis*-12 CLA-induced MFD. Selberg et al. (2004) investigated the development of plasma BHBA and NEFA and found, for both variables, significant increases on parturition day if 12 g/d *trans*-10, *cis*-12 CLA was fed starting in the transition period.

Table 18. Least square means and standard error (SE) of milk and energy variables during the first 100 trial days in multiparous cows treated with (TCLA, CLA80, CLA120) or without (CON, control) conjugated linoleic acid supplement either starting during the transition period or starting during early lactation in the transition period and in the main experiment (multiparous).

	Transition period			Early lactation			
	Treatment			Treatment			
	CON	TCLA	SE	Control	CLA80	CLA120	SE
Milk variable							
Milk, kg/d	38.1	42.3	1.61-1.70	39.1	41.7	41.0	1.4
Fat, %	3.84	3.27	0.12	4.06	3.99	3.66	0.21
Fat, kg/d	1.44	1.36	0.09	1.57	1.62	1.47	0.08
Protein, %	3.26	3.14	0.05	3.14a	3.07ab	2.97b	0.05
Protein, kg/d	1.23	1.32	0.04	1.22	1.26	1.21	0.04
Lactose, %	4.81	4.74	0.04	4.77	4.75	4.71	0.04
Lactose, kg/d	1.79	1.96	0.08	1.87	1.99	1.94	0.07
ECM, kg/d	36.8	37.7	1.8-1.9	38.4	40.1	37.9	1.4
Energy variables							
DMI, kg/d	20.2	20.9	0.8-0.9	19.3	18.6	18.6	1.0
LW, kg	649	660	17-18	662	683	669	13.6-14.1
EB, MJ NEL	-13.4	-12.8	4.3-4.5	-24.7	-35.2	-28.3	8.5-8.7

a,b different letters within a row and experiment show differences between treatment groups of an experiment

DMI= Dry matter intake; LW= Live weight; EB= Energy balance; NEL= Net energy lactation

Other studies in early or late lactation have not shown any differences in NEFA caused by treatment with CLA (Bernal-Santos et al., 2003; Moore et al., 2004; Castañeda-Gutiérrez et al., 2005, 2007; Kay et al., 2007). Odens et al. (2007), however, observed general decrease in concentrations of plasma NEFA in CLA treated groups, while glucose was increased, corresponding to simultaneously beneficial effects on EB. It remains to be clarified if these effects depend on the amount of CLA, the point of initiating CLA supplementation or if other dietary or genetic effects are responsible for differences in repartitioning and the metabolic response.

The response of primiparous cows fed the CLA product starting after parturition were not comparable to that of multiparous cows treated in the same way, nor with multiparous cows fed the CLA product during the transition period. These responses differed with respect to the synthesis of milk fat and milk yield. It is not clear which mechanisms are responsible for these actions, but suggestions have been made that the sensitivity of regulatory mechanisms in nutrient repartitioning is different in animals experiencing lactogenesis for the first time than in multiparous cows. On the contrary, Von Soosten et al., (2011), used only primiparous cows in their trial and reported CLA effects on milk fat secretion as expected for multiparous cows.

It might be suggested that increasing the amount of the MFD CLA isomer supplemented during the periparturient period would have an effect on EB and therefore alter the health and possibly also fertility variables in multiparous cows. Moore et al. (2004) observed that increasing amounts of *trans*-10, *cis*-12 CLA fed during the transition period progressively reduced the milk fat content immediately postpartum. Amounts of 10 and 15 g of *trans*-10, *cis*-12 CLA per day decreased milk fat synthesis with apparent effects at d 1 of lactation, while significant differences occurred by 5 DIM and became more pronounced as DIM increased. EB in these two groups was not as negative and returned back to positive values earlier than in the group fed 5 g of *trans*-10, *cis*-12 CLA or the control group. A low dosage of 3.75 g fed by Sigl et al., (2010) two weeks prior to parturition did not elucidate any effects on milk fat secretion in early lactation. In light of these findings, it could be speculated that supplementing 10 to 15 g of *trans*-10, *cis*-12 CLA during the transition period could overcome the health issues brought on by lactogenesis, and should proceed for three weeks of lactation at a reduced amount (5 g) of the MFD CLA isomer. However, the results on MFD generated by Moore et al. (2004) could not be supported to the same extent by Castañeda-Gutiérrez et al. (2005). They observed a significant difference ($p < 0.05$) by lactation wk 3 between the group fed 18 g of *trans*-10, *cis*-12 CLA and the control group. However, the

response in terms of milk fat synthesis was dose-dependent and occurred for both milk fat content and yield, implying benefits on EB. Providing increased amounts of *trans*-10, *cis*-12 CLA during the transition period could be followed by beneficial effects on EB, followed by reduced problems in both health and fertility, since they rely on EB (Grummer, 1993; Macmillan et al., 1996; Beam and Butler, 1999). Further studies evaluating the factors influencing the response of cows in the periparturient period on CLA-induced MFD and milk yield have to be conducted in order to predict the benefits of CLA supplementation. If these problems are overcome, CLA supplementation could be a tool to influence health and fertility in fresh lactation dairy cows.

In addition to the issues discussed above, a further aim of the study was to evaluate if supplementation of mixed CLA isomers could be utilized as a quota optimization tool by reducing the milk fat content. The results shown in Table 18 were used to calculate the potential for optimization. Based on a fictional quota of 1,000,000 kg milk with 4% fat, it was calculated that, in the transition trial, the fat quota would be sub-delivered with 1600 kg of fat in the control group equaling 28,800 l of milk using the Hansa-Milch AG (Upahl, Germany) correction factor of 0.18. In TCLA group the fat quota would be sub-delivered with 7300 kg of fat equaling 131,400 l of milk. But the raise in milk yield over-compensated the fat benefit. The statement is confirmed by the development of ECM yield respectively. Results of the long term study undermine the statement that a quota optimization is not reached by mixed CLA supplementation. Following conclusions can be drawn by the statements above:

- Feeding mixed CLA to early lactation cow's results in a milk fat depression. However, the reduction occurs with a lag in time, compared to literature data on cows in established gestation.
- The milk fat depression occurs faster in cows fed the mixed CLA product during late gestation than if the product is fed with onset of lactation
- The improvement in CLA supplementation lies in the trend for increased daily milk production with greater persistence throughout lactation. With a greater increase if the product was fed during late gestation
- A monetary validation of the long term study reveals that the improvement of 2.8 kg of milk per day in CLA-fed multiparous cows (Table 9) amounts to 854 kg for a 305 day lactation period (suggesting that the persistence in milk yield increase is held throughout 305 d of lactation). This yields €290.00 more per cow per lactation if the milk price is €0.34 per liter, averaging 2011 in Lower

Saxony. The effectivity is further improved by a reduced need of nearly 8 cows in a herd of 100 cows (10,000 liters per cow per year) to fulfill a milk quota of 1,000,000 kg. Money is saved by 8.5% lower feed costs, since DMI was identical between the trial groups; simultaneously, costs decrease for housing and the replacement of cows.

- Last but not least, it was shown that, while feeding the CLA product during the transition period, milk yield was improved. This indicates a monetary benefit when investing in early supplementation with a CLA product, particularly since fertility was numerically improved. The number of inseminations needed for a successful pregnancy was decreased by 0.5 inseminations. This led to a 26 d shorter interval between parturition and new gestation. Supplementation with mixed CLA makes the cows more efficient and provides additional value through improved fertility. The latter has to be confirmed in cows fed with a mixed CLA product starting after parturition.

CONCLUSION

It has been shown that supplementation with a CLA product during the transition period results in earlier milk fat content reductions with an earlier approach to the nadir in fresh lactating cows, as seen in cows fed the CLA product from parturition day. Less energy is repartitioned in cow milk fat synthesis in this group. However, no benefits to EB were observed in these trials as spared nutrients were repartitioned into increased milk yields with better persistence throughout lactation. The cessation of CLA supplementation was followed by a lag in the milk fat content response, which depended on the energetic status prior to CLA cessation. The amount of the *trans*-10, *cis*-12 CLA isomer incorporated into adipose tissue prior to withdrawal of the CLA product explains this lag time. The benefits of CLA product supplementation during the transition period on fertility variables remain to be verified in further trials.

REFERENCES

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutr. 23:203-227.

Bauman, D. E. and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Prod. Sci. 70:15-29.

Bauman, D. E. and J. M. Griinari. 2000. Historical perspective and recent developments in identifying the cause of diet-induced milk fat depression. Proceedings of the Cornell Nutrition Conference. pp. 191-202 Cornell University, Rochester, NY.

Baumgard, L. H., J. K. Sangster, and D. E. Bauman. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769.

Baumgard. L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278:R179-R184.

Beam, S. W., and W. R. Butler. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertility 54:411-424.

Bernal-Santos, G., J. W. Perfield II, D. M. Barbano, D. E. Bauman, and T. R. Overton. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Castañeda-Gutiérrez, E., B. C. Beefield, M. J. de Veth, N. R. Santos, R. O. Gilbert, W. R. Butler, and D. E. Bauman. 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy cows. J. Dairy Sci. 90:4253-4264.

Castañeda-Gutiérrez, E., T. R. Overton, W. R. Butler, and D. E. Bauman. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Clement, L., H. Poireier, I. Niot, V. Bocher, M. Guerre-Millo, S. Krief, B. Staels, and P. Besnard. 2002. Dietary trans-10, cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J. Lipid Res. 43:1400-1409.

De Veth, M. J., E. Castañeda-Gutiérrez, D. A. Dwyer, A. M. Pfeiffer, D. E. Putnam, and D. E. Bauman. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J. Dairy Sci. 89:4620-4631.

De Veth, M. J., S. K. Gulati, N. D. Luchini, and D. E. Bauman. 2005. Comparison of calcium salts and formaldehyde-protected conjugated linoleic Acid in inducing milk fat depression. J. Dairy Sci. 88:1685-1693.

Gervais, R., R. Spratt, M. Líonard, and P. Y. Chouinard. 2005. Lactation response of cows to different levels of ruminally inert conjugated linoleic acids under commercial conditions. Can. J. Anim. Sci. 85:231-242.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Harvatine, K. J., J. W. II Perfield, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-54.

Kay J. K., R. R. Mackle, D. E. Bauman, N. A. Thomson, and L. H. Baumgard. 2007. Effects of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. J. Dairy Sci. 90:721-730.

Liermann, T., A.-M. Pfeiffer, and F. J. Schwarz. 2008. Effects and post-effects on performance and metabolic parameters of early lactation dairy cows to dietary rumen-protected CLA and rumen protected fat. Proc. Soc. Nutr. Physiol. 17:30.

Macmillan, K. L., I. J. Lean, and C. T. Westwood. 1996. The effects of lactation on the fertility of dairy cows. Aust. Vet. J. 73:141-147.

Mir, P. S., T. A. McAllister, S. Scott, J. Aalhus, V. Baron, D. McCartney, E. Charmley, L. Goonewardene, J. Basarab, E. Okine, R. J. Weslake, and Z. Mir. 2004. Conjugated linoleic acid-enriched beef production. Am. J. Clin. Nutr. 79 (Suppl): 1270-1211.

Moore, C. E., J. K. Kay, R. J. Collier, M. J. Vanbaale, and L. H. Baumgard. 2005. Effect of supplemental conjugated linoleic acids on heat-stressed Brown Swiss and Holstein cows. J. Dairy Sci. 88:1732-1740.

Moore, C. E., H. C. Haflinger, O. B. Mendivil, S. R. Saders, D. E. Bauman, and L. H Baumgard. 2004. Increasing amounts of conjugated linoleic acid progressively reduces milk fat synthesis immediately postpartum. J. Dairy Sci. 87:1886-1895.

Odens, L. J., R. Burgos, M. Innocenti, M. J. VanBaale, and L. H. Baumgard. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. J. Dairy Sci. 90:293-305.

Pappritz, J., U. Meyer, R. Kramer, E.-M. Weber, G. Jahreis, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effects of long-term supplementation of dairy cow diets with rumen-protected conjugated linoleic acids (CLA) on performance, metabolic parameters and fatty acid profile in milk fat. Arch. Anim. Nutr. 65:89-107.

Perfield II, J. W., P. Delmonte, A. L. Lock, M. P. Yurawecz, and D. E. Bauman. 2004. Trans-10, trans-12 conjugated linoleic acid (CLA) reduces the Δ^9-desaturase index without affecting milk fat yield in lactating cows. J. Dairy Sci. 87 (Suppl.1):297(Abstract).

Perfield II, J. W., G. Bernal-Santos, T. R. Overton, and D. E. Bauman. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased trans-10, cis-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Schlegel, G., R. Ringseis, W. Windisch, F. J. Schwarz, and K. Eder. 2012. Effects of a rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows. J. Dairy Sci. 95:3905-3918.

Selberg, K. T., A. C. Lowe, C. R. Staples, N. D. Luchini, and L. Badinga. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J. Dairy Sci. 87:158-168.

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer and, M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scan. 52:16.

Von Soosten, D., U. Meyer, E.M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J. Dairy Sci. 94:2859-2879.

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