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Investigations on the effects of graded levels of rumen protected conjugated linoleic acids on dairy cow performance, fatty acid profile of milk and rumen metabolism

DISSERTATION

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ABBREVIATIONS

ACC	Acetyl CoA carboxylase
ADF	Acid detergent fiber
AGPAT	Acylglycerol phosohate acyltransferase
ALB	Albumin
AP	Amid-protected
a.p.	*Ante partum*
BCS	Body condition score
BF_3	Borontrifluoride
BFT	Back fat thickness
BHB	β-Hydroxybutyrate
BW	Body weight
CLA	Conjugated linoleic acids
CLP	Cytoplasmic lipid droplets
CM	Chylomicron
COX	Cylooxygenase
CPT	Carnitine palmitoyltransferase
Cr_2O_3	Chromium(III)oxide
CS	Calcium salts
DIM	Days in milk
DM	Dry matter
DMF	Dry matter flow
DMI	Dry matter intake
EFSA	European Food Safety Authority (Europäische Lebensmittelsicherheits-behörde)
EN	Endogenous N
EP	Endogenous crude protein
ER	Endoplasmatic reticulum
EU	European Union
FA	Fatty acid(s)
FABP	Fatty acid binding proteins
FAME	Fatty acid methyl ester
FAS	Fatty acid synthetase
FCM	Fat corrected milk
FLI	Friedrich-Loeffler-Institute
FOM	Fermented organic matter
FP	Formaldehyde-protected
FPR	Fat-to-protein-ratio
GC	Gas chromatography
GfE	German Society of Nutrition Physiology (Gesellschaft für Ernährungs-physiologie)
GPAT	Glycerol phosphate acyltransferase
HDL	High density lipoprotein
HPTLC	High-performance thin layer chromatography
i.d.	Inner diameter
Ig	Immunoglobulin
IL	Interleukin
IFN-γ	Interferon-γ
IGF-I	Insulin-like growth factor 1
LDL	Low density lipoprotein

LE	Lipid-encapsulated
Lp	Lipoprotein
LPL	Lipoprotein lipase
LS means	Least square means
LW	Live weight
ME	Metabolizable energy
MFD	Milk fat depression
MFG	Milk fat globule
MP	Microbial crude protein
MUFA	Monounsaturated fatty acids
NAN	Non-ammonia-N
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NEL	Net energy lactation
NIRS	Near infrared spectra
OM	Organic matter
PBMC	Peripheral blood mononuclear cells
PMR	Partial mixed ration
PPARγ	Peroxisome proliferator-activated receptor-γ
PUFA	Polyunsaturated fatty acids
RDP	Rumen-degradable crude protein
RNB	Ruminal N balance
p.p.	*Post partum*
RSD	Relative standard deviation
SCD	Stearoyl CoA desaturase
SCFA	Short chain fatty acids = VFA
SD	Standard deviation
SEM (SE)	Standard error of the mean
SFA	Saturated fatty acids
SREBP-1	Sterol response element-binding protein 1
SSC	Somatic cell count
SUP	Supplementation
TAG	Triacylglycerin
TMR	Total mixed ration
TNF-α	Tumor necrosis factor α
uCP	Utilizable crude protein
UCP	Uncoupling protein(s)
UDP	Rumen-undegradable crude protein
UFA	Unsaturated fatty acids
VDLUFA	Federation of Laboratories for Agricultural Researches (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten)
VFA	Volatile fatty acids = SCFA

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INTRODUCTION

Conjugated linoleic acids (CLA) is the name given to a mixture of positional and geometrical isomers of linoleic acid with two conjugated double bonds. The isomers naturally occur in ruminant derived products like milk and meat as they are intermediates of the rumen microbial metabolism of linoleic acid, while *cis*-9,*trans*-11 CLA is the most common isomer. In 1987 CLA have attracted special interest, when Ha et al. (1987) described anticancerogenic properties of CLA in studies with mice. Further investigations with other animal species showed antiatherogenic (Lee et al. 1994) and immunomodulatory (Cook et al. 1993; Miller et al. 1994) effects, alterations of body composition (Park et al. 1997) and antidiabetic properties (Houseknecht et al. 1998) of CLA. However, CLA is not a single substance and the different isomers have partially contrasting functionality. Due to their incidence and biological activity, *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA isomers are best investigated. In the late 1990s first studies with dairy cows showed that infusion of CLA into the abomasum decreased the concentration of milk fat and resulted in proportionally greater reduction of short- and medium-chain fatty acids (Loor and Herbein 1998; Chouinard et al. 1999) relative to long-chain fatty acids. In 2000 Baumgard et al. (2000) identified *trans*-10,*cis*-12 CLA as the isomer that effectively reduces milk fat synthesis. The formation of milk fat represents the highest energy expenditure compared to all milk components. Therefore, a reduction in milk fat could help to reduce the potentially occuring energy deficit at the onset of lactation. This is of special importance since a negative energy balance has negative impact on the health and reproductive performance of the cow throughout lactation. However, preliminary results regarding the effect of CLA supplementation on energy metabolism are inconsistent.

For producers, economic factors are also a motive to modify the milk fat content of their herds. The present quota system in the European Union (restriction of milk and milk fat production per year within each EU member state, proposed termination in 2014/15) encourages decreasing the milk fat yield. Up to now a lot of studies were conducted regarding the milk fat depressing potential of CLA but data on treatment and post-treatment effects, particularly after long-term supplementation of CLA, are rare. Although in humans much less is known about the physiological effects of CLA, the described beneficial effects in different animal species lead to varied efforts to increase the CLA content of ruminant derived products like meat and milk. Besides feeding diets with naturally high linoleic acid content, the direct supplementation of CLA might be a possibility to enhance the CLA content in ruminant derived products. As a prerequisite for its functionality, CLA have to be intestinally absorbed. Therefore, the process of ruminal biohydrogenation has to be avoided. Several protection

methods exist, whereby the formation of calcium salts, linkage by amide bonds, formaldehyde treatment and lipid encapsulation are the most important ones. Results from the literature indicate that none of these protection methods provide a 100% protection rate. Up to now there is a lack of information on the effects of dietary supplementation of CLA on rumen metabolism and the actual quantity of absorbable CLA entering the small intestine.

Therefore, there is a need for examinations about the actual rumen inertness of supplemental CLA, the influence on the ruminal metabolism and the resulting effects on animal performance and milk composition, especially after long-term supplementation.

BACKGROUND

1 Chemical structure of linoleic acids

Conjugated linoleic acids are a group of di-unsaturated fatty acids derived from linoleic acid (LA). The term 'conjugated' relates to the double bonds which are only separated by one single bond. The bonds can exist in *cis* or *trans* configuration, thus theoretically 14 positional isomers are conceivable. Therefore the occurrence of a lot of CLA isomers is possible, but the most important ones are the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomer. The difference between the structures of LA and the two principal CLA isomers is shown in Figure 1.

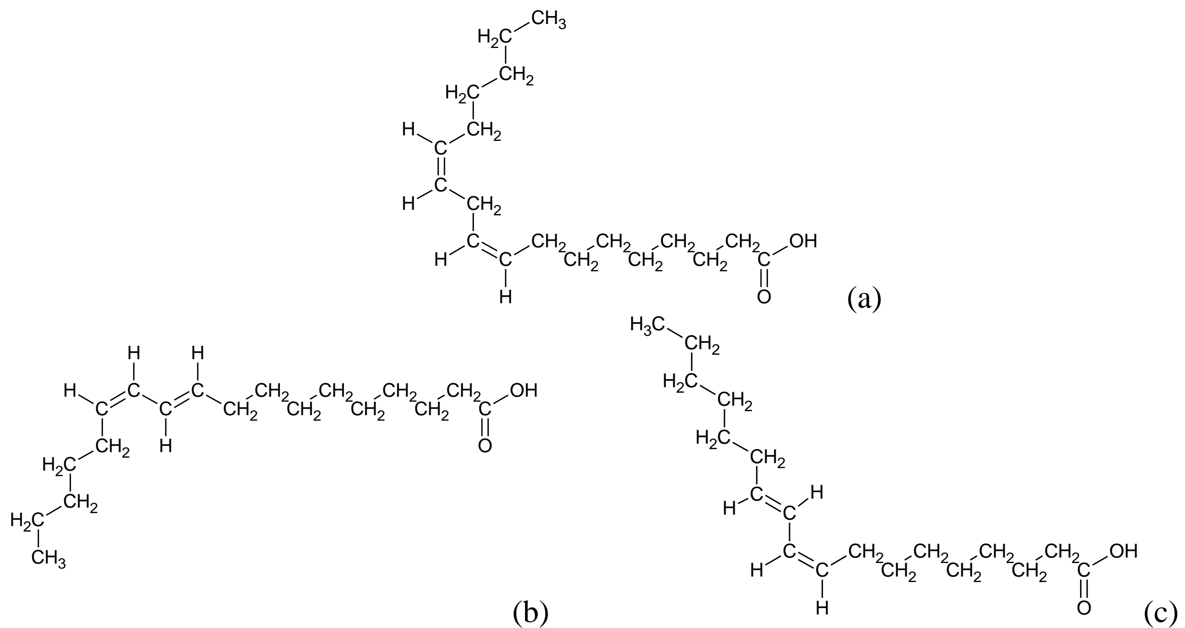

Figure 1: Structural formulas of (a) linoleic acid, (b) *trans*-10,*cis*-12 CLA and (c) *cis*-9,*trans*-11 CLA

2 Formation and metabolism of CLA

2.1 Ruminal and non-ruminal metabolism

Naturally CLA can be found in ruminant products like milk and meat, since CLA isomers are produced in the rumen during microbial biohydrogenation of dietary linoleic acid (Harfoot 1978) and in tissues through Δ9-desaturation of the rumen-derived *trans*-vaccenic acid (C18:1 *trans*) (Griinari et al. 2000) (Figure 2). More than 20 different isomers occur in milk fat (Lock and Bauman 2004), however the *cis*-9,*trans*-11 isomer, also called rumenic acid, with more than 80% comprises the main isomer in milk (Fritsche and Steinhart 1998) and meat (Mir et al. 2003). Conjugated linoleic acids and other long chain fatty acids (> C16, in part C16) in milk are derived from peripheral circulation, including fatty acids that were intestinally absorbed or mobilized from body fat depots. In consequence the fatty acid profile of milk

mainly depends on the fatty acid composition of the diet and the extent of microbial conversion of fatty acids in the rumen. The diet of ruminants contains predominantly polyunsaturated fatty acids (PUFA) as a part of plant triglycerides and glycolipids. In the rumen, two important microbial transformations of dietary lipids take place: at first the lipolysis of the plant lipids followed by the biohydrogenation of the free unsaturated fatty acids (UFA) to saturated fatty acids (SFA). The biohydrogenation process, in contrast to the ruminal digestion of fiber and proteins, is not essential to provide nutrients to ruminal microorganisms. It seems to be necessary to reduce potential negative effects of UFA on rumen fermentation. Conjugated linoleic acids are intermediates of the biohydrogenation process of linoleic acid (*cis*-9,*cis*-12 C18:2) to stearic acid (C18:0). In the first step of linoleic acid metabolism mainly the isomerisation of the *cis*-12 to the *trans*-11 double bound by means of bacterial enzymes takes place, resulting in the formation of rumenic acid (*cis*-9,*trans*-11 CLA). Other CLA isomers are formed to a smaller extent. The *trans*-10,*cis*-12 CLA isomer is a minor intermediate of ruminal linoleic acid metabolism and its flow increased in response to plant oils rich in linoleic acid (Duckett et al. 2002; Shingfield et al. 2008; Liu et al. 2011). Rumenic acid is then hydrogenated to *trans*-vaccenic acid (*trans*-11 C18:1), which is also a precursor of CLA. This monounsaturated fatty acid is formed during the ruminal metabolism of linolenic acid, too. The last step in biohydrogenation is the conversion of *trans*-vaccenic acid to stearic acid. Since the hydrogenation of *trans*-vaccenic acid requires more time than other steps of biohydrogenation, metabolism intermediates including monounsaturated fatty acids and CLA reach the small intestine together with microbial fatty acids. The fatty acids are intestinal absorbed and transported to the adipose tissue and mammary gland, where *trans*-vaccenic acid is partially metabolized to *cis*-9,*trans*-11 CLA. Stearoyl-CoA desaturase (Δ9-desaturase) catalyzes the reaction. The endogenous synthesis in the mammary gland with a contribution of >80%, is the predominant source of this CLA isomer in milk fat (Lock and Garnsworthy 2002). Besides the intake of ruminant products with the diet, endogenous synthesis of CLA is also a source of CLA in milk fat and tissue lipids of monogastrides (Loor et al. 2002; Mosley et al. 2006).

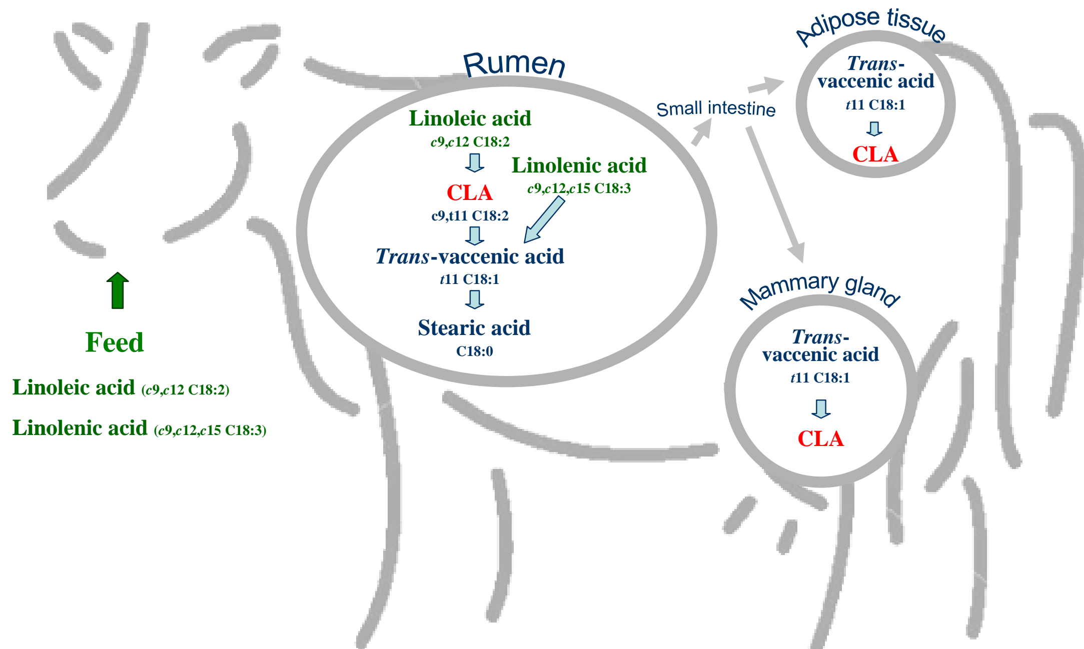

Figure 2: Key steps in the CLA metabolism of the cow

2.2 Chemical synthesis

For research purposes as well as for supplements in human and animal nutrition chemically synthesized CLA preparations are used. The production is mainly based on two processes: the alkali isomerisation of oils rich in linoleic acid or the use of special lipases. As early as 1951 Nichols et al. (1951) reported about the alkali isomerisation of linoleic acid. These early commercial syntheses focused on maximizing total CLA content and the products of alkali isomerisation containing mainly the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers together with some undesirable positional isomers of CLA as well. Today the production is mainly restricted to the *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA, owing to different purification processes. A selective synthesis of *cis*-9,*trans*-11 CLA with a purity of 83% was succeeded by Berdeaux et al. (1997) by the dehydration of methyl ricinoleate. Lie Ken Jie et al. (1997) developed a method to produce all four isomers of 9,11 C18:2 in pure form from methyl ricinoleate. A cheaper and efficient alternative compared to the common alkali isomerisation is the isomerisation by different metal catalysts using metals like nickel, platinum and ruthenium.

A chemoenzymatic method to separate *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA was reported by Chen and Sih (1998). They used a lipase which hydrolysed selective Δ9 unsaturated fatty acids. Hereby they managed a purification of 100% for the *cis*-9,*trans*-11 isomer and of 95.5% for the *trans*-10,*cis*-12 isomer.

As microbial synthesis is the natural mechanism of CLA formation it is not far to seek to synthesize CLA with the help of microbes. Applying microbial strains specialized in

enzymatic isomerisation seems to be a possibility to enrich milk products with CLA (Sieber and Collomb 2004).

3 Rumen protected CLA supplements

Increase of duodenal availability of CLA, especially of the *trans*-10,*cis*-12 isomer, being active in the mammary gland, is of great interest. In recent years a lot of studies were conducted with various CLA preparations. All of the studies conducted with dairy cows used CLA supplements containing a mixture of CLA isomers. In addition to the *trans*-10,*cis*-12 isomer mainly *cis*-9,*trans*-11 CLA but also *trans*-8,*cis*-10 CLA and *cis*-11,*trans*-13 CLA were detectable in such supplements. However, for the last three isomers no change in milk fat yield could be observed when they were abomasally infused at a dose comparable to *trans*-10,*cis*-12 CLA (Baumgard et al. 2000; Perfield et al. 2004b). To achieve an increase of duodenal available CLA, the applied CLA supplements have to be protected against the transformation by the ruminal microbial population. Furthermore, the protection method should avoid any impairment of the growth and function of the ruminal microbial population, since different polyunsaturated fatty acids have been reported to be toxic towards some rumen microorganisms (Maia et al. 2007).

The formation of calcium salts, lipid encapsulation, the linkage by amide bonds and formaldehyde treatment were the main processes that have been used to protect such fats. In literature mostly calcium salts have been applied (Table 1). But information about the actual rumen inertness of such supplements are rare. Up to now only *in vitro* or estimated data from *trans*-10,*cis*-12 CLA transfer into milk fat were available for cows and they suggested that rumen protection is not 100%.

Perfield et al. (2004a) extrapolated from the transfer efficiencies (calculated as follows: CLA isomer secretion with milk [g/d] x 100 / CLA isomer intake [g/d]) of *trans*-10,*cis*-12 CLA into milk after dietary supplementation in comparison to abomasal infusion, to the ruminal biohydrogentation of different rumen protected CLA supplements. They concluded that a large proportion of the CLA must be metabolized in the rumen or unavailable for postruminal absorption independent from the protection method. For calcium salts of CLA de Veth et al. (2005a) summarized eight studies. The range in transfer efficiency of *trans*-10,*cis*-12 CLA into milk fat was 1.9 to 7.4% and this would represent 9 to 34% protection. Across four studies realized with lipid-encapsulated CLA supplements (Perfield et al. 2004a; Castañeda-Gutiérrez et al. 2007; Odens et al. 2007; Moallem et al. 2010), the *trans*-10,*cis*-12 CLA transfer efficiency into milk fat ranged from 4.6 to 7.9%, representing a protection of 23 to 40%. Lock et al. (2004) showed by the comparison of milk fat depression (MFD) and the milk

fat content of *trans*-10,*cis*-12 CLA between ruminal and abomasal infusion of 7.8 g *trans*-10,*cis*-12 CLA/d indications for a limited rumen protection of a lipid-encapsulated CLA supplement. In an *in vitro* investigation Dehkordi et al. (2008) evaluated the effect of pelleting on the protection of a lipid-encapsulated CLA supplement against rumen biohydrogenation. Processing seems to result in a significant lower rumen protection of the supplement compared to the unprocessed fat supplement.

Table 1: Transfer efficiency of *trans*-10,*cis*-12 CLA fed in rumen-protected form into milk fat (according to de Veth et al. (2005a), modified) and total CLA secretion into milk fat

Reference	Supple-ment[§]	*Trans*-10,*cis*-12 CLA			Total CLA secretion into milk fat [% of total FAME]*
		Intake [g/d]	Secretion into milk fat [g/d]	Transfer into milk fat [%][$]	
Giesy et al. (2002)		0.0	0.47		0.58
	CS	4.3	0.53	1.4	0.57
	CS	8.6	0.61	1.6	0.61
	CS	17.3	0.74	1.6	0.70
	CS	34.5	1.26	2.3	0.86
Perfield et al. (2002)		0.0	<0.01		0.49
	CS	9.0	0.31	3.4	0.61
Bernal-Santos et al. (2003)		0.0	<0.01		0.40
	CS	9.0	0.43	4.8	0.44
Moore et al. (2004)		0.0	<0.01		0.51
	CS	12.4	0.80	6.5	1.08
	CS	25.0	1.60	6.4	1.70
	CS	37.4	2.50	6.7	2.69
Selberg et al. (2004)		0.0	0.48		0.49
	CS	15.0	0.94	3.1	0.60
Piperova et al. (2004)		0.0	0.09		0.52
	CS	4.7	0.28	4.0	0.83
Perfield et al. (2004a)[+]		0.0	<0.01		0.63
	AP	10.0	0.71	7.1	1.01
	LE	10.0	0.79	7.9	0.99
de Veth et al. (2005a)[+]		0.0	<0.01		0.87
	CS	10.0	0.36	3.6	0.94
	FP	10.0	0.79	7.9	1.34
Castañeda-Gutiérrez et al. (2005)		0.0	<0.01		0.05
	CS	9.0	0.29	3.2	0.60
	CS	18.0	0.52	2.9	0.72
Odens et al. (2007)		0.0	<0.01		0.43
	LE	30.0	2.15	7.2	2.01
Castañeda-Gutiérrez et al. (2007)		0.0	<0.01		0.46
	LE	2.4	0.13	5.4	0.52
	LE	7.1	0.35	4.9	0.52
Moallem et al. (2010)		0.0	0.21		0.38
	LE	5.0	0.44	4.6	0.51

[+] CLA supplements were placed directly into the rumen
[§] CS, Calcium salts; AP, Amid-protected; LE, Lipid-encapsulated; FP, Formaldehyde-protected
[$] Transfer to milk fat was calculated after correction of the milk fat *trans*-10,*cis*-12 CLA content of the in CLA supplemented groups for the content of this isomer in control milk fat
* if not stated as % of total fatty acid methyl esters (FAME) a fatty acid proportion of 88.6% in milk fat was assumed

Data for the transfer of *trans*-10,*cis*-12 CLA into milk fat are available in most of the dietary CLA supplementation studies and summarized in Table 1. A secretion of this isomer into milk fat between 0.13 and 2.50 g/d was observed after the supplementation of different protected CLA preparations and all studies showed a dose-dependent but small increase of the *trans*-10,*cis*-12 CLA secretion. Only small proportions of the supplemented *trans*-10,*cis*-12 CLA were transferred into milk fat. The transfer efficiency ranged from 1.4 to 7.9%. Based on abomasal infusion studies with a *trans*-10,*cis*-12 CLA transfer efficiency of on average 20% (Table 2) equated with 100% rumen inertness, this would represent 7 to 40% protection independent from the protection method.

Despite of the apparent small quantity of CLA which is duodenally available after the application of rumen protected CLA preparations, the absorbed amount of CLA seems to be sufficient to act in different body tissues as summarized in the following paragraph.

Table 2: Transfer efficiency of abomasal infused *trans*-10,*cis*-12 CLA into milk fat

	Trans-10,*cis*-12 CLA		
Reference	Infusion [g/d]	Secretion into milk fat [g/d]	Transfer into milk fat [%][$]
Baumgard et al. (2000)	10.0	2.71	27.1
Baumgard et al. (2001)	3.5	0.76	21.7
	7.0	1.44	20.6
	14.0	2.35	16.8
Baumgard et al. (2002b)	13.6	1.37	10.1
Perfield et al. (2004b)	4.0	1.09	27.1
de Veth et al. (2004)	4.2	0.72	17.0
	4.2	0.74	17.7
Perfield et al. (2006)	5.0	1.23	24.5
Kay et al. (2007)	9.3	2.18	17.2
Perfield et al. (2007)	5.0	1.00	19.9

[$] Transfer to milk fat was calculated after correction of the milk fat *trans*-10,*cis*-12 CLA content of the in CLA supplemented groups for the content of this isomer in control milk fat

4 Potential effects of dietary supplemented CLA on dairy cows

During more than three decades, hundreds of reports based on microbial, animal and human trials on the biological activities of CLA have been accumulated and reviewed in different areas of research. A lot of biologically beneficial activities have been shown for CLA and they appear to be the result of multiple interactions of the active CLA isomers with numerous metabolic signalling pathways (Moya-Camarena et al. 1999; Peterson et al. 2004). Although CLA has been shown to be metabolized like other fatty acids (Banni et al. 2001; Banni et al. 2004), it seems that the effects of CLA are most likely due to the CLA isomers itself rather than its metabolites. There are two considerable biologically active isomers of CLA:

cis-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA. The last one appears to induce particularly effects which relate to fat storage and metabolism. In dairy cows research was focused on the milk fat reducing properties of the *trans*-10,*cis*-12 isomer and the resulting effects on energy balance. The following review summarizes the common knowledge about the mechanism of action of CLA in different tissues and their relevance for dairy cows.

4.1 Milk yield, milk fat and milk protein

Milk fat requires the highest energy cost in milk production. The application of CLA in dairy cow diets reduces the milk fat content and the effect has been shown to be specific for the *trans*-10,*cis*-12 CLA isomer. This fact has been studied extensively and predominantly in short-term trials in dairy cattle (Table 3). As involved molecular mechanisms the down-regulation of mRNA expression for key lipogenic enzymes, responsible for circulating fatty acid uptake and transport, *de novo* fatty acid synthesis, desaturation of fatty acids and triglyceride synthesis in the mammary gland are well established. In a study by Baumgard et al. (2002b) a down-regulation of mRNA expression was detected for acetyl CoA carboxylase (ACC), fatty acid synthetase (FAS), stearyl CoA desaturase (SCD), lipoprotein lipase (LPL), fatty acid binding proteins (FABP), glycerol phosphate acyltransferase (GPAT) and acylglycerol phosphate acyltransferase (AGPAT) after CLA treatment. These results were supported by Harvatine and Bauman (2006) who showed a significant lower mRNA abundance for LPL and FAS and Peterson et al. (2003) who confirmed a reduced mRNA abundance for ACC, FAS, GPAT and AGPAT. The role of these enzymes in milk lipid synthesis is summarized in Figure 3. The transcription factor sterol response element-binding protein (SREBP)-1 seems to play an important role in this process. All of these lipogenic genes were SREBP-1-regulated and Peterson et al. (2004) described an inhibition of the proteolytic activation of STEBP-1 in bovine mammary epithelial cells after *trans*-10,*cis*-12 CLA treatment.

Furthermore, Gutgesell et al. (2009) investigated the expression of important FA transporters in the mammary gland of lactating rats after a dietary CLA treatment. They observed lower relative mRNA concentrations of FA translocase/CD36, fatty acid transport protein and plasma membrane FA binding protein indicating that reduced uptake of circulating NEFA into the mammary gland could also contribute to the reduction of milk triacylglycerol concentrations by dietary CLA in rats and possibly also in other species such as cows and sheep.

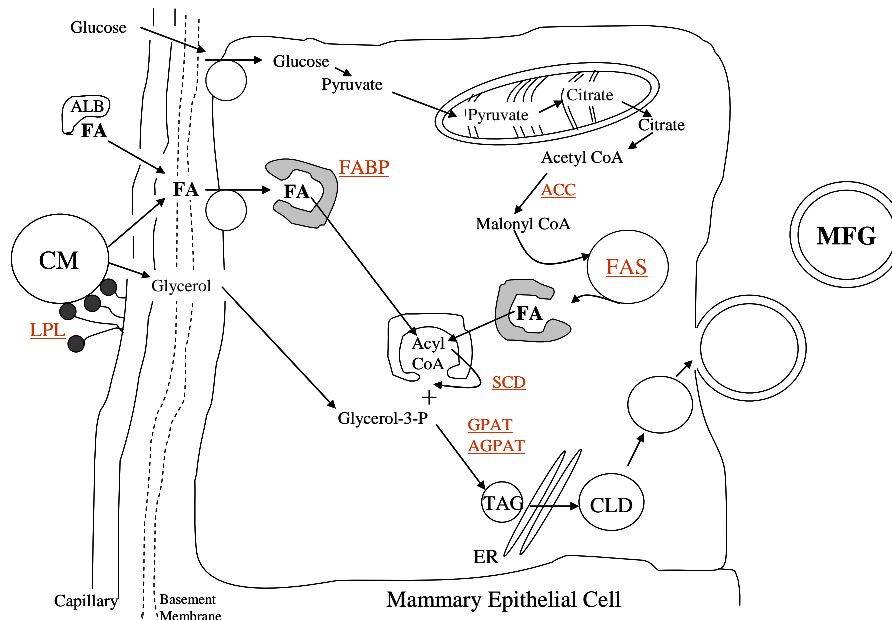

Figure 3: The role of CLA affected enzymes (marked in red) in synthesis and secretion of milk fat; adapted from Neville and Picciano (1997). ALB, Albumin; CM, Chylomicron; ER, Endoplasmatic reticulum; FA, Fatty acids; TAG, Triacylglycerin; CLD, Cytoplasmic lipid droplets; MFG, Milk fat globule; LPL, Lipoprotein lipase; FABP, Fatty acid binding proteins; ACC, Acetyl CoA carboxylase; FAS, Fatty acid synthetase; SCD, Stearoyl CoA desaturase; GPAT, Glycerol phosphate acyltransferase; AGPAT, Acylglycerol phosphate acyltransferase

An overview of the studies conducted with dietary CLA supplements is given in Table 3. Except of Castañeda-Gutiérrez et al. (2007) and Sigl et al. (2010) all investigations supplementing 3.2 to 51.6 g *trans*-10,*cis*-12 CLA/d could show significantly reduced milk fat contents. In some cases the milk fat content was reduced by more than 1%-point. But it does not always mean that a significant decrease of milk fat synthesis (= milk fat depression, MFD) occurs, since in part the lower milk fat content only resulted from a higher milk yield coincidently occurring with higher milk fat synthesis. MFD ranged from 9 to 44%.

As shown in Table 3 the majority of investigations observed no effects of CLA on protein content of milk. Only Piamphon et al. (2009), Moallem et al. (2010) and von Soosten et al. (2011) reported about a reduction of the milk protein content, whereas Medeiros et al. (2010) observed an increase most likely attributable to the relatively higher utilizable protein in the CLA diet.

In studies by Perfield et al. (2002), Giesy et al. (2002), Bernal-Santos et al. (2003), Moore et al. (2004), Selberg et al. (2004), Piperova et al. (2004), Castañeda-Gutiérrez et al. (2005), Kay et al. (2006), Odens et al. (2007), Sippel et al. (2009) and Medeiros et al. (2010) mixtures of four different CLA isomers (*cis*-9,*trans*-11 CLA; *trans*-10,*cis*-12 CLA; *trans*-8,*cis*-10 CLA; *cis*-11,*trans*-13 CLA) were used in varying amounts. As already mentioned milk fat depressing properties only could have been shown for the *trans*-10,*cis*-12 isomer.

Long-term studies during early and established lactation were only performed with CLA mixtures of the four mentioned isomers by Perfield et al. (2002) and Bernal-Santos et al. (2003). Only Castañeda-Gutiérrez et al. (2005), Castañeda-Gutiérrez et al. (2007) and Medeiros et al. (2010) took a depletion period into consideration. After supplementation over 11 weeks during the transition period and early lactation, Castañeda-Gutiérrez et al. (2005) monitored the milk fat concentration for further eight weeks. During the treatment period a significant reduction of milk fat yield of 12 and 21% after the supplementation of 9 and 18 g *trans*-10,*cis*-12 CLA/d, respectively was the only effect on milk composition. After the end of the supplementation period the differences in milk fat yield were no longer evident and no other treatment effect was observed. After 36 days of supplementation of relative low doses *trans*-10,*cis*-12 CLA (2.4 and 7.1 g/d) no effects on milk yield and composition were observed by Castañeda-Gutiérrez et al. (2007) and these variables remained unaffected within the 4 weeks post-treatment period. Medeiros et al. (2010) added 22 g *trans*-10,*cis*-12 CLA/d for 56 d in mid-lactation to the diet of the cows. Milk yield tended to be higher, milk protein yield was increased and milk fat yield decreased during the treatment period. The post-treatment period lasted for 27 days and the effects on milk yield and milk protein yield were still noticeable.

Table 3: Overview of trials with dietary CLA supplementation to dairy cow diets

Reference	***Trans*-10, *cis*-12 CLA intake [g/d]**	**Supplement**	**Animal number[$]**	**Start of supplementation [DIM]**	**Time of supplementation [d]**	**DMI [kg/d]**	**Milk yield [kg/d]**	**Milk fat content [%]**	**Milk fat yield [kg/d]**	**MFD [%][II]**	**Milk protein content [%]**	**Net energy balance [MJ NEL/d]**
Giesy et al. (2002)	0.0	CS[♦]	5	93	5	25.7	42.3	3.45	(1.46)		3.17	n.s.
	4.3					26.4	43.5	2.97*	(1.29)	(11.6)	3.17	
	8.6					26.4	47.9	2.96*	(1.42)	(2.7)	3.17	
	17.3					27.0	44.0	2.46*	(1.08)	(26.0)	3.15	
	34.5					26.4	43.1	2.29*	(0.99)	(32.2)	3.24	
Perfield et al. (2002)	0.0	CS[♦]	30	79	340	23.4	30.4	3.80	1.20		3.13	19.3
	9.0					23.1	30.8	2.90*	0.93*	22.8	3.16	24.3
Bernal-Santos et al. (2003)	0.0	CS[♦]	30	-14	154	23.5	23.5	3.60	1.57		2.77	-5.4
	9.0					23.9	23.9	3.15*	1.45	-	2.74	-2.5
Moore et al. (2004)	0.0	CS[♦]	19	-10	31	17.9	33.4	4.57	1.47		4.02	-36.8
	12.4					16.4	33.7	3.97	1.29	-	3.49	-41.0
	25.0					18.2	35.5	3.32*	1.15	-	3.76	-21.8
	37.4					16.0	34.3	3.10*	1.03*	30.0	3.68	-25.9
Selberg et al. (2004)	0.0	CS[♦]	38	-28	77	21.6	40.3	3.49	1.38		2.89	n.s.
	15.0					20.0	41.5	2.99*	1.19	-	2.82	only figure
Piperova et al. (2004)	0.0	CS[♦]	45	129	28	23.5	37.8	3.39	1.24		3.05	n.s.
	4.7					23.5	35.2	2.54*	0.95*	23.4	3.03	
Perfield et al. (2004a)[+]	0.0		3	-78	7	30.6	40.5	3.23	1.27		2.55	n.s.
	10.0	AP[◊]				31.6	42.6	2.37*	1.00*	21.1	2.51	
	10.0	LE[◊]				30.4	42.7	2.34*	0.99*	21.9	2.58	
de Veth et al. (2005a)[+]	0.0		3	mid lact	7	23.6	21.9	3.61	0.79		3.16	n.s.
	10.0	CS[◊]				23.1	20.6	2.61*	0.52*	34.4	3.38	
	10.0	FP[◊]				23.3	19.5	2.34*	0.44*	44.0	3.48	
Castañeda-Gutiérrez et al. (2005)	0.0	CS[♦]	48	-14	77	21.7	43.3	3.82	1.65		2.85	-13.8
	9.0					21.3	43.8	3.43*	1.46*	11.5	2.81	-10.5
	18.0					20.5	43.8	3.08*	1.30*	21.2	2.79	-7.1
Kay et al. (2006)	0.0	n.s.[♦]	39	-27	36	11.3	19.4	5.12	1.00		3.68	-20.5
	21.0					12.0	22.1*	3.35*	0.74*	26.0	3.60	3.9*
Odens et al. (2007)	0.0	LE[♦]	31	-9	50	17.9	35.6	4.27	1.41		3.22	-21.3
	30.0					19.3	38.2	3.16*	1.09*	22.6	3.38	-5.4
	30.0/10.0				20/30	19.8	38.5	3.49*	1.17*	17.0	3.46	-9.2

Table 3 continued

Reference	*Trans*-10, *cis*-12 CLA intake [g/d]	Supple-ment	Animal number[$]	Start of supplemen-tation [DIM]	Time of supplemen-tation [d]	DMI [kg/d]	Milk yield [kg/d]	Milk fat content [%]	Milk fat yield [kg/d]	MFD [%][II]	Milk protein content [%]	Net energy balance [MJ NEL/d]
Castañeda-Gutiérrez et al. (2007)	0.0	LE[◊]	45	20	36	21.9	41.8	3.30	1.38		2.69	-6.3
	2.4					22.1	42.9	3.24	1.39	-	2.60	-6.7
	7.1					22.0	43.4	3.10	1.35	-	2.64	-5.9
Meyer et al. (2007)	0.0	LE[◊]	5	mid lact	14	18.3	33.1	3.68	(1.22)		2.92	n.s.
	3.9					19.1	33.7	3.19*	(1.08)	(11.5)	2.92	
	8.8					18.7	34.0	2.64*	(0.90)	(26.2)	2.84	
	17.5					16.6	30.4	2.22*	(0.67)	(45.1)	3.03	
Schwarz et al. (2007)	0	LE[◊]	50	1	98	17.3	32.1	3.82	1.21		3.07	-10.6
	4					18.0	34.2	3.09*	1.05*	13.2	3.02	-4.3*
	0		53			19.0	34.8	3.66	1.28		3.16	-9.5
	4					19.0	35.9	3.00*	1.07*	16.4	3.10	-2.0*
Huang et al. (2008)	0.0		36	mid lact	28	21.5	30.3	3.53	1.07		3.18	n.s.
	n.s.	free				20.7	29.7	2.62*	0.78*	27.1	2.99	
	n.s.	CS[◊]				21.5	29.9	2.62*	0.78*	27.1	3.09	
Suksombat and Chullanandana (2008)	0.0		24 CB	mid lact	70	14.0	15.2	3.49	0.53		2.63	n.s.
	14.0					13.4	14.5	2.55*	0.37*	30.3	2.89	
Piamphon et al. (2009)	0.0	LE[◊]	5 CB	mid lact	21	12.7	15.2	4.26	0.64		3.91	n.s.
	2.0					12.5	16.0*	4.12	0.65	-	3.92	
	4.0					13.3	16.4*	3.73*	0.60	-	3.74*	
	8.0					13.5	16.0*	3.82	0.60	-	3.73*	
	16.0					12.2	15.7	3.59*	0.54	-	3.79	
Sippel et al. (2009)	0.0	CS[♦]	10	mid lact	28	15.9	25.4	3.48	0.88		3.39	-6.3
	6.4					16.6	27.1	2.84*	0.77*	12.1	3.38	-0.4*
	13.0					16.4	27.3	2.53*	0.69*	21.3	3.35	1.7*
	19.4					16.4	27.6	2.47*	0.68*	22.9	3.28	1.3*
	51.6					16.1	25.5	2.13*	0.54*	38.0	3.43	8.8*
Metzger-Petersen et al. (2009)	0.0	LE[◊]	49	6		20.1	32.9	4.04	(1.33)		3.31	n.s.
	3.3				80	20.6	35.7*	3.86	(1.38)		3.30	
	3.2				120	19.9	35.7*	3.81*	(1.36)		3.22	
Sigl et al. (2010)	0.0	CS[◊]	10 BS	-14	42	n.s.	24.5	6.10	1.49		3.81	n.s.
	10.0					n.s.	24.5	5.77	1.41	-	3.82	

Table 3 continued

Reference	*Trans*-10, *cis*-12 CLA intake [g/d]	Supple-ment	Animal number[$]	Start of supplemen-tation [DIM]	Time of supplemen-tation [d]	DMI [kg/d]	Milk yield [kg/d]	Milk fat content [%]	Milk fat yield [kg/d]	MFD [%][II]	Milk protein content [%]	Net energy balance [MJ NEL/d]
Moallem et al.	0.0	LE[◊]	42	21	77	28.5	50.6	3.36	1.68		3.08	18.0
(2010)	5.0					27.8*	52.9*	2.94*	1.53*	8.9	2.98*	17.6
Medeiros et al.	0.0	CS[♦]	30 CB	28	56	n.s.	15.2	2.90	0.44		2.84	n.s.
(2010)	22.0					n.s.	16.3	2.14*	0.35*	20.4	3.05*	
Hutchinson et al.	0.0	LE[◊]	72	1	60	17.2	26.5	4.26	1.08		3.12	n.s.
(2011)	6.0					17.3	26.5	3.78*	0.98*	9.3	3.07	only figure
von Soosten et al.	0.0	LE[◊]	25	1	42	14.8	23.4	4.40	1.02		3.42	1.8
(2011)	6.0					14.1	24.5	3.78*	0.92		3.32	-0.7
	0.0				105	18.1	25.9	4.10	1.05		3.35	20.9
	6.0					16.6	28.6	3.06*	0.87*	17.1	3.00*	15.4

* Differences between control and CLA group were significant ($p<0.05$)
[II] Milk fat depression is only stated if milk fat yield was significantly reduced after CLA treatment as defined by Bauman and Griinari (2001)
[♦] Mixture of *trans*-10,*cis*-12, *cis*-9,*trans*-11, *trans*-8,*cis*-10 and *cis*-11,*trans*-13 CLA; [◊] Mixture of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA
[$] Trials which were not conducted with Holstein Cows were stated separately: CB, Crossbred; BS, Brown Swiss
[+] CLA-supplement was placed directly into the rumen
LE, Lipid-encapsulated; CS, Calcium salts; AP, Amid-protected; FP, Formaldehyd-protected; n.s., not stated;
Values with brackets: data were not evaluated statistically in the study

4.2 Fatty acid profile of milk

In milk fat many different fatty acids occur. As mentioned above, the diet of the animals is an important factor influencing the fatty acid profile of milk since fatty acids with more than 16 C-atoms originate from feed or the mobilization of body fat. Fatty acids with less than 16 C-atoms originate from *de novo* synthesis in the mammary gland. The C16 fatty acids are from both sources. The *trans*-10,*cis*-12 CLA induced reduction in milk fat synthesis represents a decrease in the yield of most fatty acids. Since the decline is in most cases greatest for *de novo* synthesized fatty acids, the fatty acid profile changes markedly. In consequence the milk fat profile shifts toward reduced proportions of short and medium chain fatty acids (≤C16) and greater concentrations of longer chain fatty acids (>C16) as summarized in Figure 4. For <C16 fatty acids, the difference to control decreased with increasing amounts of *trans*-10,*cis*-12 CLA in the diet. The C16 fatty acids remained unaffected from CLA dosage, while with increasing amounts of *trans*-10,*cis*-12 CLA in the diet the preformed fatty acids tended to increase compared to control in milk fat.

Ruminant-derived food products, like milk but also meat are the natural source of CLA isomers in the food chain, whereas dairy products contribute the main part. The beneficial effects of CLA lead to an increasing demand in CLA enriched milk and meat. Naturally, there is a wide range of CLA content of milk. Jahreis et al. (1999) determined the highest CLA concentrations in milk of ruminants compared to the milk of monogastrides. But also among the milk samples of goat, ewe and cow they found enormous differences in the CLA levels (0.64 – 1.08% of fatty acid methyl esters (FAME)). For cow and ewe milk the highest CLA proportions and a great influence of the season were reported. Furthermore there is variation in the CLA content of milk between the individual animals under the same feeding regimen (Peterson et al. 2002). As shown, various factors influence the CLA content of milk, however, the diet will remain the most important one. Studies showed a higher CLA content in the milk of grass fed cattle (Jahreis et al. 1997; Kelly et al. 1998b; Dhiman et al. 1999a). The percentages of CLA in milk of grazing Holstein cows ranged from 0.72 to 2.39% of total FAME, while CLA concentration in the milk from silage fed Holstein cows ranged from 0.41 to 0.69% of total FAME. Likewise the addition of different vegetable oils, especially oils high in linoleic acid (Kelly et al. 1998a; Zheng et al. 2005; Rego et al. 2009) but also of fish oil (Jones et al. 2000) resulted in higher CLA concentrations in milk fat. The supplementation of sunflower oil in the trial of Kelly et al. (1998a) resulted in 2.1% CLA of total FAME. A

proportion of 2.2% CLA of total FAME was detected by Jones et al. (2000) after the supplementation of 2% fish oil (DM basis) to the diet.

As shown in Table 1 the application of rumen-protected CLA supplements is one possible way to enhance the CLA concentration in milk. In all mentioned trials the total CLA concentration of milk fat was increased by an average of 100% after the treatment with rumen-protected CLA supplements. The determined proportions of total CLA of FAME ranged from 0.05 to 0.87 and 0.44 to 2.69% % for the control and CLA supplemented cows, respectively. If increasing amounts of CLA were fed the content of these fatty acids in milk fat increased dose-dependently. The main part of total CLA in milk fat is represented by the *cis*-9,*trans*-11 isomer, whereas *trans*-10,*cis*-12 CLA was detected in very small amounts and in most cases only in the milk of the CLA supplemented cows and not in milk of control cows. Giesy et al. (2002), Selberg et al. (2004), Piperova et al. (2004) and Moallem et al. (2010) found proportions of 0.01 to 0.04% *trans*-10,*cis*-12 CLA in milk fat of cows not supplemented with CLA. After CLA supplementation proportions up to 0.24% *trans*-10,*cis*-12 CLA of milk fat were detected (Moore et al. 2004).

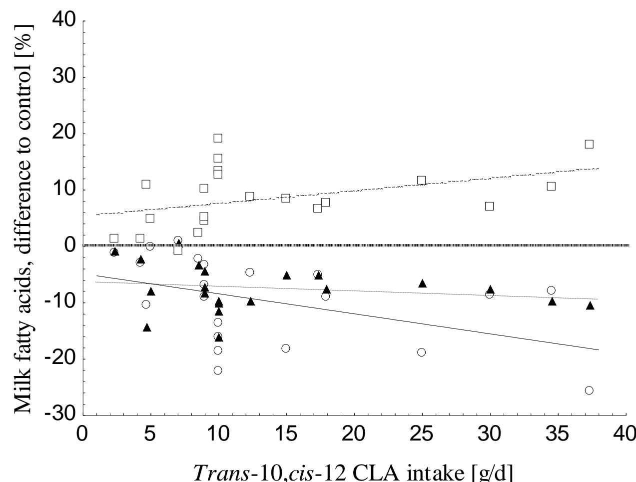

Figure 4: Secretion response of *de novo* synthesized (─○─ <C16) y=-4.8474-0.3555*x (p=0.037, r^2=0.210, RSD=8%), preformed (···□·· >C16) y=5.4193+0.2233*x (p=0.064, r^2=0.169, RSD=5%) and milk fatty acids of both sources (- -▲- - C16) y=-6.4062-0.0822*x (p=0.391, r^2=0.039, RSD=4%) to a dietary CLA supplementation; references: Giesy et al. (2002), Perfield et al. (2002), Bernal-Santos et al. (2003), Moore et al. (2004), Selberg et al. (2004), Piperova et al. (2004), Perfield et al. (2004a), de Veth et al. (2005a), Castañeda-Gutiérrez et al. (2005), Odens et al. (2007), Castañeda-Gutiérrez et al. (2007), Moallem et al. (2010)

4.3 Energy balance

The energy balance of dairy cows can be described as the difference between ingested energy and the requirement for maintenance, growth, milk production and gestation (GfE 2001). For a highly productive dairy cow a large part of ingested energy is spent for milk production. But

especially during the early stage of lactation the energy requirement for milk production exceeds the energy intake, which results in the mobilization of body reserves, mainly stored in the form of body fat. The consequential negative energy balance during this period results in a potentially higher risk for metabolic disorders and an impairment of animal health. It is believed that particularly in this sensitive stage of lactation the milk fat depressing properties of *trans*-10,*cis*-12 CLA could help to save energy and improve the energy balance of dairy cows, especially since studies indicate that dietary *trans*-10,*cis*-12 CLA doses of ≥10 g/d clearly reduce (2-25%) the energy output *via* milk as shown in Figure 5. The reduction ranged from 0.48 MJ NEL/d to 24.16 MJ NEL/d. It could be concluded that only a part of the spared milk fat energy is expended for higher milk yields.

However, except of Kay et al. (2006), Schwarz et al. (2007) and Sippel et al. (2009) in the most studies summarized in Table 3 the calculated net energy balance, if stated, remained unaffected from CLA addition to the diet. This is mostly attributed to a repartitioning of energy to higher milk yields. In the investigation of Kay et al. (2006) the grazing cows received a CLA mix of four isomers resulting in an intake of 21 g *trans*-10, *cis*-12 CLA. Supplemental CLA improved the net energy balance by 17 MJ/d. Sippel et al. (2009) fed increasing amounts of calcium salts of CLA (0, 6.4, 13.0, 19.4 and 51.6 *trans*-10,*cis*-12 CLA) to the cows and net energy balance increased linearly from -6.3 to 8.4 MJ/d in mid lactation. Also Schwarz et al. (2007) could show that net energy balance was improved after the intake of 4 g *trans*-10, *cis*-12 CLA/d.

The supplementation of a CLA mixture containing 12.4, 25.0 and 37.4 g *trans*-10,*cis*-12 CLA/d had no effect on the mean net energy balance during the first 21 d of lactation in an investigation of Moore et al. (2004). However, days to energy balance nadir were decreased by CLA for 1.5, 2.5 and 4.7 days compared to control whereby the difference was only significant for the group receiving the highest CLA dose.

The plasma concentration of non-esterified fatty acids (NEFA) closely reflects the whole animal energy balance (Bauman et al. 1988) since NEFA are liberated if adipose tissue mobilization occurs. Plasma NEFA levels for cows supplemented with CLA were decreased and their net energy balance numerically improved compared to the control animals in a study by Odens et al. (2007). In a resent study by Hutchinson et al. (2011) the peak concentration of NEFA in blood was reduced by supplemental CLA, while Moore et al. (2004) reported no effect of dietary CLA addition on NEFA plasma concentration during early lactation.

Several studies have shown that feeding CLA reduces dry matter and energy intake which has directly negative impact on the animal's energy balance. In a recent study by Moallem et al.

(2010) the average 5.0 MJ/d reduction in energy corrected milk was countered by a corresponding reduction in energy intake of the CLA treated cows, possibly caused by the higher proportion of UFA in the CLA diet. Baumgard et al. (2000) observed a trend for a lower DMI in cows abomasally infused with CLA. Selberg et al. (2004) fed cows with calcium salts of CLA and showed a trend for a lower feed intake. The abomasal infusion of 7.5 g *trans*-10,*cis*-12 CLA/d by Harvatine et al. (2009) resulted in a decreased DMI of 2.3 kg/d and the meta-analysis of CLA infusion data demonstrated a mean 1.5-kg reduction intake during CLA-induced MFD.

However, energy metabolism cannot be separated from body composition. Therefore the effects of CLA on body composition (see chapter 4.4), especially on adipose tissues have to be taken into consideration.

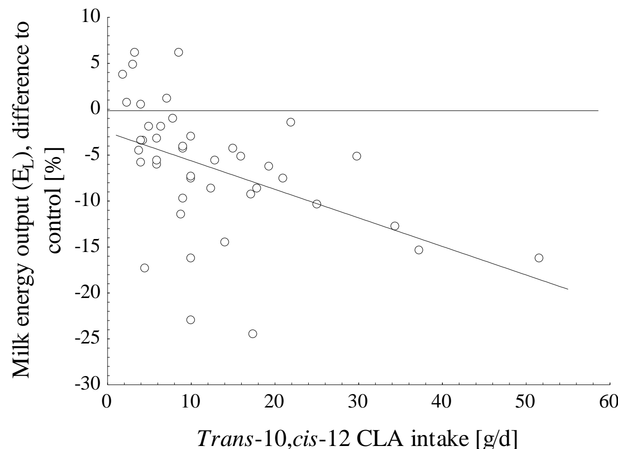

Figure 5: Relationship between *trans*-10,*cis*-12 CLA intake and the difference of milk energy output (E_L [MJ NEL/d]=(0.95+0.38*Milk fat content [%]+0.21*Milk protein content [%]) * Milk yield [kg/d]) (Tyrrell and Reid (1965)) of CLA supplemented animals relative to control; y=-2.5101-0.3107*x (p=0.002, r^2=0.265, RSD=7%); for references see Table 3, the data of Huang et al. (2008) were not included in the evaluation because the administered dose of *trans*-10,*cis*-12 CLA was not reported in the publication

4.4 Body composition

Studies with other animal species showed that CLA influences fat metabolism in mammals not only in the mammary gland by inhibiting milk fat synthesis. Investigations with mice showed that CLA alters body composition by decreasing the body fat content and increasing the body protein content (Park et al. 1997; Delany et al. 1999). An enhancement of fat-free body mass and a reduction in body fat could also be shown for rats (Stangl 2000b; Yamasaki et al. 2003b) and pigs (Ostrowska et al. 1999). However, CLA is not a single substance and the different isomers have partially different functionalities. It has been confirmed that the *trans*-10,*cis*-12 isomer is the isomer responsible for the alteration of body composition, while

the *cis*-9,*trans*-11 isomer has no effect (Park et al. 1999). However, Joseph et al. (2010) deduced from a reduced body fat mass of hamsters following 28 d of a 2% *trans*-8,*cis*-10 + *cis*-9,*trans*-11 CLA supplemented diet that this mixture is able to alter body composition. Investigations from Park et al. (1997) with growing mice receiving a diet containing 0.5% CLA-mix suggest that in part the effects on body composition appear to be due to reduced fat deposition. On the other hand, CLA induced apoptosis of adipose cells (Tsuboyama-Kasaoka et al. 2000) and reduction of levels of triglyceride in adipose cells (Yamasaki et al. 1999) indicate that CLA is able to reduce already existing body fat as well. Tsuboyama-Kasaoka et al. (2000) observed after the addition of 1% CLA-mix to the diet of mice for 5 months a redistribution of fat between adipose tissue and liver. Coincident with lower fat tissue weights the liver weight increased and histological analysis of liver revealed a panlobular macrovesikular steatosis without hepatic inflammation.

Multiple mechanisms for CLAs effect on body fat modulation have been suggested based on studies with certain animal species and model systems. These include increasing energy expenditure, modulating adipocyte metabolism and increasing fatty acid ß-oxidation.

Energy expenditure seems to be influenced after CLA intake by increased oxygen consumption and increased expression of uncoupling proteins (UCP) (Tsuboyama-Kasaoka et al. 2000). Uncoupling proteins are members of a larger family of mitochondrial anion carrier proteins and thus modulators of energy balance and metabolism. Today five members of this family are described: UCP1-5. The UCP1 is exclusively expressed in brown adipose tissue and responsible for direct heat production instead of ATP, while UCP2 and 3 are expressed in adipose tissues and skeletal muscles (Dulloo and Samec 2001). However, it seems that CLA have a species-specific effect on the expression of UCP1-3 as Ealey et al. (2002) could show that the reduced adipose depot weights after CLA treatment in mice but not in rats were attributable to alterations in UCP expression. The role of UCP4 and 5 in energy metabolism is not known (Erlanson-Albertsson 2003).

For CLA induced modulation of adipocyte metabolism, different starting points are conceivable and the results about the underlying molecular mechanisms are not well-defined. The reduction of lipid accumulation seems to be due to an inhibition of LPL (Park et al. 1997; Park et al. 1999), a key enzyme for cellular fat uptake which hydrolyzes fatty acids from circulating triacylglycerol. Furthermore it could be shown that CLA induces apoptosis in preadipocytes, possibly trough an increased expression of the cytokine tumor necrosis factor α (TNF-α) in adipose cells as shown by Tsuboyama-Kasaoka et al. (2000) in a study with mice. The effect of CLA on adipocyte differentiation was investigated by Kang et al. (2003) using

3T3-L1 preadipocytes treated with *trans*-10,*cis*-12 CLA and detecting the mRNA transcription of the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), which controls the process of adipocyte differentiation. Gene expression of PPARγ was blocked by *trans*-10,*cis*-12 CLA, leading to inhibition of adipocyte differentiation. Furthermore, the measured triglyceride content in differentiating 3T3-L1 preadipocytes indicated a decreased accumulation after the CLA treatment. These data collectively suggest that CLA, especially the *trans*-10,*cis*-12 isomer is able to decrease lipid accumulation in adipose tissue, induce apoptosis in preadipocytes and inhibit adipocyte differentiation, at least in certain species and model systems resulting in a reduction of adipose tissue mass. Investigations by Azain et al. (2000) showed that in rats a dietary supplementation of CLA leads to a reduction in fat mass which is a result of a reduction in cell size rather than in cell number.

Apparently CLA is able to increase fatty acid oxidation. For carnitine palmitoyltransferase (CPT), a rate limiting enzyme for fatty acid ß-oxidation, Martin et al. (2000) and Park et al. (1997) showed an increased activity after dietary CLA intake. Martin et al. (2000) fed a diet containing 1% *trans*-10,*cis*-12 CLA for 6 weeks to rats and showed that hepatic and adipose CPT activity was increased. In mice the dietary supplementation of 0.5% CLA-mix to the diet increased the muscle CPT in fasted mice and the fat pad CPT in fed mice, whereas the hepatic CPT remained unaffected from CLA treatment. Evans et al. (2002) treated 3T3-L1 preadipocytes with 50 µmol/L of *trans*-10,*cis*-12 CLA for 6 weeks and observed an increased oxidation of ^{14}C-oleic acid. Park and Pariza (2007) interpreted that in biological systems CLA causes fat to be preferentially used as an energy source, which in turn helps to reduce body fat deposit.

The results mostly obtained from studies with mice and rats suggest a possible influence of supplemented CLA, especially *trans*-10,*cis*-12 CLA, on fat metabolism of dairy cows as well. But it has to be considered that the CLA doses used to induce reductions in body fat are about tenfold greater than the doses applied to reduce milk fat synthesis. In general performance studies with dairy cows investigating the effects of CLA supplementation, parameters like body weight (BW), body condition store (BCS) and back fat thickness (BFT) were used to describe changes in body composition. The collection of these variables often failed to show the expected decrease in mobilization of body reserves, especially for adipose tissue after a CLA induced MFD (Perfield et al. 2002; Moore et al. 2004; Castañeda-Gutiérrez et al. 2005; Suksombat and Chullanandana 2008; Sippel et al. 2009; Medeiros et al. 2010). However, in a trial by Odens et al. (2007) CLA supplementation diminished the BW and BCS loss and

Hutchinson et al. (2011) observed an improvement of BCS after CLA treatment. But these variables seem insufficient to evaluate the mobilization of body reserves, since no redistribution is detectable and no differentiation into fat and protein mobilization or accretion is possible. Results from literature suggest that CLA induced body fat reduction is often associated with increased whole body protein, water and ash relative to controls (Park et al. 1997).

In contrast to the results obtained from investigations with mice and rats, first studies with dairy cows showed that simultaneously with a CLA induced milk fat depression the expression of key enzymes and regulators of lipid synthesis were upregulated in adipose tissue (Harvatine et al. 2009). During this trial 7.5 g/d of the *trans*-10, *cis*-12 isomer were abomasaly infused for 4 days and in this time a higher relative mRNA abundance could be shown for FASN, LPL, SCD, FABP4, SREBP1, thyroid hormone responsive spot 14 (S14) and PPARγ. The results indicate that the energy spared from the reduction in milk fat synthesis is possibly partitioned towards adipose tissue, although the CLA supplemented animals had a significant lower DMI. A current dietary study from von Soosten et al. (2011) with primiparous dairy cows showed after a CLA treatment of 6 g/d for 105 d *post partum* that the mobilization of the retroperitoneal adipose depot was decelerated during the first 42 days in milk (DIM). This finding coincides with the expectation of energy sparing. But for the same animals Akter et al. (2011) observed a numeric decreased adipocyte size of retroperitoneal fat depot after 42 DIM and a significant decrease after 105 DIM for the CLA animals compared to control. Furthermore, after CLA treatment adipocyte sizes of tailhead, omental and mesenteric fat depots were reduced after 105 DIM and omental and mesenteric after 42 DIM as well. The authors concluded that CLA have lipolytic or antilipogenic effects, or both on adipose tissue. These findings differ from the results of Harvatine et al. (2009), perhaps due to the most likely lower amount of CLA that was duodenal available. Contrary to the findings for mice, no changes in liver weight (von Soosten et al. 2011) or the concentrations of glycogen and triglycerides in the liver (Bernal-Santos et al. 2003) were found for CLA treated cows.

Taken together the results obtained from investigations with dairy cows were inconsistent which demand further research.

4.5 *Insulin resistance*

Insulin resistance is defined as a state in which normal concentrations of insulin produce a less than normal biological response. Two forms are described: a decreased insulin sensitivity attributed to a high dose of insulin required to produce a half-maximal response in blood glucose concentration and a decreased insulin responsiveness associated with a low maximal blood glucose response to a high dose of insulin (Kahn 1978). Insulin has numerous roles in the metabolism of carbohydrates, lipids and proteins. During late gestation (transition period) and early lactation the provision of glucose for uterine or mammary utilization is a metabolic priority for the dairy cow. Lowered responsiveness and sensitivity of extrahepatic tissues, like adipose and muscle tissue to insulin and a high gluconeogenesis rate in liver are adaption mechanisms of the cow to meet the increasing glucose requirement *post partum*. Thereby a better insulin-independent GLUT-1 mediated glucose uptake of placenta and mammary gland is possible (Bell and Bauman 1997). Changes in metabolic status and the often lacking supply of nutrients in this period predispose dairy cattle to develop hepatic lipidosis and ketosis. Such metabolic disorders were highly associated with insulin resistance of peripheral tissues (Oikawa and Oetzel 2006; Hayirli 2006). There is evidence in literature that dietary CLA supplementation causes insulin resistance in rodents and humans. In particular the application of *trans*-10,*cis*-12 CLA or a mixture of the *trans*-10,*cis*-12 and the *cis*-9,*trans*-11 isomer have been shown to increase insulin resistance (Tsuboyama-Kasaoka et al. 2000; Risérus et al. 2002; Halade et al. 2009). Nevertheless, Choi et al. (2004) showed in a trial with rats that both isomers were able to decrease insulin resistance.

In most studies with dairy cows the plasma concentrations of insulin or glucose were determined after the long term dietary intake of CLA to draw conclusions of insulin resistance but no effects were detected (Perfield et al. 2002; Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castañeda-Gutiérrez et al. 2005; Sigl et al. 2010; Lin et al. 2010; Hutchinson et al. 2011). Only in a trial by Odens et al. (2007) the supplementation of a CLA mixture of four isomers slightly increased (11%) circulating glucose levels, possibly indicating a decrease in insulin sensitivity and giving a reason for higher milk yields of CLA treated cows. Baumgard et al. (2002a) determined the basal glucose concentration and glucose response to an insulin challenge after the short-time abomasal infusion of 13.6 g *trans*-10,*cis*-12 CLA/d and observed no effect. In a current *in vivo* investigation of Haarstrich (2011) dairy cows were fed 50 and 100 g of a CLA supplement containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA. Animals receiving the highest CLA dose showed a reduction of whole body insulin sensitivity; however, peripheral insulin response appears not to be affected.

4.6 Reproduction performance

A negative energy balance during early lactation is a common problem in dairy cow management and associated with health problems that can impact milk production (Drackley 1999) and reproduction performance (Lucy et al. 1992; Wlodarek et al. 2011). The relationship between energy balance and *post partum* reproductive activity is confirmed by longer intervals to first ovulation in cows with more days to the energy balance nadir (Beam and Butler 1999). Collard et al. (2000) showed a significant positive relationship between the number of days with negative net energy balance and occurrence of reproductive problems.

Since there is evidence in literature that especially the *trans*-10,*cis*-12 CLA is able to influence the net energy balance of early lactating dairy cows by reducing milk fat yield (Moore et al. (2004), see chapter 4.3), investigations about CLA effects on reproductive variables are of great interest. In one of the first studies with dietary CLA supplementation to dairy cows by Perfield et al. (2002) a mixture of four CLA isomers was fed to pregnant animals (200 d prior to calving) and gestation length, process of parturition and average birth weight were included in the evaluation. No differences were observed between the treatment groups for the mentioned variables. Bernal-Santos et al. (2003) examined the influence of a CLA supplementation in the first 20 weeks of lactation on days to first ovulation, conception and maintenance of pregnancy and all numerical changes in reproductive variables were positive. Even Castañeda-Gutiérrez et al. (2005) investigated these variables after 11 weeks of CLA treatment *post partum* in a dose response study (0, 9 and 18 g *trans*-10,*cis*-12 CLA/d). In this study just positive numerical changes were observed for the CLA groups for days to first ovulation and the proportion of cows pregnant before 185 DIM, but the effect was more pronounced for the medium dose of CLA. The percentage of pregnant cows before 126 DIM was numerically greater compared to control and similar for both CLA groups. A recent investigation by Medeiros et al. (2010) confirmed the positive trends without showing clearly significant results for earlier *post partum* ovulation, time to conception and overall success of pregnancy after CLA treatment, whereas Moallem et al. (2010) observed no beneficial effect on reproduction. Hutchinson et al. (2011) fed a 50:50 mix of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA resulting in a daily intake of 6 g of each isomer. For the CLA supplemented cows services per conception tended to be reduced but there was no effect on the *post partum* interval to first ovulation.

Regarding the mode of action of CLA, besides the possible improvement of energy status by CLA the positive influence of these fatty acids may relate to the impact of reproductive hormones, since it had been shown that dietary fatty acids alter the synthesis of prostaglandins (Mattos et al. 2000). To evaluate the mechanism of action of CLA isomers on reproduction in

dairy cows Castañeda-Gutiérrez et al. (2007) fed two different CLA mixtures: CLA75:25 providing 7.1 g *cis*-9,*trans*-11 CLA and 2.4 g *trans*-10,*cis*-12 CLA/d and CLA50:50 providing 7.1 g of each isomer/d during the interval from 20 (±1) DIM to 56 (±1) DIM. There was no treatment effect on net energy balance. However, insulin-like growth factor 1 (IGF-I) was greater, progesterone during the early luteal phase and the estradiol:progesterone ratio in the follicular fluid tended to be greater in cows supplemented with CLA50:50. The results suggest that CLA may improve endocrine signals that can be beneficial for reproduction. These benefits seem to be associated with the *trans*-10,*cis*-12 isomer. Moreover, the improvement of reproduction parameters seems to be independent from energy sparing effects of CLA.

In a long-term supplementation study by Onnen-Lübben (2009) the cows received graded amounts of CLA (10 and 20 g/d of a mix consisting of equal amounts of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA) from the first to the 182. DIM. In estrus cycle and early pregnancy the effect of CLA on corpus luteum function was evaluated. The graded supplementation with CLA had almost no effect on corpus luteum sizes, luteal blood flow areas, luteal cells/mm^2 and progesterone levels. Only the IGF-I plasma concentration increased significantly for the highest supplementation group in estrus cycle and for both CLA groups during early pregnancy. However, in a recent *in vitro* study May et al. (2011) detected that CLA decreases the prostaglandin synthesis by luteal cells via attenuation of cyclooxygenase (COX-2) gene expression.

In all reviewed studies the authors noted that the validity of results is limited by the number of cows. De Veth et al. (2009) combined data from 5 controlled studies in which CLA had been supplemented to early-lactation dairy cows (Bernal-Santos et al. 2003; Castañeda-Gutiérrez et al. 2005; de Veth et al. 2005b; Castañeda-Gutiérrez et al. 2007; Mann et al. 2007) and three of these studies were already mentioned above. The association of CLA with time to first ovulation, time to conception (survival analysis) and overall success of pregnancy (logistic regression) were evaluated for 212 animals which received 0 to 63.2 g CLA/d (0 – 18.3 g *trans*-10,*cis*-12 CLA/d). The data showed that the probability of cows becoming pregnant increased and time to ovulation as well as time to first conception decreased after *trans*-10,*cis*-12 CLA supplementation independent of CLA dosage, whereas the optimal dose predicted to be 10.1, 8.0 and 10.5 g/d, respectively.

4.7 Immune function

Immune suppression often occurs during the transition period of dairy cows. Metabolic disorders, such as milk fever and ketosis, exacerbate immune suppression at this production stage. Studies have shown that CLA might have immune enhancing properties. Furthermore there is evidence that the two principal isomers, *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA, have distinct functionalities (O`Shea et al. 2004). However, findings in literature are often contrary and the knowledge is lacking concerning the mechanism by which CLA act. For fatty acids, including CLA different modes of action are conceivable: the regulation of arachidonic acid metabolism, possibly in part by competing with linoleic acid in the biosynthesis of arachidonic acid (Ha et al. 1987), by modifications of the membrane fluidity as well as by the transcriptional regulation of gene expression e.g. by PPAR (Kang et al. 2007). By this means, CLA are able to influence the production of different mediators of the immune system like cytokines (Hayek et al. 1999; Kelley et al. 2002; Yamasaki et al. 2003a; Ramírez-Santana et al. 2009), immunoglobulins (Sugano et al. 1998; Yamasaki et al. 2003a; Corino et al. 2009) and eicosanoids (Sugano et al. 1997; Sugano et al. 1998; Stachowska et al. 2009) and to affect the metabolism of different immune cells (Hayek et al. 1999; Kang et al. 2007). In general it is anticipated that the addition of CLA to the diet resulted in an enhanced immune response.

The impact of CLA on the immune system of ruminants, in particular dairy cows, is not well investigated. Only a few studies exist. Castro et al. (2006) observed that the serum IgG concentration after parturition in goats was increased after the addition of 12 g CLA mix/kg feed to the diet from the third month of gestation, whereby the colostrum IgG concentration remained unaffected. Lambs consuming a diet supplemented with 2.5 and 10 g CLA mix/kg feed showed no treatment effect on the immune response to ovalbumine vaccine (Terré et al. 2011). Odens et al. (2007) detected the IgG concentration in colostrum of dairy cows fed 9 days *post partum* with 30 g/d of a CLA mixture and no differences to control were observed. A recent *ex vivo* investigation of Renner et al. (2011) examined the effects of the supplementation of 6 g CLA/d to the diet of dairy cows on proliferation and cytokine expression of peripheral blood mononuclear cells (PBMC) and splenocytes. The function of bovine PBMC was not affected in this trial, but the ability to stimulate splenocytes treated with Concanavalin A was reduced after CLA feeding. Results of the examination of cytokine expression (IL-4, IL-10, IL-12, TNF-α, IFN-γ) were rather inconsistent. Hussen et al. (2011) found that feeding 10 g CLA/d to dairy cows caused changes in the composition of PBMC whereby the CD4/CD8 ratio was decreased. Furthermore a reduction of the IgG1 and IgG2 concentration in serum was observed, indicating an effect on B-cell differentiation into

plasma cells. The proliferation of bovine PBMC was inhibited after an *in vitro* incubation with *cis*-9,*trans*-11 CLA in a trial by Hussen and Schuberth (2011).

5 Potential effects of CLA on human health

The natural daily CLA intake for humans is difficult to record as CLA content of foodstuffs fluctuates and the intake is influenced by many factors like age, sex and home country. By determining the CLA quantities in foods, Fritsche and Steinhart (1998) calculated a mean intake of 0.35 g CLA/d for women and 0.43 g CLA/d for men in Germany, where 0.24 and 0.28 g/d, respectively, originate from milk and dairy products. Although the effects of CLA supplementation on human health are not well described, results summarized in Table 4 lead to varied efforts to increase CLA in foodstuffs. Nevertheless, dietary CLA supplements for humans are available on the market and dosages of approximately 3 g CLA/d are recommended. The manufacturers advertise with a reduction of total body fat and an increase of lean body mass. The addition of such supplements to the diet would potentially lead to a 10-fold higher CLA intake as compared to the natural intake.

There is evidence that the different CLA isomers have different or even opposite biological effects (Pariza 2004). Most supplements contain *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA at a ratio of 1:1, while with more than 80% the *cis*-9,*trans*-11 isomer is the most abundant CLA isomer in food (Fritsche and Steinhart 1998). Therefore the supplementation of 3 g CLA preparation leads to extremely high intakes of the *trans*-10,*cis*-12 isomer. However, recent long-term clinical studies have reported that the supplementation of 3.4 g of a CLA mixture/d for 12 – 24 months is well tolerated in healthy, overweight humans (Gaullier et al. 2004; Gaullier et al. 2005). If adverse events associated with CLA supplementation were reported, they were rated as “mild” or “moderate”, showing evidence for safety in humans. Since only a few long-term clinical trials have been performed, CLA supplements are further considered critically.

Various physiological effects of CLA are described in literature in dependence of animal species, applied dosage and the CLA isomers used (Table 4). Details concerning the potential effects of CLA on immune function, body composition and insulin resistance were already given in chapter 4, since these effects are of great interest for dairy cows, too.

There is a growing interest in the antiatherogenic properties of CLA, as lifestyle-related diseases, such as hyperlipidemia and arteriosclerosis are widespread in industrialized countries. Different animal models have been frequently used to show that CLA causes a serum lipoprotein profile considered to be less atherogenic (Lee et al. 1994; Stangl 2000a) and that animals fed the CLA-containing diet exhibited less atherosclerotic plaque formation (Lee

et al. 1994; Kritchevsky et al. 2000). Thereby, the *cis*-9,*trans*-11 isomer seems to be ineffective (Gavino et al. 2000) and the *trans*-10,*cis*-12 isomer seems to be the active isomer affecting blood lipid levels in hamsters (de Deckere et al. 1999). However, animal studies have failed to clearly show beneficial effects of supplementation with CLA on markers of atherosclerosis like plasma lipid profile (Stangl et al. 1999; Munday et al. 1999; Kostogrys et al. 2011). The underlying mechanisms of action are only poorly understood. Ringseis and Eder (2009) summarized results from *in vitro* studies dealing with the effects of CLA isomers and CLA mixtures on functional properties of cells contributing to atherosclerotic lesion development. They concluded that the CLA exert several beneficial actions in endothelial cells, smooth muscle cells and monocyte-derived macrophages through the activation of nuclear PPAR.

During the 1980s, CLA was identified as a new anticancerogen from fried ground beef, which inhibits tumor development (Ha et al. 1987). Further animal studies showed that CLA reduced the development of chemical induced tumors in different tissues (Ip et al. 1991; Belury et al. 1996). The inhibitory effects of CLA on the growth of various human cancer cells including skin, colorectal and breast have also been studied *in vitro (*Shultz et al. 1992).

Table 4: History of discovery of the physiological effects of CLA (according to Kraft (2003), modified)

Physiologic effects of CLA	References
anticancerogenic	Ha et al. (1987)
immunemodulating	Cook et al. (1993)
antiatherogenic	Lee et al. (1994)
alteration of body composition	Park et al. (1997)
bone mass-modulating	Seifert and Watkins (1997)
antidiabetic	Houseknecht et al. (1998)
antithrombotic	Truitt et al. (1999)

Only a few clinical trials investigating the effects of CLA (naturally occurring or supplemented) in humans are available in literature.

Results of both *in vitro* and *in vivo* studies have shown that the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomer have anticarcinogenic effects in a range of human breast cells and tumors as summarized by Kelley et al. (2007). The few epidemiological human studies have yielded far less conclusive results than studies conducted in animals. In a case-control study in France no association between CLA content in breast adipose tissue and the relative risk of breast cancer could be shown (Chajès et al. 2002). Four studies have investigated dietary intake of CLA and postmenopausal breast cancer; a prospective cohort with Swedish women (Larsson et al. 2009) and the results of the Western New York Exposures and Breast Cancer Study (McCann

et al. 2004) provide no evidence of a protective effect of CLA against breast cancer development, the Netherlands cohort study showed a weak, positive association between CLA intake and breast cancer risk (Voorrips et al. 2002). A Finnish case-control study showed a 60% reduction in risk associated with higher CLA intakes (Aro et al. 2000). Results of the Swedish mammography cohort study suggested that high intakes of high-fat dairy products and CLA may reduce the risk of colorectal cancer (Larsson et al. 2005).

An informative review of clinical trials on humans about the effects of CLA supplements or CLA-enriched products (0.7-4.5 g mixtures of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA/d) on total body weight, body composition, plasma lipid profile, glycemia, insulinemia, insulin sensitivity, lipid oxidation and inflammation was published by Salas-Salvadó et al. (2006). They summarized 21 studies published between 2000 and 2005 which were controlled with placebo and concluded that human studies on how CLA affects body composition and metabolism are not consistent and there is not enough evidence to show that CLA has an effect on these variables in humans. Furthermore they observed that in some of these trials the intake of CLA mixtures had adverse effects on lipid profile (decrease of HDL cholesterol, increase of Lp(a)), glucose metabolism (decrease of insulin sensitivity), lipid oxidation, inflammation, or endothelial function. During the past 5 years human studies continue to report inconsistent effects of CLA on human health. An opinion of the European Food Safety Authority (EFSA) from 2010 addresses the scientific substantiation of health claims in relation to an equimolar mixture of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA and contribution to the maintenance or achievement of a normal body weight, increase in lean body mass, increase in insulin sensitivity, protection of DNA, proteins and lipids from oxidative damage, and contribution to immune defences by stimulation of production of protective antibodies in response to vaccination (EFSA 2010). The Panel concludes that a cause and effect relationship has not been established between the consumption of CLA and the mentioned claimed effects.

SCOPE OF THE THESIS

Up to now a lot of studies were conducted regarding the milk fat depressing properties of CLA, especially *trans*-10,*cis*-12 CLA in dairy cow diets. However, the data available from literature about treatment and post-treatment effects of CLA, particularly after long-term supplementation, were rare and the results regarding energy metabolism were inconsistent. Additionally, literature data did not clearly demonstrate if CLA supplementation is a potent method to increase the CLA content in milk. Furthermore, there is a lack of information about the effects of dietary supplementation of CLA on rumen metabolism and about the actual quantity of absorbable CLA in the duodenum, as it is assumed that the protection is not 100%.

Considering these gaps of knowledge on CLA effects and metabolism, the aim of this thesis is to answer the following questions:

1. Which effects does the long-term addition of lipid-encapsulated CLA to dairy cow diets have on performance and variables of energy metabolism depending on dosage of CLA preparation?
2. Are there post-treatment effects observable after a long-term CLA supplementation?
3. What is the impact of a CLA supplementation on rumen fermentation and duodenal nutrient flow?
4. How much of the supplemented amount of *trans*-10,*cis*-12 CLA is actually available at the duodenum? How strong is the actual rumen protection of the lipid-encapsulated CLA preparation?
5. How does supplemental CLA alter the fatty acid profile of milk? Does the dietary CLA supplementation lead to higher CLA contents in milk fat?

Two experiments concerning the CLA effects on dairy cows were carried out to answer these questions. The lipid-encapsulated CLA supplement (Lutrell® pure, BASF SE, Ludwigshafen, Germany) was fed in two different dosages: 50 g (CLA-1) or 100 g (CLA-2)/d containing 10% of each CLA isomer (*trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA). In the control group (CON) the CLA in the diet were substituted by stearic acid to reach similar fat intakes.

In the first study, cows were supplemented with CLA for 182 days *post partum*. Long-term and post-treatment effects of CLA on performance, fatty acid profile in milk fat, calculated net energy balance and plasma levels of non esterified fatty acids (NEFA) and

ß-hydroxybutyrate (BHB) as variables meaningful for energy metabolism were examined (**Paper I**).

In a second experiment the CLA supplement was fed to ruminally and duodenally fistulated dairy cows to determine the duodenally available amount of the *trans*-10,*cis*-12 isomer. Furthermore, the effects of supplemental CLA on rumen metabolism, the duodenal nutrient flow and the fatty acid profile of milk fat were investigated (**Paper II**).

Paper I

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

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Abstract

The supplementation of conjugated linoleic acids (CLA) to the diet of dairy cows represents an opportunity to reduce the content of milk fat. Therefore, CLA has the potential beneficial effect of reducing energy requirements of the early lactating cow. The present study aimed at the examination of long-term and post-treatment effects of dietary CLA intake on performance, variables of energy metabolism like plasma levels of NEFA and BHB, and fatty acid profile in milk fat. Forty-six pregnant German Holstein cows were assigned to one of three dietary treatments: 1) 100 g/d of control fat supplement (Control), 2) 50 g/d of control fat supplement and 50 g/d of CLA supplement (CLA-1) and 3) 100 g/d of CLA supplement (CLA-2). The lipid-encapsulated CLA supplement consisted of approximately 10% of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA each. The experiment started 1 d after calving and continued for about 38 weeks, divided into a supplementation (26 weeks) and a depletion period (12 weeks). Over the first 7 weeks of treatment, 11 and 16% reductions in dry matter intake compared to control were observed for the cows fed the CLA supplements, respectively. Consequently, the calculated net energy balance was lower for these two CLA-groups compared to the control. Plasma levels of NEFA and BHB remained unaffected. Later in lactation the highest CLA supplementation resulted in a reduction of milk fat content by 0.7%-points. However, no reduction in milk fat yield accordingly no milk fat depression (MFD) could be shown. The *trans*-10, *cis*-12 CLA in milk fat increased with increasing dietary CLA supplementation in a dose-dependent manner. The proportion of C16 in milk fat was decreased by the highest CLA supplementation. With exception of an increase of plasma glucose level in the CLA-2 group no post-treatment effects were observed. Overall, under the conditions of the present study no improvement of the calculated net energy balance by CLA supplementation could be shown for the entire evaluation period.

Keywords: conjugated linoleic acid; dairy cow; dry matter intake; energy balance; fatty acid

1 Introduction

During the transition period the energy intake of dairy cows is often inadequate to meet the requirements for maintenance and milk synthesis and the animals compensate this gap through the mobilization of adipose depots. In consequence, they experience a negative energy balance (Drackley 1999). In order to prevent potential negative consequences on health and reproduction, the objective of prepartal feeding is an optimal body condition of the cow at time of parturition. Furthermore, the saving of energy at the onset of lactation can cause an improvement of the energy balance. The milk fat represents the major energy cost in

production of milk components and is the most variable component in milk of ruminants. In addition to diet induced reduction of milk fat production (milk fat depression (MFD)) by low fiber diets or marine oil diets (Bauman and Griinari 2003), the use of dietary conjugated linoleic acid (CLA) supplements is known to be a possibility to reduce the milk fat yield in dairy cows (Bernal-Santos et al. 2003, Selberg et al. 2004, Castaneda-Gutierrez et al. 2005). After abosomal infusion of pure CLA isomers, *trans*-10, *cis*-12 CLA was identified as the main isomer responsible for MFD (Baumgard et al. 2000). The involved mechanisms are based on the inhibition of the mammary expression of enzymes necessary for the synthesis of milk fat (Baumgard et al. 2002b). Due to a CLA induced reduction of milk fat yield the fatty acid pattern of milk changed. The *trans*-10, *cis*-12 CLA supplementation resulted in a more dramatic decrease in fatty acids originating from *de novo* synthesis (<C16) compared with preformed fatty acids (>C16) (Chouinard et al. 1999, Baumgard et al. 2000, Kraft et al. 2001, Shingfield et al. 2009). In a study by Sippel et al. (2009) who fed up to 51 g *trans*-10, *cis*-12 CLA/d for 4 weeks in mid lactation the milk fat content decreased dose-dependently by up to 39% while milk yield and other milk components were unaltered. Consequently, the calculated net energy balance was improved for the treatment groups. Moore et al. (2004) and Odens et al. (2007) observed after feeding of 37 and 30 g *trans*-10, *cis*-12 CLA for 3 and 6 weeks p.p., respectively, a decrease in milk fat content as well, but the concomitant numeric increase in milk yield resulted in an unaltered calculated net energy balance. Post-supplementation effects were only determined after short-term supplementation (9 weeks p.p.) of CLA from Castaneda-Gutierrez et al. (2005) and they observed no influence of CLA on the performance parameters after end of supplementation.

The milk fat depressing properties of CLA, especially *trans*-10, *cis*-12 CLA, and their effects on fatty acid pattern of milk fat of dairy cows are well documented. However, long-term effects and post-treatment effects after long-term supplementation have not been investigated yet and the results regarding energy metabolism were inconsistent. Therefore, the present study aimed at the examination of these effects on performance, fatty acid profile in milk fat, calculated net energy balance and plasma levels of non esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) as variables meaningful for energy metabolism.

2 Materials and methods

2.1 Animals, treatments and experimental design

The study was carried out at the experimental station of the Friedrich-Loeffler-Institute (FLI) in Braunschweig. The experiment was conducted according to the European Community

regulations concerning the protection of experimental animals and the guidelines of the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Germany, File Number 33.14.42502-04-071/07). Forty-six pregnant German Holstein cows were assigned to one of three dietary treatments according to the mean live weight (627 ±9 kg, only cows), mean number of lactation (1.9 ±0.1) and milk yield of previous lactation (5797 ± 122 kg, 200-day milk yield): (1) 100 g/d of control fat preparation (CON, n=15); (2) 50 g/d of CLA preparation and 50 g/d of the control fat preparation (CLA-1, n=15); (3) 100 g/d of CLA preparation (CLA-2, n=16). Control fat and CLA preparation were added to the diet in a rumen-protected form. CLA supplementation was started one day after calving and continued for 26 weeks (supplementation period). The experimental period started 3 weeks *ante partum* (a.p.) and lasted until 12 weeks after end of supplementation (depletion period).

During the treatment period the cows were fed a partial mixed ration (PMR) for *ad libitum* consumption consisting of 37% concentrate and 63% silage (60% maize silage, 40% grass silage based on dry matter (DM) content). The composition of the concentrate is presented in Table 1. During the supplementation period each cow additionally received 4 kg concentrate from the concentrate station (TYPE RIC, Insentec, B.V., Marknesse, The Netherlands). The diets were formulated to meet the nutritional requirements of the cows stated by the German Society of Nutrition Physiology (GfE 2001). The concentrate which was included in the PMR consisted of the same components as the concentrate provided by the concentrate station (concentrate CON and CLA) but without the fatty acid supplements (Table 1). Supplemental CLA was included in the diet as a rumen-protected commercial CLA preparation (Lutrell® pure, BASF SE, Ludwigshafen, Germany) and added to the concentrate provided by the concentrate station. The two predominant CLA isomers were *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA (analyzed proportion of both isomers: 12% of total fatty acid methyl esters (FAME)). The concentrate fed to the control group contained a rumen-protected fat preparation (Silafat®, BASF SE, Ludwigshafen, Germany) in which the conjugated linoleic acids were substituted by a corresponding amount of stearic acid (Table 2). During the depletion period the cows received only the PMR, without extra concentrate. The cows were housed in group pens according to their feeding group. The PMR was provided in 15 self-feeding stations (TYPE RIC, Insentec, B.V., Marknesse, The Netherlands) per group. The cows had free access to water.

Table 1: Components and chemical composition of concentrates and partial mixed ration (PMR) (means ± SD).

	Concentrate		PMR
	CON (n=5)	CLA (n=5)	(n=12)
Components [%]			
Wheat	38.50	38.50	
Dried sugar beet pulp	29.00	29.00	
Rapeseed meal	20.00	20.00	
Soybean meal	6.50	6.50	
Soybean oil	1.00	1.00	
Calcium Carbonate	0.50	0.50	
Mineral feed*	2.00	2.00	
CLA supplement	-	2.50	
Control fatty acid supplement	2.50	-	
Dry matter [g/kg]	889 ± 10	887 ± 11	426 ± 20
Nutrients [g/kg DM]			
Total ash	71 ± 5	74 ± 3	69 ± 4
Crude protein	187 ± 1	187 ± 5	118 ± 10
Ether extract	59 ± 3	53 ± 4	32 ± 3
Crude fibre	88 ± 6	89 ± 4	193 ± 11
Acid detergent fibre	123 ± 12	124 ± 10	225 ± 15
Neutral detergent fibre	258 ± 10	256 ± 5	425 ± 17
Energy♦ [MJ NEL/kg DM]	8.8	8.8	6.8
Trans-10, *cis*-12 CLA+ [g/kg DM]	0.02	2.25	0.01

Notes: * Per kg mineral feed: 140 g Ca; 120 g Na; 70 g P; 40 g Mg; 6 g Zn; 5. 4 g Mn; 1 g Cu; 100 mg I; 40 mg Se; 5 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D_3; 1500 mg vitamin E; ♦ Calculation based on nutrient digestibilities measured with wethers (GfE 1991); + Calculation based on analyzed concentrations in concentrates and silages; DM, Dry matter; NEL, Net energy lactation

Table 2: Fatty acid profile of the fat supplements.*

Fatty acid [% of total FAME♦]	CON	CLA
C16:0	10.89	10.89
C18:0	87.30	50.31
C18:1 *cis*-9	<0.01	10.66
Conjugated linoleic acid (CLA)		
C18:2 *cis*-9, *trans*-11	0.06	11.99
C18:2 *trans*-10, *cis*-12	0.02	11.88
Other CLA	0.15	0.95
Other FA	1.58	3.32

Notes: * Supplemental CLA was included in the concentrate portion as a rumen-protected CLA preparation, for the control group conjugated linoleic acids were substituted by stearic acid; ♦ FAME, Fatty acid methyl ester

2.2 Sample collection

Each cow was equipped with an ear transponder recording continuously the daily individual water intake and feed intake. Representative concentrate samples were taken once, grass and maize silage samples twice a week, while PMR samples were collected daily and pooled over approximately 4 weeks.

Cows were milked at 5.30 h in the morning and 15.30 h in the afternoon and the individual milk yield was recorded by the milking system. Milk samples for the analysis of milk composition were taken twice a week in the morning and in the afternoon of the same day.

The milk samples were conserved with bronopol and stored at 8 °C until they were analyzed. For the analysis of the fatty acid profile in milk fat, milk samples of 100 ml were collected twice a day at week 1, 4, 12 p.p., at the end of CLA supplementation, and at week 2 and 10 after terminating the CLA supplementation. The samples were stored at -20 °C until they were freeze dried. The live weight was automatically recorded daily.
The blood samples were obtained from the *Vena jugularis externa*. The date of sampling depended on the time relative to calving (3, 2 and 1 weeks a.p.; 1 day and 1, 2, 3, 5, 7, 10, 15, and 20 weeks p.p.) and the end of CLA supplementation (26 weeks p.p., 1, 2, 4, 6, 8 and 10 weeks after the end of supplementation). Body condition score (BCS) of each animal was recorded at each blood sampling time using a five-point system (Edmonson et al. 1989) by two persons, and the average of these two scores was the assigned value. Back fat thickness (BFT) of each cow was measured at the sacral region by using ultrasound at 3 weeks a.p., 1 day, 3, 10, 15 weeks p.p., at the of supplementation and 2, 6 and 10 weeks after end of supplementation (Staufenbiel 1997). The measuring point was on a line between the upper range of *tuber coxae* and *tuber ischiadicum*.
As the investigations presented in this paper are part of a comprehensive project, additional samples were taken for further analysis. The corresponding results are not presented here, but a systematic effect on the recorded performance parameters cannot be ruled out.

2.3 Analysis

The silage and PMR samples were dried at 60 °C for 72 h. All feed samples were ground to pass through a sieve with 1 mm pore size for analyzing the contents of nutrients according to the methods of the VDLUFA (Bassler 1976).
Milk samples were analyzed for fat, protein, lactose and the somatic cell count using an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 500, Foss Electric, Hillerød, Denmark).
Before analyzing the fatty acid profile in milk fat, the milk samples were heated up to 40 °C and homogenized by Ultra Turrax (T25, JANKE & KUNKEL, IKA®-Labortechnik, Germany) treatment. Afterwards, morning and evening milk were mixed according to their milk yields and freeze dried. The fat-extraction of freeze dried milk was accomplished according to SOXHLET listed in VDLUFA (Bassler 1976). Total milk fat was converted into their FAME by the use of sodium methoxide as catalyst.
The lipid content of the feed samples was extracted according to Folch et al. (1957). After this a transesterification with Boron trifluoride (BF_3) followed, to gain FAME. The emerged

extracts were purified by thin-layer chromatography (SIL G-25 UV_{254}, MACHERY-NAGEL, Germany).
All sample FAME extracts were analyzed via gas chromatography (GC-17A Version 3, Schimadzu, Japan) equipped with an auto sampler and flame ionisation detector. Two different GC procedures were necessary to analyze the FAME profile of these samples. The first GC method determined the identity and general FA profile from 4 to 25 carbon length FA using a medium polarity column (DB-225ms, 60 m x 0.25 mm, i.d.; 0.25 µm; J&W Scientific, Germany). The second GC method separated the *cis* and *trans* isomers of C18:1 using a high polarity column (Select™ FAME, 200 m x 0.25 mm, i.d.; 0.25 µm; VARIAN Inc., Germany). Various reference standards were used as FAME mix to identify FA peaks: No. 463, 674, (NU-CHEK PREP, INC., Elysian, U.S.), BR2, BR4, ME 93 (Larodan; Malmö, Sweden), Supelco® 37 Component FAME Mix, PUFA No. 3, conjugated linoleic acid, linoleic-, linolenic- and octadecenoic acid methyl ester mix (Supelco; Bellefonte, U.S.).
The results were expressed as percentage values in % of total FAME.

The centrifugation of plasma (Heparin and EDTA) and serum was performed after sampling and the samples were stored at -80°C for further analysis. Plasma NEFA and glucose levels were determined by enzymatic analysis using commercial kits (NEFA HR(2) R1+R2 Set, WAKO Chemicals GmbH, Neuss, Germany; Hexokinase Fluid 5+1, mti diagnostics GmbH, Idstein, Germany). Plasma concentrations of BHB were quantified using a commercial kit (RANBUT, RB 1008; Randox Laboratories GmbH, Wülfrath, Germany). These procedures were performed using the Cobas Mira Plus Chemistry Analyzer (F. Hoffmann-La Roche Ltd, Basel, Switzerland).

2.4 Calculations

Net energy lactation (NEL) of the used feedstuffs was calculated by using the nutrient digestibilities from the studies with the wethers according to GfE (1991). Fat-corrected milk (FCM) was estimated according to (Gaines 1928):

$$\text{FCM [kg/d]} = ((\text{milk fat [\%]} \cdot 0.15) + 0.4) \cdot \text{milk yield [kg/d]} \qquad (1)$$

The net energy balance was calculated by subtracting the daily requirement for maintenance (GfE 1991) and the daily requirement for milk production (Tyrrell and Reid 1965) from the daily energy intake.

$$\text{Maintenance requirement [MJ NEL/d]} = 0.293 \cdot \text{Live weight [kg]}^{0.75} \quad (2)$$

$$\text{Energy content of milk [MJ NEL/kg]} = (0.95 + 0.38 \cdot \text{Milk fat [\%]} + 0.21 \cdot \text{Milk protein [\%]}) + 0.07 \quad (3)$$

$$\text{Requirement for milk production [MJ NEL/d]} = \text{Energy content of milk [MJ NEL/kg]} \cdot \text{Milk yield [kg/d]} \quad (4)$$

Daily feed intake, live weight and milk yield values were condensed to weekly means before data analysis. For calculation of net energy balance and yields of milk components the weekly means were used. The proportions of fatty acids in milk fat which were lower than the detection limits were considered as zero in evaluating the data.

2.5 Statistical analysis

Results are presented as least square means (LS means) and standard error (SE) of the mean. All data were analyzed by a one-way ANOVA followed by the Tukey test. Differences were considered to be significant at $p<0.05$, trends were declared at $p<0.1$. All analyses were performed with SAS (Software package, version 9.1, SAS Institute, Cary, NC, USA).

Owing to technical conditions immediately after calving the evaluation of performance parameters and milk components was not possible for the first two weeks p.p.. To examine the distinct physiological stages during lactation in more detail the supplementation period was separated for analysis into two periods: Period 1: week 2 – 7 and Period 2: week 8 – 26.

Milk yield, milk components, dry matter intake (DMI), net energy intake, calculated net energy balance and change in live weight were analyzed by using the PROC MIXED procedure with a compound symmetry covariance structure. The model contained supplementation and week of lactation as fixed factors and the interaction between both factors. The individual cow effects resulting from the frequent measurements in the course of the experiment were considered by the repeated procedure.

Change of BCS and BFT, plasma levels of NEFA, BHB and glucose as well as milk fatty acid profiles were analyzed using the PROC MIXED procedure. Supplemental CLA was

considered as a fixed effect. The influence of the individual cow on the data was considered in the model as random effect.

3 Results

Out of 46 cows, 43 completed the full 38 weeks of treatment. Two cows were removed from the trial due to sickness (distorsion (week 8, CON), displacement of abomasum (week 26, CLA-1)), and one cow had to be taken out of the experiment due to serious mastitis problems (week 26, CLA-1).

Only slight differences in the contents of nutrients were detected between the two different concentrate feeds (Table 1). The concentration of the *trans*-10, *cis*-12 CLA isomer in concentrates and silages was calculated based on the analyzed concentrations in these feed components. In the CLA concentrate the concentration of *trans*-10, *cis*-12 CLA amounted to 2.25 g/kg DM. Thus, CLA-1 and CLA-2 group had an intake of 4 and 8 g *trans*-10, *cis*-12 CLA/d, respectively. The analyzed proportion of *trans*-10, *cis*-12 CLA in the applied rumen-protected CLA preparation amounted to 12% of total FAME (Table 2).

Performance data for the three periods are presented in Table 3. As expected for a cow trial including nearly the complete lactation period, most measured parameters markedly changed over the lactation period. The development of the dry matter intake (DMI) over the course of the trial is shown in Figure 1. The recorded DMI in the 2nd and 3rd week *ante partum* was similar in the three groups (CON: 13.98 kg/d, CLA-1: 14.50 kg/d, CLA-2: 13.95 kg/d). During the whole treatment period each cow daily consumed the 4 kg of concentrate feed containing the respective fat supplements.

In Period 1 of supplementation the control group consumed significantly more total DM (11% - 16%) than the two CLA groups. There was a significant supplementation x week of lactation interaction during the whole supplementation period because cows fed CLA had a more pronounced increase of DM intake in the second week of lactation. No differences in the DMI were observed between the treatment groups in Period 2 of supplementation and depletion period.

At the beginning of recording the milk yield there were no significant treatment differences (Figure 2). After the third week of lactation the cows fed CLA had a steeper increase in milk yield compared to the control group resulting in a significant supplementation x week of lactation interaction. Overall, the contents of fat and protein, content and yield of lactose and somatic cell count (SCC) were not affected by treatment.

Over Period 2 of supplementation, CLA supplementation caused a 7 and 12% dose dependent significant reduction in milk fat content of CLA-1 and CLA-2 group, respectively (Figure 3). After finishing the supplementation the milk fat content of the previously CLA-groups reached the same level like the control group. There was a significant supplementation x week of lactation interaction due to the reduction of milk fat content in the first weeks of lactation and the increase after end of supplementation. A tendency in reduction of milk protein content (5%, p=0.088) was observed for the highest supplementation group (CLA-2). There was a significant supplementation x week of lactation interaction in Period 2 of supplementation because of the increase of milk protein content in CLA-2 group. The control group had a significant higher NEL intake in Period 1 of supplementation in comparison to the two CLA groups. Live weight (LW) remained unaffected by the CLA supplementation and all groups showed an almost linear increase of the live weight with similar weekly LW gains. Calculated net energy balance declined significantly (CLA-1: -27 MJ/d; CLA-2: -23 MJ/d) by CLA feeding compared with controls in the first weeks of lactation (Period 1) (Figure 4). After 7 weeks of lactation the animals of the CLA-groups reached a balanced energy balance, whereas the control group had a positive calculated net energy balance during the entire evaluation period.

Table 3: Milk yield and milk composition and energetic variables in Period 1 (early lactation, week 2 - 7 of lactation), Period 2 (until end of CLA supplementation, week 8 - 26 of lactation) and Period 3 (depletion period, 12 weeks) (LS means ± SE).

	Supplementation groups			*p*-value		
Parameter	CON	CLA-1	CLA-2	SUP	Week	SUP x Week
PERIOD 1	(n=15)	(n=15)	(n=16)			
DMI [kg/d]	21.1^{a} ±0.7	18.5^{b} ±0.7	17.8^{b} ±0.7	0.003	<0.001	<0.001
Milk yield [kg/d]	30.4 ±2.1	32.4 ±2.1	30.9 ±2.0	0.768	<0.001	0.035
Milk fat						
[%]	4.43 ±0.15	4.48 ±0.15	4.24 ±0.14	0.492	<0.001	0.024
[kg/d]	1.37 ±0.12	1.48 ±0.12	1.32 ±0.11	0.630	0.306	0.380
FCM [kg/d]	33.6 ±2.6	36.0 ±2.6	32.7 ±2.6	0.653	0.015	0.802
Milk protein						
[%]	3.12 ±0.05	3.11 ±0.05	3.14 ±0.04	0.897	<0.001	0.849
[kg/d]	0.94 ±0.06	1.00 ±0.06	0.96 ±0.06	0.735	0.001	0.260
Milk lactose						
[%]	4.81 ±0.04	4.77 ±0.04	4.81 ±0.04	0.790	<0.001	0.216
[kg/d]	1.46 ±0.10	1.56 ±0.10	1.49 ±0.09	0.719	<0.001	0.076
SCC [log10/ml]	4.85 ±0.13	5.06 ±0.13	4.93 ±0.12	0.511	0.011	0.876
Net energy intake [MJ/d]	142.8^{a} ±4.3	126.3^{b} ±4.3	121.9^{b} ±4.2	0.003	<0.001	<0.001
Live weight [kg]	526 ±16	549 ±16	531 ±15	0.587	<0.001	0.500
Energy balance [MJ/d]	14.9^{a} ±5.4	-12.3^{b} ±5.4	-8.3^{b} ±5.2	0.002	<0.001	0.039
PERIOD 2	(n=14)	(n=15)	(n=16)			
DMI [kg/d]	21.6 ±0.6	22.4 ±0.6	21.2 ±0.6	0.404	0.033	0.050
Milk yield [kg/d]	30.2 ±1.6	34.2 ±1.6	33.0 ±1.6	0.223	<0.001	0.672
Milk fat						
[%]	4.11^{a} ±0.13	3.81^{ab} ±0.13	3.60^{b} ±0.12	0.021	<0.001	0.562
[kg/d]	1.23 ±0.07	1.30 ±0.07	1.19 ±0.07	0.515	<0.001	0.239
FCM [kg/d]	30.9 ±1.7	33.7 ±1.7	31.4 ±1.7	0.484	<0.001	0.314
Milk protein						
[%]	3.24 ±0.05	3.14 ±0.05	3.09 ±0.05	0.088	<0.001	0.044
[kg/d]	0.97 ±0.05	1.07 ±0.05	1.01 ±0.05	0.352	<0.001	0.850
Milk lactose						
[%]	4.81 ±0.03	4.79 ±0.03	4.83 ±0.03	0.683	<0.001	0.111
[kg/d]	1.45 ±0.07	1.64 ±0.07	1.59 ±0.07	0.193	<0.001	0.825
SCC [log10/ml]	4.85 ±0.12	5.08 ±0.12	4.97 ±0.11	0.412	0.187	0.796
Net energy intake [MJ/d]	144.3 ±3.8	148.8 ±3.8	141.7 ±3.7	0.410	0.003	0.018
Live weight [kg]	567 ±15	591 ±15	576 ±15	0.521	<0.001	0.987
Energy balance [MJ/d]	15.3 ±2.7	10.4 ±2.6	10.8 ±2.5	0.359	<0.001	0.031
PERIOD 3	(n=14)	(n=13)	(n=16)			
DMI [kg/d]	19.2 ±0.5	19.6 ±0.5	18.9 ±0.5	0.621	<0.001	0.136
Milk yield [kg/d]	24.0 ±1.1	25.3 ±1.1	24.5 ±1.0	0.665	<0.001	0.999
Milk fat						
[%]	4.16 ±0.12	4.21 ±0.12	4.26 ±0.11	0.849	<0.001	<0.001
[kg/d]	0.99 ±0.05	1.07 ±0.05	1.04 ±0.05	0.568	<0.001	0.011
FCM [kg/d]	24.7 ±1.2	26.5 ±1.2	25.8 ±1.1	0.518	<0.001	0.432
Milk protein						
[%]	3.38 ±0.05	3.34 ±0.05	3.34 ±0.05	0.803	<0.001	0.153
[kg/d]	0.80 ±0.03	0.84 ±0.03	0.81 ±0.03	0.680	<0.001	0.923
Milk lactose						
[%]	4.73 ±0.03	4.74 ±0.03	4.73 ±0.03	0.963	<0.001	0.273
[kg/d]	1.14 ±0.05	1.2 ±0.05	1.16 ±0.04	0.612	<0.001	0.999
SCC [log10/ml]	4.97 ±0.12	5.10 ±0.12	5.11 ±0.11	0.637	0.003	0.759
Net energy intake [MJ/d]	124.5 ±3.4	127.0 ±3.4	122.3 ±3.2	0.610	0.391	0.164
Live weight [kg]	609 ±16	627 ±16	613 ±15	0.688	<0.001	0.980
Energy balance [MJ/d]	12.1 ±2.1	8.9 ±2.1	7.4 ±1.9	0.251	<0.001	0.586

Notes: ^{ab} Values with different superscripts within one period and within one are significantly different ($p < 0.05$); CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA supplement/d; CLA-2, Cows fed 100 g of the CLA supplement/d; SUP, Supplementation; FCM, 4% fat corrected milk; SCC, Somatic Cell Count; DMI, Dry matter intake

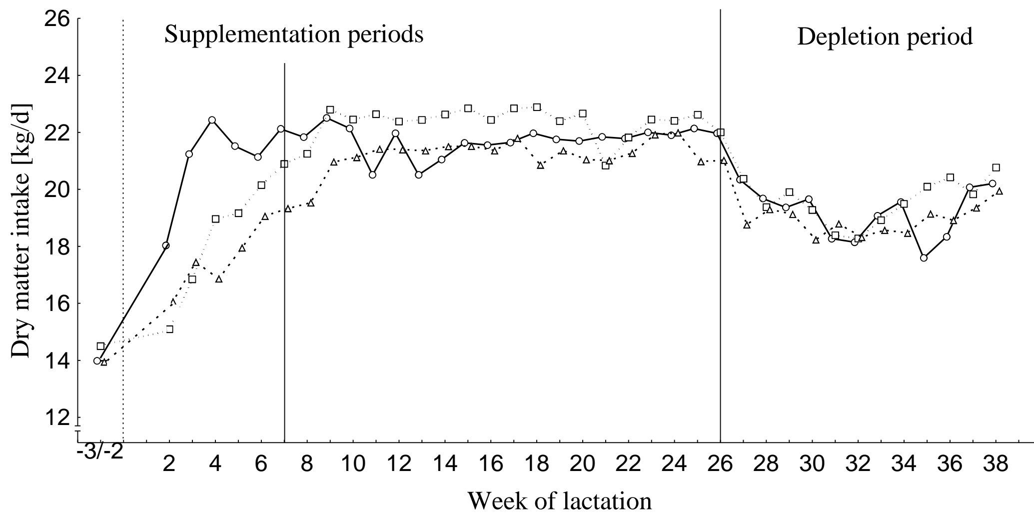

Figure 1: Development of dry matter intake (means) in supplementation and depletion periods for the feeding groups
Control group (n=15/14), CLA-1 group (n=15/13), CLA-2 group (n=16)

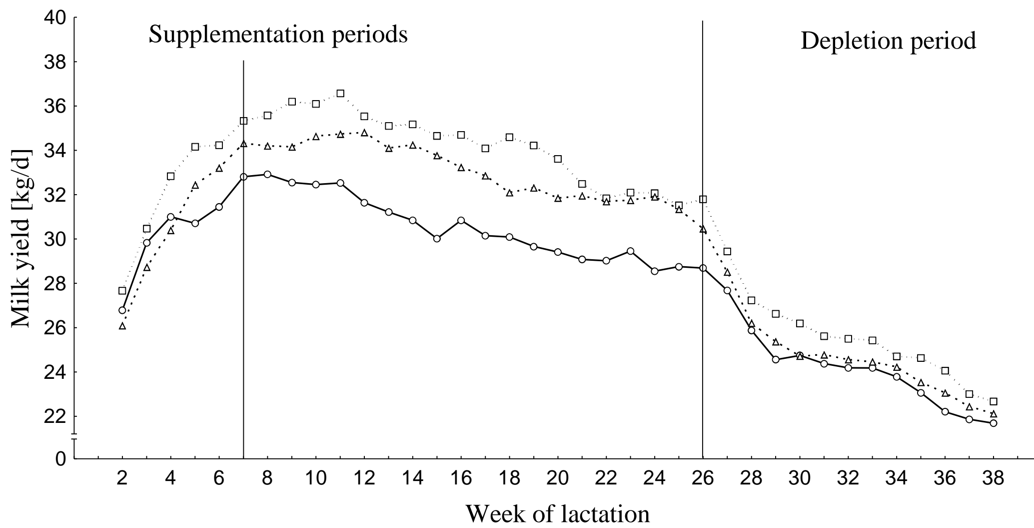

Figure 2: Development of milk yield (means) in supplementation and depletion periods for the feeding groups
Control group (n=15/14), CLA-1 group (n=15/13), CLA-2 group (n=16)

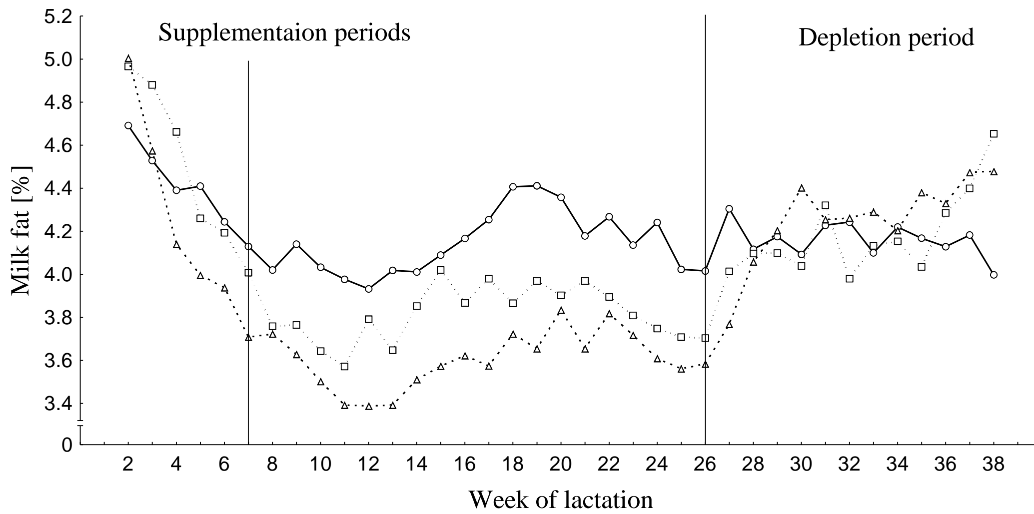

Figure 3: Development of milk fat content (means) in supplementation and depletion periods for the feeding groups
Control group (n=15/14), CLA-1 group (n=15/13), CLA-2 group (n=16)

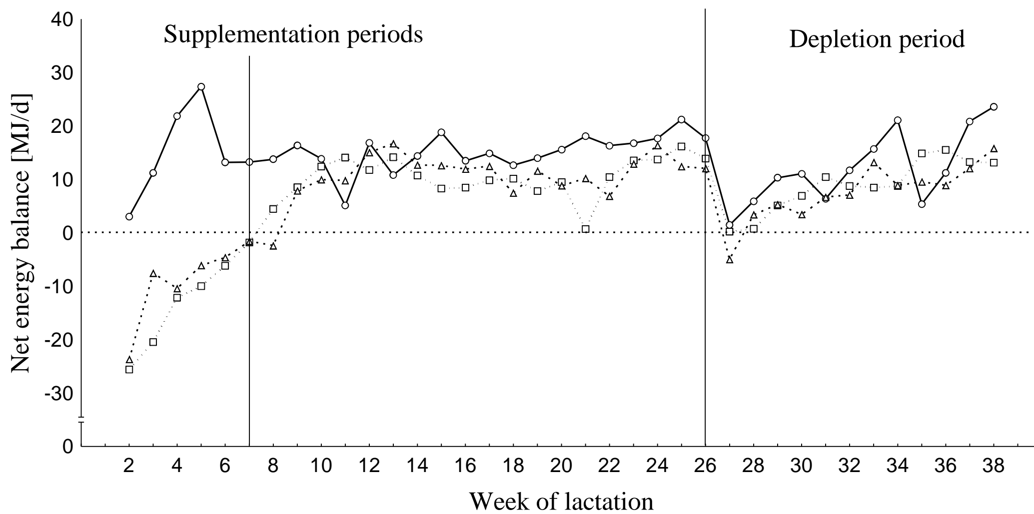

Figure 4: Development of net energy balance (means) in supplementation and depletion periods for the feeding groups
Control group (n=15/14), CLA-1 group (n=15/13), CLA-2 group (n=16)

Table 4 shows the effects of supplementing rumen-protected CLA on indicators of energy metabolism. BCS and BFT remained unaffected by CLA supplementation. Plasma concentrations of glucose, BHB and NEFA did not differ between treatments over the 26 week supplementation period, although there was a trend (p=0.057) for lower plasma NEFA concentrations in the CLA-2 group over Period 2 of supplementation. In the depletion period plasma NEFA and BHB concentrations remained unaffected by treatment, whereas the plasma glucose concentration was significantly higher in the CLA-2 group.

Table 4: Body condition score (BCS), Back fat thickness (BFT) and plasma metabolites in Period 1 (early lactation, week 2 - 7 of lactation), Period 2 (until end of CLA supplementation, week 8 - 26 of lactation) and Period 3 (depletion period, 12 weeks) (LS means ± SE).

	Supplementation groups			*p*-value
Parameter	CON	CLA-1	CLA-2	SUP
PERIOD 1	(n=15)	(n=15)	(n=16)	
BCS*	3.08 ±0.04	3.10 ±0.04	3.11 ±0.04	0.899
BFT [cm]	2.55 ±0.07	2.71 ±0.07	2.62 ±0.07	0.262
Plasma metabolites				
Glucose [mmol/l]	3.5 ±0.1	3.6 ±0.1	3.7 ±0.1	0.282
NEFA [µEq/l]	700 ±62	659 ±60	693 ±58	0.877
BHB [mmol/l]	0.6 ±0.1	0.7 ±0.1	0.7 ±0.1	0.862
PERIOD 2	(n=14)	(n=15)	(n=16)	
BCS*	2.99 ±0.06	2.99 ±0.06	3.08 ±0.06	0.442
BFT [cm]	2.32 ±0.07	2.39 ±0.07	2.44 ±0.06	0.455
Plasma metabolites				
Glucose [mmol/l]	3.7 ±0.1	3.6 ±0.1	3.8 ±0.1	0.273
NEFA [µEq/l]	289 ±21	250 ±20	217 ±20	0.057
BHB [mmol/l]	0.5 ±0.03	0.5 ±0.03	0.5 ±0.03	0.340
PERIOD 3	(n=14)	(n=13)	(n=16)	
BCS*	3.04 ±0.06	3.03 ±0.06	3.05 ±0.06	0.975
BFT [cm]	2.24 ±0.08	2.35 ±0.08	2.26 ±0.08	0.557
Plasma metabolites				
Glucose [mmol/l]	3.7^{b} ±0.1	3.9^{ab} ±0.1	3.9^{a} ±0.1	0.027
NEFA [µEq/l]	245 ±27	319 ±26	306 ±25	0.121
BHB [mmol/l]	0.4 ±0.02	0.4 ±0.02	0.4 ±0.02	0.996

Notes: ^{ab} Values with different superscripts within one period and within one row are significantly different ($p < 0.05$); * Use of a body condition scoring chart with a scale from one (under conditioned) to five (over conditioned); CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; SUP, Supplementation; NEFA, Non esterified fatty acids; BHB, ß-Hydroxybutyrate

For the analysis of fatty acid profile of milk fat (Table 5) the trial was separated in supplementation and depletion period. The addition of the CLA supplement did not alter the proportion of *de novo* synthesized (<C16) and preformed (>C16) milk fatty acids, but reduced significantly the proportion of C16:0 and C16:1 in total (originating from both sources) in CLA-2 group during the supplementation period. The proportion of *trans*-10, *cis*-12 CLA in milk fat increased significant and dose-dependently in the CLA groups, but the actual increase was minimal in level. The proportion of *cis*-9, *trans*-11 CLA remained unaffected by CLA supplementation. In the depletion period no differences in the fatty acid profile in milk fat were observed between the 3 treatment groups.

Table 5: Fatty acid profile in milk fat in supplementation period (4 dates) and depletion period (2 dates) (LS means ± SE).

Fatty acid	Supplementation period			*p*-value	Depletion period			*p*-value
[% of total FAME]	CON (n=15/14)	CLA-1 (n=15)	CLA-2 (n=16)	SUP	CON (n=14)	CLA-1 (n=13)	CLA-2 (n=16)	SUP
C4:0	3.50 ±0.08	3.56 ±0.08	3.61 ±0.07	0.598	3.30 ±0.08	3.25 ±0.08	3.26 ±0.07	0.889
C6:0	2.37 ±0.06	2.42 ±0.06	2.43 ±0.06	0.727	2.69 ±0.07	2.64 ±0.07	2.75 ±0.07	0.502
C8:0	1.31 ±0.05	1.34 ±0.05	1.37 ±0.04	0.709	1.45 ±0.05	1.47 ±0.05	1.55 ±0.04	0.255
C10:0	2.91 ±0.13	3.00 ±0.13	3.08 ±0.13	0.685	3.20 ±0.13	3.36 ±0.13	3.52 ±0.12	0.199
C12:0	3.12 ±0.14	3.16 ±0.14	3.28 ±0.14	0.692	3.48 ±0.14	3.65 ±0.14	3.82 ±0.13	0.223
C14:0	10.02 ±0.29	10.27 ±0.29	10.77 ±0.28	0.168	11.30 ±0.23	11.45 ±0.23	11.83 ±0.21	0.213
C14:1	0.92 ±0.05	0.87 ±0.05	0.89 ±0.05	0.841	1.22 ±0.07	1.24 ±0.07	1.14 ±0.06	0.523
C15:0	1.78 ±0.08	1.74 ±0.08	1.76 ±0.08	0.944	1.88 ±0.06	1.78 ±0.06	1.86 ±0.06	0.453
C16:0	30.41^{a} ±0.49	29.39^{ab} ±0.49	28.62^{b} ±0.47	0.039	34.76 ±0.72	34.01 ±0.72	33.03 ±0.67	0.224
C16:1	2.06 ±0.11	2.00 ±0.11	1.90 ±0.10	0.573	2.27 ±0.12	2.27 ±0.12	2.10 ±0.11	0.494
C17:0	1.62 ±0.05	1.60 ±0.05	1.60 ±0.05	0.960	1.34 ±0.04	1.29 ±0.04	1.33 ±0.04	0.696
C18:0	10.22 ±0.34	10.91 ±0.34	10.96 ±0.33	0.219	8.56 ±0.37	8.78 ±0.37	9.21 ±0.35	0.438
C18:1 *trans*	2.15 ±0.15	2.27 ±0.15	2.27 ±0.14	0.811	1.92 ±0.10	1.79 ±0.10	1.77 ±0.10	0.549
C18:1 *cis*-9	20.92 ±0.63	20.60 ±0.63	20.57 ±0.61	0.910	17.16 ±0.61	17.66 ±0.61	17.51 ±0.57	0.835
C18:2 *trans*-9, *trans*-12	0.30 ±0.02	0.32 ±0.02	0.33 ±0.02	0.700	0.07 ±0.01	0.08 ±0.01	0.07 ±0.01	0.424
C18:2 *cis*-9, *cis*-12	1.90 ±0.06	1.99 ±0.06	1.97 ±0.06	0.574	1.30 ±0.06	1.34 ±0.06	1.27 ±0.05	0.702
Conjugated linolic acid (CLA)								
C18:2 *cis*-9, *trans*-11	0.57 ±0.04	0.60 ±0.04	0.58 ±0.04	0.868	0.59 ±0.03	0.54 ±0.03	0.51 ±0.03	0.166
C18:2 *trans*-10, *cis*-12	0.004^{c} ±0.001	0.02^{b} ±0.001	0.03^{a} ±0.001	<0.001	0.00	0.00	0.00	
Other CLA	0.17 ±0.01	0.16 ±0.01	0.16 ±0.01	0.628	0.09 ±0.004	0.08 ±0.004	0.08 ±0.003	0.219
C18:3	0.41 ±0.02	0.41 ±0.02	0.42 ±0.02	0.788	0.27 ±0.01	0.29 ±0.01	0.28 ±0.01	0.542
C20:0	0.13 ±0.005	0.13 ±0.005	0.14 ±0.005	0.449	0.13 ±0.01	0.14 ±0.01	0.14 ±0.01	0.260
Other	3.21 ±0.10	3.24 ±0.10	3.26 ±0.09	0.979	3.02 ±0.06	2.89 ±0.06	2.97 ±0.06	0.475
Summation								
<C16	26.55 ±0.66	26.98 ±0.66	27.74 ±0.64	0.419	29.35 ±0.59	29.64 ±0.59	30.55 ±0.55	0.301
C16	32.46^{a} ±0.49	31.37^{ab} ±0.49	30.53^{b} ±0.48	0.025	37.03 ±0.76	36.27 ±0.76	35.13 ±0.71	0.191
>C16	40.97 ±0.97	41.66 ±0.97	41.73 ±0.94	0.826	33.62 ±0.97	34.08 ±0.97	34.32 ±0.91	0.870

Notes: ^{abc} Values with different superscripts within one period in the rows are significantly different ($p < 0.05$); CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; SUP, Supplementation; FAME, Fatty acid methyl ester

4 Discussion

In short term studies (<10 weeks) the effects of dietary supplemented CLA, especially of the *trans*-10, *cis*-12 isomer, on performance and metabolic parameters of dairy cows are well investigated. However, data regarding the long-term effects of CLA supplementation on dairy cows is still rare as most examinations were conducted either during the transition period and early lactation (Moore et al. 2004, Selberg et al. 2004, Castaneda-Gutierrez et al. 2005, Odens et al. 2007) or during a few weeks in mid lactation (Giesy et al. 2002, de Veth et al. 2005, Suksombat and Chullanandana 2008, Piamphon et al. 2009, Sippel et al. 2009, Moallem et al. 2010). Only Perfield et al. (2002) and Bernal-Santos et al. (2003) performed studies over a 20-week of lactation treatment period but their CLA supplement was a mixture of four isomers, including *trans*-8, *cis*-10 CLA, *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA and *cis*-11, *trans*-13 CLA. The aim of the present study was to investigate the long term effects of *trans*-10, *cis*-12 CLA on dairy cows. Each animal of the CLA groups received 5 or 10 g of *trans*-10, *cis*-12 CLA per day from 1 d p.p. to the 26th week of lactation. The *cis*-9, *trans*-11 isomer, which did neither affect the rates of milk fat synthesis in a study by Baumgard et al. (2002a) nor the lipid metabolism of lactating cows, was present at almost the same proportion due to the manufacturing process of the CLA preparation. To determine possible post-supplementation effects the animals were observed for further 12 weeks.

We aimed at an individual dietary intake of 5 and 10 g *trans*-10, *cis*-12 CLA/d in group CLA-1 and CLA-2, respectively. However, in both groups the calculated intakes, based on the analyzed concentrations in the concentrates, were approximately 20% lower than expected (4 and 8 g *trans*-10, *cis*-12 CLA/d, respectively).

In the present study the DMI of the two CLA groups was significantly reduced by 12 and 16% in early lactation. The similar dry matter intakes (DMI) in the three groups in the 2nd and 3rd week *ante partum* showed that the differences in the first weeks are not ascribable to differences before the start of the trial. Furthermore, we measured that each animal consumed the 4 kg of concentrate feed which included the fat supplement. Therefore the sensory properties are unaccountable for the decrease in DMI. The lower DMI concerns the PMR, which consisted of the same components for all three groups. Data concerning the effects of dietary supplemented CLA on the feed intake of dairy cows during the first weeks of lactation are mostly consistent and in contrast to our findings. Moore et al. (2004) and Castaneda-Gutierrez et al. (2005) worked with supplements consisting of various CLA isomers and different *trans*-10, *cis*-12 CLA concentrations (9, 12, 25 and 37 g/d). Even with such high concentrations no influence on DMI in early lactation was observed. Piamphon et al. (2009)

reported a slightly increased DMI when 8 g *trans*-10, *cis*-12 CLA was added to the diet during mid lactation. However, after the abomasal infusion of 10 g *trans*-10, *cis*-12 CLA/d Baumgard et al. (2000) found a tendency towards a reduced DMI (p=0.07), while abomasal infused *cis*-9, *trans*-11 CLA did not affect the DMI. Thus, a CLA related transitory depression in DMI seems to be possible. Mechanisms behind this effect are not yet understood but seem to be regulated at the metabolic level since the CLA containing supplements were consumed completely by all cows. In a trial of Moallem et al. (2010) DMI was significant reduced by 2.5% after the dietary supplementation of 5 g *trans*-10, *cis*-12 CLA and the authors supposed a relation to the saturation of the fatty acids in the diet. With the energy content of feed being nearly similar, the significant lower net energy intake of the CLA-groups during the first weeks of lactation can be explained by the lower DMI of these animals in this time.

Neither milk yield nor FCM yield was significantly affected by CLA supplementation. This finding is in accordance with the literature (Giesy et al. 2002, Perfield et al. 2002, Perfield et al. 2004, Sippel et al. 2009) where the CLA-groups exhibited an apparent but also not significant increase in milk yield of 13%, 4%, 5% and 9%, respectively.

The dietary addition of CLA during early lactation induced a gradual reduction in milk fat percentage similar to results in studies by Bernal-Santos et al. (2003), Selberg et al. (2004) and Castaneda-Gutierrez et al. (2005) and the decrease was significant for the highest dose (CLA-2) in Period 2 of supplementation. The differences between CLA-groups and control group were already indicated at the end of the Period 1 of supplementation. The reason for the lack of a CLA response in milk fat during the first weeks of lactation is unknown. However, it can be excluded that there was any difference in uptake of *trans*-10, *cis*-12 CLA by the mammary gland, because the fatty acid analysis indicated that the isomer was consistently transferred to milk fat throughout the supplementation period (data not shown). An abomasal infusion of *trans*-10, *cis*-12 CLA in studies with cows during established lactation resulted in an immediate reduction in milk fat content (Kraft et al. 2000, Baumgard et al. 2000, Baumgard et al. 2001). Bernal-Santos et al. (2003) and Castaneda-Gutierrez et al. (2005) assumed that the early lactating mammary gland is less sensitive and responsive to CLA, because of the markedly alterations of many key enzymes and biochemical pathways at the onset of lactation. These conclusions were supported by Moore et al. (2004). They supplemented different doses of *trans*-10, *cis*-12 CLA ranging from 12 to 37 g/d in the first three weeks of lactation and observed a decline of milk fat content and yield immediately *post partum* when a high dose was fed. However, in the current trial no MFD, as defined by

Bauman and Griinari (2000), could be observed, because milk fat yield was unaltered in the treatment groups. Due to the higher milk yield in the CLA-1 and CLA-2 group of 4 and 3 kg on average respectively, a dilution effect can be assumed.

Milk protein tended to be lower after CLA supplementation in the current experiment in mid lactation while the milk protein yield remained unaffected, but all values are within a physiological range. The reduction of milk protein content could be explained as a result of diluting. With the exception of Piamphon et al. (2009) who found a significant reduction, other studies have shown that the milk protein content was not altered by the dietary supplementation of similar CLA doses (Giesy et al. 2002, Bernal-Santos et al. 2003, Perfield et al. 2004, Castaneda-Gutierrez et al. 2005). However, the abomasal infusion of 10 g *trans*-10, *cis*-12 CLA resulted in a significant reduction of milk protein content while the infusion of similar amounts of the *cis*-9, *trans*-11 isomer had no effect on milk protein (Baumgard et al. 2000).

The SCC was determined to get an impression about the udder health of the animals. Generally the SCC was within the normal range beside some individual variations when mastitis occurred. We couldn't observe distinguished effects of CLA on the mastitis occurrence (Con: eight, CLA-1: seven, CLA-2: ten cows with mastitis). The intake of the CLA supplement had no effect on SCC in accordance with the literature (Perfield et al. 2002, Bernal-Santos et al. 2003, Odens et al. 2007).

Related to an expected MFD and the resulting lower energy requirement for milk production, it could be assumed that the net energy balance is improved after dietary CLA intake. Odens et al. (2007) observed in the CLA-groups a decrease in milk fat synthesis and in consequence an improved net energy balance status. Furthermore, the improved plasma levels of the NEFA and glucose in their investigations supported these findings. As well as by Moore et al. (2004) eventually the numeric higher milk yield prevented an improvement of the calculated net energy balance. Despite the enhanced milk yield the energy conservation owing to the MFD resulted in an improvement of the calculated net energy balance in an investigation by Schwarz et al. (2007). Moreover, the CLA treated cows reached positive net energy balance sooner in their investigations. In the present examination the control group had a positive calculated net energy balance during the entire evaluation period. In contrast, the CLA-groups showed a negative estimated net energy balance (-10.3 MJ/d) immediately *post partum*. The variation can be directly attributed to the lower dry matter intake as there were no significant differences in live weight, milk yield and milk contents. In Period 2 of supplementation the

energy spared from the reduction in milk fat was probably used to increase milk synthesis so that calculated net energy balance was unchanged.

Apparently, the cows of the CLA-groups were able to utilize the energy better than the control cows in Period 1 of supplementation as there are no differences in milk yield and milk fat content in this time and the plasma levels of NEFA and BHB, as indicators for a higher adipose tissue mobilization, were not increased at the same time. In addition BCS and BFT were not altered after CLA supplementation. With the available data no explanation can be given for these findings. Therefore, further studies are of interest to obtain more information about the influence of CLA on the energy metabolism of the lactating cow.

Bauman and Griinari (2000) described an alteration of milk fatty acid composition in relation with MFD and stated that the decline is greatest for *de novo* synthesized fatty acids. In the current study, we did not observe a MFD so that dietary supplements of CLA resulted in only slight changes of the fatty acid profile of milk fat. The origin of milk fatty acids differs in dependency on carbon chain length: fatty acids synthesized *de novo* (<C16), fatty acids from the uptake of preformed fatty acids (>C16) and fatty acids from both sources (C16) (McGuire and Bauman 2002). In the current trial only the proportion of C16 was significantly decreased after CLA-2 treatment, which is in agreement to previous results from Castaneda-Gutierrez et al. (2005). Without supplementation the *trans*-10, *cis*-12 CLA isomer is from ruminal origin and represents an intermediate of the ruminal biohydrogenation of linoleic acid. Hence, diet induced changes in the small proportion of this fatty acid in milk are possible. This isomer, which is known to be responsible for MFD (Baumgard et al. 2000) was consistently transferred to milk fat (data not shown) and the proportion was increased dose dependently throughout the treatment period which is in accordance with the literature (Giesy et al. 2002, Moore et al. 2004, Castaneda-Gutierrez et al. 2005, Piamphon et al. 2009). The predominant source (>80%, (Lock and Garnsworthy 2002)) of *cis*-9, *trans*-11 CLA, the main CLA isomer in milk fat, is the endogenous synthesis in the mammary gland from *trans*-11 C18:1 as the rumen origin substrate and with Δ9-Desaturase as the key enzyme. The proportion of this isomer remained unaffected from CLA supplementation. However, the abomasal infusion of a CLA mixture with *cis*-9, *trans*-11 CLA or the single isomer resulted in an obvious increase in the content of this fatty acid in milk (Chouinard et al. 1999, Baumgard et al. 2000).

For the post-treatment period no differences were observed in milk production, in milk components or milk fatty acid profile because of treatment. After the end of CLA supplementation the differences in milk yield were no longer evident. In accordance to short-term studies with abomasal infusions of CLA (Chouinard et al. 1999, Baumgard et al. 2000,

Shingfield et al. 2009) the milk fat content returned to levels similar to the control group when the CLA supplementation was terminated. Castaneda-Gutierrez et al. (2005) observed the cows for an 8 week post-treatment period after 9 weeks of supplementation and the results were comparable. Supplemental CLA slightly (5.4%) increased circulating glucose levels in the post-treatment period. Odens et al. (2007) observed an increase of plasma glucose level in the CLA-groups (30 g *trans*-10, *cis*-12 CLA/d) during treatment and concluded a decrease in insulin sensitivity for these animals. In contrast Perfield et al. (2002), Bernal-Santos et al. (2003) and Moore et al. (2004), which worked with lower CLA concentrations comparable to the current trial, could not show any changes of plasma glucose level when CLA was supplemented.

5 Conclusions

For unknown reasons supplementing the diets with CLA resulted in a decreased DMI and consequently in a compromised calculated net energy balance in early lactation (week 1 – 7 p.p.), but milk yield, yield of milk components, BCS and BFT as well as plasma levels of NEFA and BHB remained unaffected. However, the used amounts of supplemented CLA were ineffective in realizing the expected MFD, which questions the justification for a supplementation. The fatty acid profile in milk changed after CLA supplementation, but the amount of total CLA remained unaffected. Taken together, these results suggest an increased efficiency of metabolizable energy which, however, could not be measured by the used methods. Therefore, further research is of interest to obtain more information about the influence of CLA on feed intake regulation and energy metabolism of the lactating cow.

References

Bassler R.E..1976. Die chemische Untersuchung von Futtermitteln, Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). Darmstadt.

Bauman DE, Griinari JM. 2000. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest Prod Sci. 70: 15-29.

Bauman DE, Griinari JM. 2003. Nutritional regulation of milk fat synthesis. Annu Rev Nutr. 23: 203-227.

Baumgard LH, Corl BA, Dwyer DA, Bauman DE. 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 Anim Sci. 80: 1285-1293.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA, Bauman DE. 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 LH, Corl BA, Dwyer DA, Saebo A, Bauman DE. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am J Physiol Regul Integr Comp Physiol. 278: R179-R184.

Baumgard LH, Sangster JK, Bauman DE. 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.

Bernal-Santos G, Perfield JW, Barbano DM, Bauman DE, Overton TR. 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.

Castaneda-Gutierrez E, Overton TR, Butler WR, Bauman DE. 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.

Chouinard PY, Corneau L, Barbano DM, Metzger LE, Bauman DE. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J Nutr. 129: 1579-1584.

de Veth MJ, Gulati SK, Luchini ND, Bauman DE. 2005. Comparison of calcium salts and formaldehyde-protected conjugated linoleic acid in inducing milk fat depression. J Dairy Sci. 88: 1685-1693.

Drackley JK. 1999. Biology of dairy cows during the transition period: the final frontier. J Dairy Sci. 82: 2259-2273.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. 1989. A body condition scoring chart for Holstein dairy cows. J Dairy Sci. 72: 68-78.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 226: 497-509.

Gaines W. 1928. The energy basis of measuring milk yield in dairy cows. Illinois Agric Exp Sta Bull. 308: 401-438.

GfE. 1991. Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. J Anim Physiol Anim Nutr. 65: 229-234.

GfE. 2001. Empfehlungen zur Energie- und Nährstoffaufnahme der Milchkühe und Aufzuchtrinder. Frankfurt am Main: DLG Verlag.

Giesy JG, McGuire MA, Shafii B, Hanson TW. 2002. Effect of dose of calcium salts of conjugated linoleic acid (CLA) on percentage and fatty acid content of milk fat in midlactation Holstein cows. J Dairy Sci. 85: 2023-2029.

Kraft J, Lebzien P, Flachowsky G, Möckel P, Jahreis G. 2000. Duodenal infusion of conjugated linoleic acid mixture influences milk fat synthesis and milk CLA content in dairy cows. Occ Publ of Brit Soc Anim Sci. 25, 143-147.

Kraft J, Griinari J, Mockel P, Jahreis G. 2001. Effects of CLA calcium salts on CLA content in milk lipids. Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier, 8. Symposium, 26 und 27 September, 2001, Jena/Thuringen, Germany.

Lock AL, Garnsworthy PC. 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. Anim Sci. 74: 163-176.

McGuire MA, Bauman DE. 2002. Encyclopedia of Dairy Sciences. Milk Biosynthesis and Secretion: 1828-1834.

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

Moore CE, Hafliger HC, III, Mendivil OB, Sanders SR, Bauman DE, Baumgard LH. 2004. Increasing amounts of conjugated linoleic acid (CLA) progressively reduces milk fat synthesis immediately postpartum. J Dairy Sci. 87: 1886-1895.

Odens LJ, Burgos R, Innocenti M, VanBaale MJ, Baumgard LH. 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.

Perfield JW, Bernal-Santos G, Overton TR, Bauman DE. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J Dairy Sci. 85: 2609-2617.

Perfield JW, Lock AL, Pfeiffer AM, Bauman DE. 2004. Effects of amide-protected and lipid-encapsulated conjugated linoleic acid (CLA) supplements on milk fat synthesis. J Dairy Sci. 87: 3010-3016.

Piamphon N, Wachirapakorn C, Wanapat M, Navanukraw C. 2009. Effects of protected conjugated linoleic acid supplementation on milk fatty acid in dairy cows. Asian Australas J Anim Sci. 22: 49-56.

Schwarz FJ, Lierman T, Pfeiffer AM. 2007. Effect of rumen-protected conjugated linoleic acid in combination with propylene glycol or rumen protected fat on performance and metabolic parameters of early lactation dairy cows. Proceedings of the 2nd EAAP International Symposium on Energy and Protein Metabolism and Nutrition 155-156.

Selberg KT, Lowe AC, Staples CR, Luchini ND, Badinga L. 2004. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and *trans*-octadecenoic acids. J Dairy Sci. 87: 158-168.

Shingfield KJ, Saebo A, Saebo PC, Toivonen V, Griinari JM. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J Dairy Sci. 92: 4317-4329.

Sippel MA, Spratt RS, Cant JP. 2009. Milk production responses of primiparous and multiparous dairy cows to dose of conjugated linoleic acid consumed in rumen inert form. Can J Anim Sci. 89: 393-399.

Staufenbiel R. 1997. Evaluation of body condition in dairy cows by ultrasonographic measurement of back fat thickness. Prakt Tierarzt. 78: 87-92.

Suksombat W, Chullanandana K. 2008. Effects of soyean oil or rumen protected conjugated linoleic acid supplementation on accumulation of conjugated linoleic acid in dairy cows` milk. Asian-Aust J Anim Sci. 21: 1271-1277.

Tyrrell HF, Reid JT. 1965. Prediction of the energy value of cow's milk. J Dairy Sci. 48: 1215-1223.

Paper II

Duodenal availability of conjugated linoleic acids after supplementation to dairy cow diets

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Summary
The objective of the present study was to investigate the effects of a lipid-encapsulated CLA preparation on rumen metabolism and the actual post-ruminal bioavailability of the applied CLA isomers. In the rumen, the CLA supplementation modified the molar proportions of VFA. In Period CLA-1 the rumen fermentation shifted towards more butyric acid at the expense of acetic acid. The highest CLA supplementation resulted in increased amounts of isobutyric, isovaleric and valeric acid. The apparent ruminal digestibility of starch increased in Period CLA-2. The ruminal protein degradation was higher after CLA supplementation, while the efficiency of the use of the RDP for microbial protein synthesis declined. The duodenal flow of *trans*-10,*cis*-12 CLA amounted to 16 and 5% of the intake in Periods CLA-1 and CLA-2, respectively. The transfer of *trans*-10,*cis*-12 CLA from duodenum into milk was 36 and 48% in Periods CLA-1 and CLA-2, respectively. Overall, the observed effects of the supplementation of lipid-encapsulated CLA on the parameters of rumen metabolism were negligible. The actual low post-ruminal bioavailability of *trans*-10,*cis*-12 CLA suggest that most of the applied fat supplement was biohydrogenated.

Running title: CLA in dairy cow diets

Keywords: CLA / Dairy cow / Rumen / Duodenal fatty acid flow

1 Introduction

CLA supplements are used in dairy cow diets to reduce the milk fat yield to save energy, especially at the onset of lactation, and to change the fatty acid pattern of milk fat. For this purpose, the CLA have to be intestinally absorbed. Therefore, several methods are applied to protect such supplements against ruminal digestion processes and avoid any impairment of the growth and function of the ruminal microbial population. The main processes that have been used to protect fats are the formation of calcium salts, linkage by amide bonds, formaldehyde treatment and lipid encapsulation. Calcium salts are most frequently applied to prevent the ruminal metabolism of special CLA isomers and to protect them from rumen biohydrogenation. De Veth et al. (2005b) concluded from the results of eight studies examining the transfer efficiency of *trans*-10,*cis*-12 CLA into milk fat using calcium salts of CLA, that only 15 to 17% of the CLA isomers were protected from rumen biohydrogenation. Comparable investigations with lipid-encapsulated CLA supplements are rare. Perfield et al. (2004a) calculated a transfer of 7.9% *trans*-10,*cis*-12 CLA into milk after a dietary addition of lipid-encapsulated CLA, representing a protection rate of 36%. These results suggest a

ruminal biohydrogenation of major proportions of the supplemented CLA. Dehkordi et al. (2008) conducted *in vitro* investigations on the effects of processing on the protection of lipid-encapsulated CLA supplements against rumen biohydrogenation. They concluded that the protection of this supplement, in the processed and unprocessed form, is lower than 50% and a thermal/mechanical processing of the supplement may even increase its susceptibility to rumen biohydrogenation. However, there is a lack of information on the effects of dietary supplements of rumen-protected CLA on rumen metabolism and about the actual quantity of absorbable CLA in the small intestine. Therefore, the present study investigates the effects of lipid-encapsulated CLA preparations on parameters of rumen metabolism and on duodenal availability of the supplemented CLA isomers and other long-chain fatty acids by examining the ruminal and duodenal fluid of double-fistulated cows.

2 Material and methods

2.1 Animals, treatments and experimental design

The study was carried out at the experimental station of the Friedrich-Loeffler-Institute (FLI) in Braunschweig with a total of six German Holstein pluriparous dairy cows. The experiment was conducted according to the European Community regulations concerning the protection of experimental animals and the guidelines of the LAVES (Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany, File Number: 33.11.42502-04-057/07). Each animal was fitted with a large rubber cannula in the dorsal sac of the rumen (inner diameter: 10 cm) and a simple T-shaped plastic cannula at the proximal duodenum close to the pylorus (inner diameter: 2 cm). At the beginning of the experiment the cows had an average live weight of 624 (±79) kg. Due to the limited number of animals, one non-lactating cow was used, while the other five cows were 196 (± 93) days in milk (DIM) at the beginning of the trial. Two cows were dried off before the end of the trial in preparation for calving.

The cows were kept in a tethered stable using neck straps and equipped with an individual trough for each cow. They had free access to water. Forage (60 % maize and 40 % grass silage based on dry matter (DM) content) was offered at 05.30 h and 15.30 h, shortly after each cow had consumed 500 g of concentrate feed (Table 1). Except for the non-lactating cows, the amount of forage offered was adjusted to the expected intake of each cow in order to reach nearly *ad libitum* intake but to avoid feed refusals. To prevent excessive fattening of the dry cow, the forage amount was restricted to cover the maintenance requirement. The experiment was split into three treatments: (1) 100 g/d of control fat preparation (CON);

(2) 50 g/d of CLA preparation and 50 g/d of control fat preparation (CLA-1); (3) 100 g/d of CLA preparation (CLA-2). The CLA and the control fat preparations were thoroughly mixed with the components of the concentrate feed and thereafter gently pelleted. The CLA supplement (Lutrell® pure, BASF SE, Ludwigshafen, Germany) consisted of approximately 10% of *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA. The CLA, in the form of fatty acid methyl esters, was coated with hydrogenated vegetable fats consisting of palmitic and stearic acid linked to glycerin. In the control fat preparation (Silafat®, BASF SE, Ludwigshafen, Germany), the CLA was substituted with a corresponding amount of stearic acid (Table 2). To avoid possible carryover effects of CLA on the control treatment, each cow was subjected to the three treatments in the same order: CON, CLA-1 and CLA-2. Each treatment period consisted of four weeks in which two weeks of adaptation to the respective diets were followed by two weeks of sample collection.

Table 1: Composition of the concentrates [g/kg dry matter].

	Treatment		
Component	CON	CLA-1	CLA-2
Rapeseed meal		184	
Soybean meal		60	
Wheat		354	
Dried sugar beet pulp		267	
Soybean oil		10	
Calcium Carbonate		5	
Mineral feed*		20	
CLA supplement	-	50	100
Control fatty acid supplement	100	50	-

*Per kg mineral feed: 140 g Ca; 120 g Na; 70 g P; 40 g Mg; 6 g Zn; 5. 4 g Mn; 1 g Cu; 100 mg I; 40 mg Se; 5 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D_3; 1500 mg vitamin E

Table 2: Fatty acid profile of the fat supplements*.

Fatty acid [% FAME#]	CON	CLA
C16:0	10.89	10.89
C18:0	87.30	50.31
cis-9 C18:1	<0.01	10.66
Conjugated linoleic acid (CLA)		
cis-9,*trans*-11 C18:2	0.06	11.99
trans-10,*cis*-12 C18:2	0.02	11.88
Other CLA	0.15	0.95
Other	1.58	3.32

*Supplemental CLA was included in the concentrate portion as a rumen-protected CLA preparation; #Fatty acid methyl ester

2.2 Sample collection

Cows were weighed on the day before the experiment started and on the last day of the experiment. Lactating cows were milked daily at 05.45 h in the morning and 15.45 h in the afternoon and the individual milk yield was recorded daily during the third and fourth week. Milk samples for the analysis of milk composition were taken twice in the third and fourth week in the morning and afternoon of the same day. For the analysis of the fatty acid (FA) profile in milk fat, milk samples of 100 ml were collected twice a day at two times in Week three of each experimental period. They were stored at -20 °C until they were freeze dried. The milk samples used for the analysis of milk composition were conserved with bronopol and stored at 8 °C until they were analyzed. On one day of Week three, rumen fluid was collected for NH_3-N and volatile fatty acids (VFA) analysis through the rumen fistula using a hand vacuum pump. Sampling times were at 05.30 h just before feeding, and then 30, 60, 90, 120, 180 and 360 min after the beginning of feeding. At Week four of each treatment period, four 100 ml-chyme samples were taken at two-hourly intervals through the duodenal cannula over a five-day period, and once in this week rectally collected feces of each cow was sampled. The pH-values of duodenal chyme samples were determined with a glass electrode (pH525, WTW, Weilheim, Germany). The duodenal chyme sample with the lowest pH-value of the four samples of an individual cow was added to the daily pooled sample of this cow and stored at -18 °C (Rohr et al. 1984). For duodenal flow measurements, Cr_2O_3 was mixed with wheat flour (ratio 1:4) and used as a marker. Beginning 10 days before the duodenal sampling period, 50 g of the marker were mixed into the rumen every 12 h. Starting one day before and during the sampling period, 25 g were given every 6 h.

During the duodenal chyme sampling week, samples of concentrate and forage were collected daily and pooled weekly. Feed samples were dried at 60 °C for 72 hours. Feces samples and, after quantification of the nitrogen concentration, the daily duodenal chyme samples, were freeze dried. All dried samples were ground through a 1-mm screen for further analysis.

To determine the metabolizable energy (ME) of the diet, balance studies with four wethers were carried out for maize and grass silage, following the standard procedure described by the GfE (1991).

2.3 Analysis

All feed samples were analyzed for crude nutrients and starch according to the methods of the "Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten" (Bassler

1976). The determination of ADF and NDF was performed according to Goering and Van Soest (1970).

Milk samples were analyzed for fat, protein and lactose using an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 500, Foss Electric, Hillerød, Denmark).

Immediately after rumen fluid collection, pH-values were measured as described above. Afterwards, the ruminal fluid was centrifuged (30074 x g). In the supernatant NH_3-N was determined according to DIN 38406-E5-2 (1998).

For analysis of VFA in rumen fluid, a gas chromatograph (Hewlett Packard 5580, Avondale, PA,USA) equipped with a flame ionization detector as described by Geissler et al. (1976) was used.

Before freeze drying, the nitrogen concentration of thawed daily duodenal chyme was quantified by the Kjeldahl method.

Cr_2O_3 was analyzed by atomic absorption spectrophotometry according to Williams et al. (1962) and used to calculate the duodenal and fecal DM flow. Daily duodenal DM flows were applied to generate one pooled sample per cow per week for further analysis. The concentrations of crude fiber, NDF, ADF and starch in the pooled duodenal chyme samples were quantified according to the analyzing methods for feedstuff.

Before analyzing the FA distribution in milk fat, the milk samples were heated to 40 °C and homogenized using the Ultra Turrax treatment (T25, JANKE & KUNKEL, IKA®-Labortechnik, Germany). Afterwards, the morning and evening milk were mixed according to their milk yields and freeze dried. The fat-extraction of freeze dried milk was accomplished according to SOXHLET listed in "Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten" (Bassler 1976). Total milk fat was converted into its fatty acid methyl esters (FAME) by the use of sodium methoxide as catalyst.

The lipid content of the feed, pooled duodenal chyme and feces samples was extracted according to Folch et al. (1957). Afterwards the samples were incubated with Boron trifluoride (BF_3) to gain the FAME. The emerged extracts were purified by thin-layer chromatography (SIL G-25 UV_{254}, MACHERY-NAGEL, Germany).

All sample FAME extracts were carried out by gas chromatography (GC-17A Version 3, Schimadzu, Japan) equipped with an auto sampler and flame ionisation detector. Two different GC procedures were necessary to analyze the FAME distribution of these samples. The first GC method determined the identity and general FA distribution from 4 to 25 carbon length FA using a medium polarity column (DB-225ms, 60 m x 0.25 mm, inner diameter (i.d.); 0.25 µm; J&W Scientific, Germany). The second GC method separated the *cis* and

trans isomers of C18:1 using a high polarity column (Select™ FAME, 200 m x 0.25 mm, i.d.; 0.25 µm; VARIAN Inc., Germany). Various reference standards were used as FAME mix to identify FA peaks: No. 463, 674, (NU-CHEK PREP, INC., Elysian, U.S.), BR2, BR4, ME 93 (Larodan; Malmö, Sweden), Supelco® 37 Component FAME Mix, PUFA No. 3, conjugated linoleic acid, linoleic-, linolenic- and octadecenoic acid methyl ester mix (Supelco; Bellefonte, U.S.). The results were expressed as percentage of total FAME.

For the quantification of FA in feed, duodenal chyme and feces, the percentage of FA in the fat has to be determined. Therefore the lipid classes of the extracted samples were separated by high-performance thin layer chromatography (HPTLC, Silica gel 60 F254, Merck KGaA, 64271 Darmstadt, Germany) and subsequently detected densitometrically. The method used is in accordance with Olsen and Henderson (1989) with the following modifications: Lipid standards were obtained from Sigma-Aldrich, Germany (Cholesterol, Tripalmitin, Palmitic acid methyl ester, cholesteryl palmitate) and Larodan Fine Chemicals AB, Sweden (Glycero-3-Phosphatidylcholine, Octadecanoic acid). The HPTLC plates were pre-developed in chloroform : methanol (1:1 *v*/*v*) to remove impurities. The plates were activated at 50 °C for 45 min. Lipid samples and lipid standards were added to the plate using Linomat IV (CAMAG, Switzerland). Hexane : diethyl ether : glacial acetic acid (80:20:1 by volume) served as the solvent system. The plates were dried at 50 °C for 30 min. The detection of lipid classes was achieved by immersing the plate into a solution of cupric sulphate and phosphoric acid using the Chromatogram Immersion Device III (CAMAG, Switzerland). The plates were read out by a TLC scanner 3 (CAMAG, Switzerland) and the results were analyzed using the CATS software (Version 4.05, CAMAG, Switzerland).

The proportion of microbial N of non-ammonia-N (NAN) in the daily pooled duodenal chyme samples was estimated by using near infrared spectra (NIRS) according to Lebzien and Paul (1997).

2.4 Calculations

The concentrations of milk fat, milk protein and milk lactose were calculated as a weighted mean corresponding to the milk yield.

Daily duodenal/fecal dry matter flow (DMF), required for the calculation of duodenal nutrient flow, duodenal/fecal FA flow and the apparent ruminal digestibility, was estimated as following:

$$DMF\ [kg/d] = \frac{Chromium\ application\ [mg/d]}{Duodenal/fecal\ chromium\ concentration\ [mg/g\ DM]} \div 1000$$

The daily duodenal/fecal flow of organic matter (OM) and nutrients were estimated by multiplying their chyme concentrations with the DMF. The apparent ruminal digestibility of OM and nutrients was calculated by subtracting their flow percentages of intake from 100%.
The mean ammonia proportion of total N in duodenal chyme was randomly checked and significant differences from the average value (4.9%) as defined by Riemeier et al. (2004) could not be shown. Thus, non-ammonia-N (NAN) was calculated by subtracting this amount of ammonia N from total N at the duodenum. The fraction of microbial N of NAN at the duodenum estimated with NIRS was multiplied by the NAN flow to obtain the flow of microbial N and protein (MP) respectively (N*6.25).
The utilizable crude protein (uCP) at the duodenum was estimated according to Lebzien and Voigt (1999):

$$uCP\ [g/d] = NAN\ \ flow\ at\ the\ duodenum\ [g/d] * 6.25 - endogenous\ CP\ (EP)\ [g]$$

where $EP\ [g] = endogenous\ N\ (EN)\ [g] * 6.25$ and $EN\ [g] = 3.6 * DMF\ [kg]$

(Brandt and Rohr 1981)

Rumen-undegradable crude protein (UDP), rumen-degradable crude protein (RDP), microbial OM and fermented organic matter (FOM) were calculated with the following equations:

$$UDP\ [g/d] = 6.25 * (NAN\ \ flow\ at\ the\ duodenum\ [g/d] - Microbial\ N\ [g/d]) - EP\ [g/d]$$

$$RDP\ [g/d] = CP\ intake\ [g/d] - UDP\ [g/d]$$

$$Microbial\ OM\ [kg/d] = 11.8 * Microbial\ N\ [kg/d]$$ (Schafft 1983)

$$FOM\ [kg/d] = OM\ intake\ [kg/d] - (Duodenal\ OM\ \ flow\ [kg/d] - Microbial\ OM\ [kg/d])$$

ME of forage was calculated by using the nutrient digestibilities from the studies with wethers according to GfE (1991), whereas table values were used for concentrates (DLG 1997).
For the quantification of the *trans*-10,*cis*-12 CLA isomer in milk, the glycerol content of the milk was calculated as described by Schauff et al. (1992), who assumed that milk fat consists nearly completely of triacylglycerides. Thus, milk fat was comprised of on average 88.6% FA and 11.4% of glycerol.
The total amounts of FA in feed, duodenal chyme and feces samples were obtained by using a multiplication factor of 0.950, 0.936 and 0.888, respectively, derived from the quantities of lipid classes analyzed by the HPTLC procedure.

2.5 Statistical analysis

The SAS software package (Version 9.1, SAS Institute, Cary, NC, USA) was used to analyze the data by a one-way ANOVA followed by the Tukey test. If not mentioned otherwise, results are presented as least square means (LS means) with standard error of the mean (SEM). Significant differences were considered if F-statistics revealed $p<0.05$.

Intakes, FA distribution in milk, duodenal chyme and feces as well as rumen and duodenal variables were analyzed using the PROC MIXED procedure. The model contained the period as a fixed factor, and the fact that a cow had to be used in several periods for different treatments was considered in all the models by using the random statement for the individual cow effect. Additionally, for rumen variables, the model contained the time after starting the feeding as fixed factor. To allow for differences resulting from various stages of lactation, the OM intake was applied as a fixed regressive component in all the models except for the nutrient and fatty acid intakes and the *trans*-10,*cis*-12 CLA transfer to milk.

3 Results

The energy content of the feedstuffs was very similar during all three periods (Table 3). The differences in the contents of nutrients were due to crude nutrient variations of the silages (data not shown), since the silos in which grass and maize silage were ensiled changed over the course of the experiment. The intake of the crude nutrients varied between the three periods dependent on the composition of the silages and different DM intakes (Table 4). The content of the *trans*-10,*cis*-12 CLA isomer in the diet was calculated based on the analyzed concentrations in maize silage, grass silage and concentrate. During the CON, CLA-1 and CLA-2 period the cows received 0.01, 0.24 and 0.64 g *trans*-10,*cis*-12 CLA/kg DM, respectively.

Table 3: Nutrient composition and energy content of the different diets.

		Treatment[♦]	
[g/kg DM]	CON	CLA-1	CLA-2
Organic matter	932	931	928
Nutrients			
Crude protein	122	139	133
Ether extract	34	39	41
Crude fiber	183	167	179
Neutral detergent fiber[§]	410	387	400
Acid detergent fiber[§]	213	198	209
Starch	246	238	239
Trans-10,*cis*-12 CLA*	0.01	0.24	0.64
Metabolizable energy [MJ/kg DM][#]	10.3	10.4	10.4

[♦]CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; [§]free of residual ash; *Calculation based on analyzed concentrations in concentrate and forage; [#]Calculation for forage based on nutrient digestibilities measured with weathers and for concentrates tabular values were used

Table 4: Nutrient intakes by lactating and non-lactating cows (LS means ± SEM)

[kg/d]	Treatment♦						*p*-value
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Dry matter	13.89[a]	±0.72	13.87[a]	±0.72	12.61[b]	±0.73	<0.001
Organic matter	12.94[a]	±0.67	12.92[a]	±0.67	11.70[b]	±0.68	<0.001
Ether extract	0.47[c]	±0.02	0.53[a]	±0.02	0.51[b]	±0.02	<0.001
N	0.27[b]	±0.02	0.31[a]	±0.02	0.27[b]	±0.02	<0.001
Neutral detergent fiber	5.70[a]	±0.30	5.37[b]	±0.30	5.05[c]	±0.30	<0.001
Acid detergent fiber	2.95[a]	±0.15	2.75[b]	±0.15	2.64[c]	±0.15	<0.001
Starch	3.41[a]	±0.17	3.30[b]	±0.17	3.01[c]	±0.17	<0.001

[abc] Values with different superscripts in the rows are significantly different ($p<0.05$)

♦CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d

The ruminal parameters are presented as LS means over the whole sampling time, as no significant effects or trends (Tukey method) at single measurement times were found between CON, CLA-1 and CLA-2 (Table 5). There were no significant treatment differences regarding ruminal pH-value, ammonia concentration and total VFA concentration. The molar proportion of acetic acid was significantly declined after supplementation of 50 g CLA preparation/d, whereas the molar proportion of butyric acid was enhanced in this period. The CLA supplementation had no effect on the molar proportion of propionic acid. A significant and dose-dependent increase was observed for the molar proportion of isobutyric acid and isovaleric acid, while the molar proportion of valeric acid increased significantly after CLA supplementation but not in a dose-dependent manner.

Table 5: Effect of CLA supplementation on ruminal parameters averaged over sampling times (just before and 30, 60, 90, 120, 180, 360 minutes after the first feeding in the morning) (LS means ± SEM).

	Treatment♦						*p*-value	
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		CLA	Time
pH	6.23	±0.10	6.23	±0.09	6.40	±0.11	0.221	<0.001
NH_3 [mmol/L]	6.81	±1.08	7.69	±1.07	6.70	±1.13	0.114	<0.001
VFA* [mol %]:								
Acetic acid	64.2[a]	±1.1	62.1[b]	±1.1	63.9[ab]	±1.2	0.004	<0.001
Propionic acid	19.2	±0.7	19.3	±0.7	17.9	±0.8	0.114	<0.001
Isobutyric acid	0.7[b]	±0.1	0.7[b]	±0.1	0.9[a]	±0.1	0.001	<0.001
Butyric acid	13.1[b]	±0.4	14.4[a]	±0.4	13.7[ab]	±0.5	0.001	<0.001
Isovaleric acid	1.1[b]	±0.2	1.2[b]	±0.2	1.5[a]	±0.2	0.003	0.086
Valeric acid	1.7[b]	±0.4	2.2[a]	±0.4	2.2[a]	±0.4	<0.001	<0.001
Acetic acid : Propionic acid	3.4	±0.2	3.4	±0.2	3.7	±0.2	0.384	<0.001
VFA [mmol/L]	102.8	±5.2	100.4	±5.1	92.2	±6.0	0.334	<0.001

[ab] Values with different superscripts in the rows are significantly different ($p< 0.05$)

♦CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; *Volatile fatty acids

As shown in Table 6, the CLA supplementation had no influence on the duodenal nutrient flow. The proportion of FOM to OM intake was not altered by CLA. The addition of CLA did not affect the apparent ruminal digestibility of OM, NDF and ADF. Only an improved apparent ruminal digestibility of starch was observed after the supplementation of 100 g CLA preparation/d.

Table 6: Effect of CLA supplementation on nutrient flow at the duodenum, ruminal fermented organic matter (FOM) and apparent ruminal digestibility (LS means ± SEM).

	Treatment[♦]						*p*-value
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Duodenal flow [kg/d]							
Organic matter (OM)	7.45	±0.15	7.40	±0.15	7.21	±0.15	0.466
Neutral detergent fiber[§]	2.97	±0.09	2.78	±0.09	2.83	±0.09	0.311
Acid detergent fiber[§]	1.67	±0.06	1.57	±0.06	1.57	±0.06	0.341
Starch	0.44	±0.06	0.40	±0.06	0.29	±0.06	0.068
Fermented organic matter (FOM) [%]	6.41	±0.14	6.29	±0.14	6.35	±0.14	0.627
FOM of OM intake [%]	52.3	±1.1	51.4	±1.1	52.0	±1.1	0.672
Apparent ruminal digestibility [%]							
Organic matter (OM)	39.0	±1.3	39.5	±1.3	41.1	±1.3	0.424
Neutral detergent fiber[§]	43.9	±1.6	47.6	±1.6	47.9	±1.6	0.175
Acid detergent fiber[§]	39.1	±2.1	42.2	±2.1	44.4	±2.2	0.161
Starch	86.1[b]	±1.7	87.3[ab]	±1.7	90.9[a]	±1.7	0.046

[ab] Values with different superscripts in the rows are significantly different ($p < 0.05$)
[♦]CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; [§]free of residual ash

The effects of CLA supplementation on nitrogen flow at the duodenum and microbial synthesis in the rumen are presented in Table 7. Even though the amount of N and non-ammonia-nitrogen (NAN) at the duodenum was not influenced by CLA, the proportion of duodenal N related to N intake was significantly reduced by approximately 25% after CLA treatment. Moreover, the CLA supplementation resulted in a decline in the proportion of ruminally undegraded feed protein (UDP) in relation to crude protein (CP) intake. Regarding the parameters of ruminal microbial synthesis, only the amount of microbial protein (MP) related to ruminally degraded protein (RDP) was significantly reduced after CLA supplementation. The other parameters of the microbial synthesis remained unaffected by CLA.

Table 7: Effect of CLA supplementation on nitrogen flow at the duodenum and efficiency of ruminal microbial synthesis (LS means ± SEM).

	Treatment[♦]						*p*-value
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Nitrogen flow:							
N [g/d]	226	±13	203	±13	188	±14	0.168
N of N intake [%]	88.5[a]	±4.4	66.5[b]	±4.4	65.9[b]	±4.5	0.005
NAN[§] [g/d]	215	±13	193	±13	179	±13	0.168
uCP[†] [g/d]	1136	±78	1000	±78	919	±79	0.171
UDP[#] [g/d]	274	±29	229	±29	214	±29	0.302
UDP per CP intake [%]	17.1[a]	±1.5	12.0[b]	±1.5	12.0[b]	±1.5	0.036
Microbial synthesis:							
MP* [g/d]	862	±54	771	±54	705	±55	0.135
MP per FOM[Δ] [g/kg]	137	±9	125	±9	112	±9	0.174
MP per ME[‡] [g/MJ]	6.4	±0.4	5.7	±0.4	5.2	±0.4	0.099
MP per RDP[††] [g/g]	0.63[a]	±0.04	0.47[b]	±0.04	0.45[b]	±0.04	0.015

[ab] Values with different superscripts in the rows are significantly different ($p < 0.05$)

[♦]CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; [§]Non-ammonia-nitrogen; [‡]Metabolizable energy; [†]Utilizable crude protein, [#]Ruminally undegraded feed protein; *Microbial crude protein; [Δ]Fermented organic matter; [††]Ruminally degraded protein

Intakes of individual and total FA are presented in Table 8. Accompanied by the higher fat intake, an on average 59 g/d significant higher total FA intake was observed in both CLA periods. Differences in the intake of stearic acid (C18:0), oleic acid (C18:1) and CLA reflected the fatty acid composition of the fat supplements. Cows fed the control fat supplement consumed more C18:0 but less C18:1 and CLA than the animals fed the CLA preparation (CLA-1, CLA-2). Furthermore, the difference in the fatty acid composition of the silages is an influencing factor. In the CLA periods, the intake of C16:0, non-conjugated C18:2 and of C18:3 fatty acids was significantly higher compared to the control period. The animals consumed 0.1, 3.1 and 8.0 g *trans*-10,*cis*-12 CLA and 0.2, 3.1 and 7.8 g *cis*-9,*trans*-11 CLA per day in Period CON, CLA-1 and CLA-2, respectively. The intake of total C18 was 42 g/d higher in the CLA periods compared to the control.

Table 8: Intake of selected fatty acids (FA) and total FA of lactating and non-lactating cows (LS means ± SEM)

	Treatment[♦]						*p*-value
Intake [g/d]	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Total FA	429.1[b]	±21.2	486.9[a]	±21.2	490.0[a]	±21.2	<0.001
C16:0	71.6[c]	±3.9	83.0[b]	±3.9	85.1[a]	±3.9	<0.001
C18:0	93.7[a]	±0.6	77.3[b]	±0.6	47.3[c]	±0.6	<0.001
cis C18:1	52.7[c]	±3.1	66.5[b]	±3.1	72.8[a]	±3.1	<0.001
trans C18:1	0.51[c]	±0.03	0.61[b]	±0.03	0.62[a]	±0.03	<0.001
Non-conjugated C18:2	120.7[c]	±7.6	145.4[b]	±7.6	154.8[a]	±7.6	<0.001
Conjugated linoleic acid (CLA)							
trans-10, *cis*-12 C18:2	0.1[c]	±0.01	3.1[b]	±0.01	8.0[a]	±0.01	<0.001
cis-9, *trans*-11 C18:2	0.2[c]	±0.01	3.1[b]	±0.01	7.8[a]	±0.01	<0.001
Total CLA	0.7[c]	±0.04	6.8[b]	±0.04	16.6[a]	±0.04	<0.001
C18:3 (n-3/n-6)	63.8[c]	±4.3	77.2[b]	±4.3	81.5[a]	±4.3	<0.001
Total C18	332.1[b]	±15.7	373.8[a]	±15.7	373.6[a]	±15.7	<0.001

[abc] Values with different superscripts in the rows are significantly different ($p<0.05$)

[♦]CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d

Table 9 shows the effects of supplementing lipid-encapsulated CLA on the duodenal flows of total and individual FA. The total FA flow to the duodenum averaged 667 g/d and was unaffected by the diet. Significantly smaller amounts of C16:0 reached the duodenum in Period CLA-2 compared to the control. The flow of C18:0 was dose-dependently and significantly reduced by 9 and 14% in the two CLA periods, respectively. The CLA supplemented diet increased the flows of *cis* C18:1, *trans* C18:1, non-conjugated C18:2, total CLA and of the two CLA isomers *trans*-10,*cis*-12 and *cis*-9,*trans*-11. In Period CLA-2 the flow of C18:3 (n-3/n-6) was significantly increased compared to the control. The flow of total C18 was with 539 g/d highest for the control period and significantly reduced by 5% for the CLA-2 cows. The flow of total FA to the duodenum relative to total FA intake was significantly and dose-dependently reduced for cows fed the CLA supplement. A significantly higher flow (% of intake) of *trans*-10,*cis*-12 CLA was observed in Period CLA-1 compared to the control. In Table 10 the excretion of FA in the feces is presented. The fecally excreted amount of *cis* C18:1 was highest for animals supplemented with 100 g CLA preparation/d. The excretion of *trans* C18:1 increased dose-dependently when CLA was added to the diet. Total CLA excretion and content of individual CLA isomers in the feces was highest for the cows in Period CLA-1. The apparent post-ruminal digestibility of *trans* C18:1 in the CLA-2 period was significant lower compared to the CON period. For the *trans*-10,*cis*-12 CLA isomer the highest apparent post-ruminal digestibility was observed in Period CLA-2.

Due to the great influence of the stage of lactation and the small number of lactating cows in Period CLA-2, data concerning milk yield and milk composition were not evaluated statistically. The milk yield of the lactating cows was 20.2 kg/d, 19.2 kg/d and 22.2 kg/d for

the CON (n=5), CLA-1 (n=5) and the CLA-2 period (n=3), respectively. The milk fat yield was reduced by 11.1 and 14.8% for Period CLA-1 (0.72 kg/d) and CLA-2 (0.69 kg/d), respectively, compared to the CON period (0.81 kg/d). The milk protein yield was similar among treatments with 0.63 kg/d, 0.63 kg/d and 0.65 kg/d for the CON, CLA-1 and CLA-2 period, respectively.

Table 9: Effect of CLA supplementation on the fatty acid (FA) flow to the duodenum (LS means ± SEM).

	Treatment[♦]						*p*-value
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Flow to the duodenum [g/d]							
Total FA	682.9	±11.9	668.9	±11.8	649.4	±12.1	0.056
C16:0	91.9[a]	±1.7	86.3[ab]	±1.7	84.1[b]	±1.7	0.015
C18:0	441.7[a]	±13.5	402.7[b]	±13.4	377.9[c]	±13.8	<0.001
cis C18:1	31.5[c]	±2.6	39.2[a]	±2.6	37.3[b]	±2.6	0.012
trans C18:1	54.2[b]	±6.6	68.5[a]	±6.5	74.0[a]	±6.7	0.005
Non-conjugated C18:2	10.1[b]	±1.6	17.2[a]	±1.6	17.4[a]	±1.6	0.007
Conjugated linoleic acid (CLA)							
trans-10,*cis*-12 C18:2	0.0[b]	±0.1	0.5[a]	±0.1	0.4[a]	±0.1	0.001
cis-9,*trans*-11 C18:2	0.1[b]	±0.1	0.5[a]	±0.1	0.4[a]	±0.1	0.004
Total CLA	0.2[b]	±0.3	2.1[a]	±0.3	1.6[a]	±0.3	<0.001
C18:3 (n-3/n-6)	0.9[b]	±0.3	1.8[ab]	±0.3	2.4[a]	±0.3	0.008
Total C18	539.1[a]	±9.9	531.8[ab]	±9.8	509.9[b]	±10.0	0.017
Flow to the duodenum [% of intake]							
Total FA	162[a]	±2	139[b]	±2	129[c]	±3	<000.1
Non-conjugated C18:2	9	±1	12	±1	11	±1	0.081
trans-10,*cis*-12 CLA	0[b]	±2	16[a]	±2	5[b]	±2	<0.001
C18:3 (n-3/n-6)	2	±0.3	2	±0.3	3	±0.3	0.052

[abc] Values with different superscripts in the rows are significantly different ($p<0.05$)

[♦]CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d

Table 10: Effect of CLA supplementation on the fatty acid (FA) excretion with feces and the post-ruminal digestibility (LS means ± SEM).

	Treatment♦						*p*-value
	CON (n=6)		CLA-1 (n=6)		CLA-2 (n=6)		
Excretion with feces [g/d]							
Total FA	288.4	±20.4	254.4	±20.3	252.5	±20.5	0.401
C16:0	36.6	±3.0	32.8	±3.0	36.4	±3.0	0.617
C18:0	208.9	±16.6	170.7	±16.5	146.2	±16.7	0.056
cis C18:1	5.5[b]	±1.0	7.9[ab]	±1.0	9.7[a]	±0.1	0.042
trans C18:1	4.5[c]	±0.7	7.3[b]	±0.7	10.2[a]	±0.7	<0.001
Non-conjugated C18:2	3.4	±0.9	3.9	±0.9	5.4	±0.9	0.326
Conjugated linoleic acid (CLA)							
trans-10,*cis*-12 C18:2	0.0[b]	±0.03	0.4[a]	±0.03	0.1[b]	±0.04	<0.001
cis-9,*trans*-11 C18:2	0.2[b]	±0.1	0.4[a]	±0.1	0.3[ab]	±0.1	0.047
Total CLA	0.4[b]	±0.1	1.2[a]	±0.1	0.7[ab]	±0.2	0.003
C18:3 (n-3/n-6)	0.7	±0.1	0.8	±0.1	0.9	±0.1	0.581
Total C18	223.4	±17.0	191.4	±17.0	173.1	±17.1	0.148
Apparent post-ruminal digestibility [%]*							
Total FA	58	±3	62	±3	61	±3	0.623
C16:0	60	±3	62	±3	57	±3	0.558
C18:0	53	±4	57	±4	62	±4	0.345
cis C18:1	81	±2	81	±2	75	±2	0.110
trans C18:1	91[a]	±1	89[ab]	±1	86[b]	±1	0.014
Non-conjugated C18:2	64	±6	77	±6	71	±6	0.305
Conjugated linoleic acid (CLA)							
trans-10,*cis*-12 C18:2	-1[b]	±7	18[b]	±7	69[a]	±7	<0.001
cis-9,*trans*-11 C18:2	-71	±43	17	±43	9	±43	0.310
Total CLA	-39	±25	41	±25	45	±25	0.054
C18:3 (n-3/n-6)	23	±10	53	±10	59	±10	0.055
Total C18	59	±3	64	±3	66	±3	0.278

[abc] Values with different superscripts in the rows are significantly different ($p<0.05$)
♦CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; *The apparent post-ruminal digestibility of FA was calculated as: (FA duodenal flow [g/d] – FA excretion with feces [g/d]) * 100 / FA duodenal flow [g/d]

The milk yield did not correlate with the percentages of FAME in milk fat (Spearman's rank correlation). Therefore the FA distribution in milk fat was evaluated statistically (Table 11). The CLA supplementation altered the FA profile in milk predominantly in Period CLA-1. The percentages of C8:0, C10:0 and C16:0 were significantly reduced by the supplementation of 50 g CLA-preparation/d. The percentage of C16:1 was significantly higher in Period CLA-1 compared to Period CLA-2. The percentages of total *trans* C18:1 and *trans*-9,*trans*-12 C18:2 were increased significantly in the CLA-1 period, while the percentage of C18:3 (n-3/n-6) increased significantly and in a dose-dependent manner. The addition of the CLA supplement increased the proportion of *trans*-10,*cis*-12 CLA significantly and dose-dependently, but the actual amount of this fatty acid in milk remained very low in all periods. Even the *cis*-9,*trans*-11 CLA, being the main CLA isomer in milk fat, was significantly increased after CLA supplementation. Thus, a significant increase of the total proportion of CLA in milk fat was observed for the supplementation periods. When milk FA were grouped based on their

origin, no effect was observed for *de novo* synthesized FA (<C16) compared to control, while the proportion of FA absorbed from blood into the mammary gland increased significantly in Period CLA-1. The proportion of FA of both sources (C16) was significantly decreased after the addition of 50 g CLA preparation/d to the diet.

The transfer of *trans*-10,*cis*-12 CLA from feed to milk was significantly higher in the CLA-1 period compared to the control. Six percent of the consumed *trans*-10,*cis*-12 CLA were transferred to milk. Compared to the control, 36 and 48% of the duodenally available *trans*-10,*cis*-12 CLA were transferred into the milk in Period CLA-1 and CLA-2, respectively.

Table 11: Effect of dietary supplementation of CLA to dairy cows on fatty acid (FA) profile of milk and the *trans*-10,*cis*-12 CLA transfer into milk (LS means ± SEM).

	Treatment♦						*p*-value
Fatty acid [% of total FAME*]	CON (n=5)		CLA-1 (n=5)		CLA-2 (n=3)		
C4:0	3.35	±0.24	3.22	±0.23	3.86	±0.35	0.128
C6:0	3.28	±0.37	2.48	±0.34	2.64	±0.65	0.188
C8:0	1.52^{a}	±0.09	1.26^{b}	±0.08	1.33^{ab}	±0.16	0.026
C10:0	3.07^{a}	±0.19	2.62^{b}	±0.18	2.69^{ab}	±0.33	0.025
C12:0	3.29	±0.19	2.92	±0.18	2.80	±0.32	0.054
C14:0	11.85	±0.39	11.53	±0.36	11.32	±0.63	0.495
C14:1	1.56	±0.19	1.60	±0.18	1.28	±0.24	0.268
C15:0	1.10	±0.10	1.13	±0.10	0.93	±0.13	0.180
C16:0	31.51^{a}	±1.19	28.44^{b}	±1.10	31.47^{ab}	±2.05	0.005
C16:1	2.55^{ab}	±0.44	2.59^{a}	±0.44	1.78^{b}	±0.49	0.031
C17:0	0.56	±0.05	0.58	±0.04	0.48	±0.07	0.262
C18:0	7.24	±0.86	8.18	±0.80	7.90	±1.47	0.415
trans C18:1	3.79^{b}	±0.53	5.27^{a}	±0.51	4.76^{ab}	±0.79	<0.001
cis-9 C18:1	18.42	±0.74	20.44	±0.70	17.60	±1.27	0.070
trans-9,*trans*-12 C18:2	0.08^{b}	±0.01	0.10^{a}	±0.01	0.10^{ab}	±0.01	0.005
cis-9,*cis*-12 C18:2	1.59	±0.20	1.66	±0.19	2.07	±0.27	0.209
Conjugated linoleic acid (CLA)							
trans-10,*cis*-12 C18:2	<0.01^{c}	±0.01	0.03^{b}	±0.01	0.06^{a}	±0.01	<0.001
cis-9,*trans*-11 C18:2	0.74^{b}	±0.07	0.99^{a}	±0.07	1.16^{a}	±0.11	<0.001
Total CLA	0.85^{b}	±0.08	1.18^{a}	±0.08	1.36^{a}	±0.12	<0.001
C18:3 (n-3/n-6)	0.25^{b}	±0.03	0.37^{a}	±0.03	0.44^{a}	±0.05	<0.001
C20:0	0.11	±0.01	0.11	±0.01	0.11	±0.02	0.911
Other	2.36^{b}	±0.14	2.62^{a}	±0.14	2.31^{ab}	±0.21	0.005
Summation							
<C16	30.65	±0.98	28.14	±0.90	28.07	±1.74	0.064
C16	34.34^{a}	±1.49	31.24^{b}	±1.41	33.26^{ab}	±2.36	0.007
>C16	35.76^{b}	±1.74	41.04^{a}	±1.61	37.09^{ab}	±3.14	0.018
Trans-10,cis-12 CLA transfer to milk [%]°							
of intake	0^{b}	±1	6^{a}	±1	3^{ab}	±1	0.005
of duodenal flow	0^{b}	±5	36^{a}	±5	48^{a}	±7	<0.001

[abc] Values with different superscripts in the rows are significantly different ($p < 0.05$)
♦CON, Cows fed the fat supplement without CLA; CLA-1, Cows fed 50 g of the CLA-supplement/d; CLA-2, Cows fed 100 g of the CLA-supplement/d; *Fatty acid methyl ester; °The transfer rates were calculated deducting the values of control

4 Discussion

Due to the variations in the nutrient composition of the silages during the three periods, it cannot be ruled out that these variations account for some of the observed effects (Table 3). The intended individual intake was 0 g, 5 g and 10 g *trans*-10,*cis*-12 CLA/d in period CON, CLA-1 and CLA-2, respectively. However, intakes in Period CLA-1 and CLA-2, based on the actual DM intake and the analyzed concentrations in the concentrates were with 3.1 g and 8.0 g *trans*-10,*cis*-12 CLA/d lower than calculated. Since the analyzed proportion of *trans*-10,*cis*-12 CLA in the supplement corresponded to the data supplied by the manufacturer (Table 2: 11.88% of total FAME), we cannot exclude the possibility that the process of pelleting reduced the amount of FA, although the concentrate was manufactured at the Institute`s own feed mill which enabled gentle pelleting with low temperature, steam and pressure.

To determine a possible influence of dietary CLA on ruminal fermentation, concentrations of VFA and NH_3, as products of carbohydrate and protein degradation, were measured. *In vitro* experiments showed that unsaturated free FA are more inhibitory towards VFA production than SFA (Chalupa et al. 1984). The total VFA concentration and the pH-value in rumen fluid were not affected by CLA in the present trial. The effects on the molar proportions of VFA were not dose-dependent and the differences were small and of unknown biological significance. The unaffected acetic acid-to-propionic acid ratio suggests that the CLA supplementation had no adverse effects on cellulolytic activity. Huang et al. (2009) reported that the addition of 1% of CLA in the diet of sheep, as free acid or calcium salt, did not change the concentrations of total VFA and did not even influence the proportions of individual VFA in the ruminal fluid. In accordance with our results, the ruminal concentration of NH_3, as a marker for alterations of protein metabolism in the rumen, also remained unaffected by dietary CLA.

To our knowledge there are no other investigations on the effects of the addition of CLA in a free or in a protected form on the duodenal flow, the apparent ruminal digestibilities of nutrients and the N-metabolism in rumen. It is not clear if the enhanced apparent ruminal digestibility of starch in Period CLA-2 should be attributed to an influence of CLA on the microbes, or to the differences in the composition of the forage, or to forage quality fed in this period.

The amount of UDP in relation to CP intake was decreased by approximately 30% for the two CLA treatments. Thus higher quantities of MP in duodenal chyme and higher concentrations of NH_3 in rumen fluid could be expected. But MP decreased numerically, and the ruminal

NH_3 concentration remained unaffected by dietary CLA supplementation. Related to RDP, MP was on average about 27% lower after CLA supplementation compared to the control, suggesting a declined efficiency of the use of RDP for MP synthesis. Since there was no difference in the portion of fermented organic matter (FOM) in the rumen, it can be assumed that the energy needed for MP synthesis was available for the microbes. Furthermore, the ruminal N balance (RNB) was positive at all sampling times (data not shown), hence an adequate supply of microorganisms with N was achieved for the three periods. Therefore an N deficiency can be excluded as the cause for the decreased efficiency in MP synthesis in the CLA treated animals. Obviously the rumen microbes did not use the extra N by the degraded CP. It is possible that the ruminal absorption of ammonia was higher when cows received the CLA supplemented diet or that less of urea-N was recycled to the rumen and more N was excreted compared to the control period, but the NH_3 concentration in blood and the daily N excretion was not examined in the present trial. In the literature, there is no evidence for an influence of dietary added lipids on ammonia absorption or urea-N recycling in the rumen. Doreau and Ferlay (1995) reviewed the effect of dietary lipids on the ruminal nitrogen metabolism and showed that in most studies the lipid supplementation did not result in any variation in the ammonia concentration in the rumen, and that there was no relationship between FA supplementation and NAN flow.

Due to the *de novo* FA synthesis of ruminal microbes, the duodenal flow of total FA was higher than the total FA intake for all three treatments. However, the total fatty acid flow did not differ between the three treatments despite the different total FA intakes. It seems that the microbial FA synthesis was adversely affected by the slightly higher FA intake or the CLA intake in Periods CLA-1 and CLA-2. After the addition of CLA to the diet, the duodenal flow of the SFA C16:0 and C18:0 decreased, while the flow of all UFA increased. This effect may be explainable with the higher intakes of UFA in these two periods.

CLA originates from ruminal biohydrogenation of linoleic acid and serves as a substrate for the reduction to C18:1 and C18:0. In the lipid-encapsulated form, the CLA should be resistant to biohydrogenation, and the duodenal flow of CLA should increase distinctly after the supplementation of the CLA preparation. But the duodenal flows of 0.5 and 0.4 g/d of the *trans*-10,*cis*-12 CLA isomer in Period CLA-1 and Period CLA-2, respectively, suggest a low ruminal protection rate. Based on an intake of 3.1 and 8.0 g *trans*-10,*cis*-12 CLA/d, only 16 and 5% of the CLA consumed reached the duodenum, and were thus rumen-protected in Periods CLA-1 and CLA-2, respectively. The different protection rates between the CLA-1 and CLA-2 treatment cannot be explained under the conditions of the present experiment. Up

to now studies evaluating the degree of rumen inertness of CLA supplements were either conducted *in vitro* (Dehkordi et al. 2008) or the rumen-inertness was estimated from the transfer efficiency of *trans*-10,*cis*-12 CLA from feed into milk. For calcium salts of CLA, de Veth et al. (2005a) summarized eight studies. The range in transfer efficiency of *trans*-10,*cis*-12 CLA into milk ranged from 1.9 to 7.4%. That would represent a protection rate of 9 to 34%, which is in accordance with the current results. Perfield et al. (2004b) showed in an overview that the addition of amide-protected and formaldehyde-protected CLA resulted in a transfer of 7.1 and 7.0% *trans*-10,*cis*-12 CLA into milk, which is equivalent to 36 and 35% of protection, respectively. Across four studies realized with lipid-encapsulated CLA supplements (Perfield et al. 2004c; Castañeda-Gutiérrez et al. 2007; Odens et al. 2007; Moallem et al. 2010), the *trans*-10,*cis*-12 CLA transfer efficiency into milk ranged from 4.6 to 7.9%, and the resulting protection rate was, with rates of 23 to 40%, higher than the present results.

The transfer efficiencies of *trans*-10,*cis*-12 CLA from duodenum to milk (CLA-1: 36%, CLA-2: 48%) were higher than previously reported from abomasal infusion studies (mean of 22%) (de Veth et al. 2004). In a dietary study without any CLA supplementation, the duodenal flow of *trans*-10,*cis*-12 CLA amounted to an average of 0.12 g/d (Piperova et al. 2002). Here 72% were transferred into milk on average. In two dietary studies it was shown that the duodenal flow of natural *trans*-10,*cis*-12 CLA originated from ruminal biohydrogenation ranged from 0.01 to 0.57 g/d (Flachowsky et al. 2006; Liu et al. 2011) and the transfer efficiencies of duodenal *trans*-10,*cis*-12 CLA were higher than 100%, probably due to analytical inaccuracies. However, the data suggest that the absorption of *trans*-10,*cis*-12 CLA after dietary supplementation or abomasal infusion is lower compared to the absorption of naturally formed *trans*-10,*cis*-12 CLA.

Dehkordi et al. (2008) inferred from a 24 h *in vitro* incubation trial that the protection of *trans*-10,*cis*-12 CLA in the used supplement was around 47%, and that the processing of the CLA supplement increased its sensitivity to rumen biohydrogenation. It is possible that the gentle pelleting in the current trial adversely influenced the rumen inertness of the applied fat supplement and resulted in a large part of *trans*-10,*cis*-12 CLA being biohydrogenated in the rumen.

Resulting from rumen biohydrogenation high proportions of saturated fatty acids reach the small intestine. In the small intestine the main absorption processes occur, and the total FA flow leaving the small intestine is generally considered to be close to total fecal flow (Doreau and Ferlay 1994). However, due to the bacterial colonization of the large intestine, microbial

fatty acid synthesis and biohydrogenation take place as well. Doreau and Ferlay (1994) described a dependency of fatty acid synthesis on the substrate supply in the large intestine. In the present trial only the apparent digestion in the whole intestine was recorded. But the results should not be over- interpreted, since the total fecally excreted FA amounts were only calculated from the chromium concentration of a single fecal sample. Except for total C18:1 and *trans*-10,*cis*-12 CLA, the CLA supplementation did not influence the apparent post-ruminal digestion of FA. The declined digestibility of C18:1 in Period CLA-2 was possibly due to the abundant supply of this FA in the gut. The digestibility of *trans*-10,*cis*-12 CLA in the gut differed between Period CLA-1 and CLA-2 by 51%. But this difference represents a quantity of only 0.2 g.

Even if the data concerning milk yield and milk composition were not evaluated statistically, their magnitude should be compared with the literature here. Sippel et al. (2009) compared the CLA-induced milk fat depression reported in seven feeding trials using different doses of *trans*-10,*cis*-12 CLA. By plotting the depression in daily milk fat yield over the dosages of CLA they obtained an equation for the expected milk fat depression after the dietary consumption of CLA. Applying their equation to the present study, the dietary addition of 3.1 and 8.0 g *trans*-10,*cis*-12 CLA/d should result in a milk fat depression of 6.5 and 14.0% in Periods CLA-1 and CLA-2, respectively. The actual reduction of milk fat yield was, with 11.1% in the CLA-1 period, slightly higher compared to the estimated value when 3.1 g *trans*-10,*cis*-12 CLA/d were fed. In Period CLA-2 the actual reduction of milk fat yield of 14.8% corresponds to the estimated value.

Corresponding to the findings of Bauman and Griinari (2001); Perfield et al. (2002b) and Maxin et al. (2010), the milk fat proportion of C8:0, C10:0 and C16:0 decreased, and of fatty acids >C16, increased in Period CLA-1 in the current trial. In accordance with Moore et al. (2004) the proportion of *trans*-10,*cis*-12 CLA in milk fat increased dose-dependently after treatment. The *cis*-9,*trans*-11 CLA is the main CLA isomer occurring naturally in milk fat and is predominantly (>80%) *de novo* synthesized in the mammary gland (Lock and Garnsworthy 2002). In contrast to earlier findings by Castaneda-Gutierrez et al. (2007); Piamphon et al. (2009) and Pappritz et al. (2011) the proportion of this fatty acid in milk fat increased after dietary treatment with a mixture of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA. However, this corresponds to results by Perfield et al. (2002a), who observed an increase of the *cis*-9,*trans*-11 CLA content in milk after feeding a mixture of 4 CLA isomers (*trans*-8,*cis*-10; *cis*-9,*trans*-11; *trans*-10,*cis*-12; *cis*-11,*trans*-13 CLA).

5 Conclusions

Only minor changes of rumen metabolism could be observed after dietary supplementation of lipid-encapsulated CLA under the conditions of the present study. The actual duodenal availability of *trans*-10,*cis*-12 CLA was very low (CLA-1: 16%, CLA-2: 5%), suggesting a low protection rate of the applied lipid-encapsulated CLA supplement. It could be assumed that rumen microorganisms were partially exposed to the applied lipid-encapsulated fatty acids, but the consequences on rumen metabolism were negligible.

References

Bassler, R. E.1976.Die chemische Untersuchung von Futtermitteln, VDLUFA.,Darmstadt.

Bauman,D.E. and Griinari,J.M.2001.Regulation and nutritional manipulation of milk fat: low-fat milk syndrome.Livest Prod Sci.70(1-2):15-29.

Brandt,M. and Rohr,K.1981.Beiträge zur Quantifizierung der N-Umsetzung in den Vormägen von Milchkühen. 1. Bestimmung des Mikrobenstickstoffs im Duodenalchymus mit Hilfe von ^{15}N.Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde.46:39-59.

Castaneda-Gutierrez,E., Benefield,B.C., de Veth,M.J., Santos,N.R., Gilbert,R.O., Butler,W.R. and Bauman,D.E.2007.Evaluation of the Mechanism of Action of Conjugated Linoleic Acid Isomers on Reproduction in Dairy Cows.J Dairy Sci.90(9):4253-4264.

Castañeda-Gutiérrez,E., Benefield,B.C., de Veth,M.J., Santos,N.R., Gilbert,R.O., Butler,W.R. and Bauman,D.E.2007.Evaluation of the Mechanism of Action of Conjugated Linoleic Acid Isomers on Reproduction in Dairy Cows.J Dairy Sci.90(9):4253-4264.

Chalupa,W., Rickabaugh,B., Kronfeld,D. and vid Sklan,S.1984.Rumen Fermentation In Vitro as Influenced by Long Chain Fatty Acids.J Dairy Sci.67(7):1439-1444.

de Veth,M.J., Griinari,J.M., Pfeiffer,A.M. and Bauman,D.E.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(4):365-372.

de Veth,M.J., Gulati,S.K., Luchini,N.D. and Bauman,D.E.2005b.Comparison of Calcium Salts and Formaldehyde-Protected Conjugated Linoleic Acid in Inducing Milk Fat Depression.J Dairy Sci.88(5):1685-1693.

de Veth,M.J., Gulati,S.K., Luchini,N.D. and Bauman,D.E.2005a.Comparison of Calcium Salts and Formaldehyde-Protected Conjugated Linoleic Acid in Inducing Milk Fat Depression.J Dairy Sci.88(5):1685-1693.

Dehkordi,S.K., Vlaeminck B, Hostens M, Opsomer G and Fievez V.2008.In vitro rumen biohydrogenation od trans-10, cis-12 conjugated linoleic acid in a lipid-encapsulated (LE-CLA) supplement incorporated or not in a processing pellet.Comm Appl Biol Sci.73(1):119-122.

DLG.1997.Futterwerttabellen Wiederkäuer.DLG-Verlags-GmbH,Frankfurt am Main (Germany).

Doreau,M. and Ferlay,A.1994.Digestion and utilisation of fatty acids by ruminants.Animal Feed Science and Technology.45(3-4):379-396.

Doreau,M. and Ferlay,A.1995.Effect of Dietary Lipids on Nitrogen-Metabolism in the Rumen - A Review.Livestock Production Science.43(2):97-110.

Flachowsky,G., Erdmann,K., Huther,L., Jahreis,G., Mockel,P. and Lebzien,P.2006.Influence of roughage/concentrate ratio and linseed oil on the concentration of trans-fatty acids

and conjugated linoleic acid in duodenal chyme and milk fat of late lactating cows.Archives of Animal Nutrition.60(6):501-511.

Folch,J., Lees,M. and Stanley,G.H.S.1957.A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues.J Biol Chem.226(1):497-509.

Geissler,C., Hoffmann,M. and Hickel,B.1976.Ein Beitrag zur gaschromatographischen Bestimmung flüchtiger Fettsäuren.Arch Tierernahr.26:123-129.

GfE.1991.Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern.J Anim Physiol Anim Nutr.65:229-234.

Goering, HK and Van Soest, PJ.1970.Forage fiber analyses (Apparatus, reagents, procedure and some applications).Agric. Handbook No 379.ARS-USDA,Washington DC.

Huang,Y., Schoonmaker,J.P., Oren,S.L., Trenkle,A. and Beitz,D.C.2009.Calcium salts of CLA improve availability of dietary CLA.Livest Sci.122(1):1-7.

Lebzien,P. and Paul,C.1997.Use of near-infrared reflectance spectroscopy for the estimation of the microbial portion of non-ammonia-nitrogen in the duodenum of dairy cows.Anim Feed Sci Tech.68(3-4):225-233.

Lebzien,P. and Voigt,J.1999.Calculation of utilizable crude protein at the duodenum of cattle by two different approaches.Arch Anim Nutr.52(4):363-369.

Liu,S.J., Bu,D.P., Wang,J.Q., Sun,P., Wei,H.Y., Zhou,L.Y. and Yu,Z.T.2011.Effect of ruminal pulse dose of polyunsaturated fatty acids on ruminal microbial populations and duodenal flow and milk profiles of fatty acids.J Dairy Sci.94(6):2977-2985.

Lock,A.L. and Garnsworthy,P.C.2002.Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk.Anim Sci.74:163-176.

Maxin,G., Glasser,F. and Rulquin,H.2010.Additive effects of trans-10, cis-12 conjugated linoleic acid and propionic acid on milk fat content and composition in dairy cows.Journal of Dairy Research.77(3):295-301.

Moallem,U., Lehrer,H., Zachut,M., Livshitz,L. and Yacoby,S.2010.Production performance and pattern of milk fat depression of high-yielding dairy cows supplemented with encapsulated conjugated linoleic acid.animal.4(04):641-652.

Moore,C.E., Hafliger,H.C., III, Mendivil,O.B., Sanders,S.R., Bauman,D.E. and Baumgard,L.H.2004.Increasing Amounts of Conjugated Linoleic Acid (CLA) Progressively Reduces Milk Fat Synthesis Immediately Postpartum.J Dairy Sci.87(6):1886-1895.

Odens,L.J., Burgos,R., Innocenti,M., VanBaale,M.J. and Baumgard,L.H.2007.Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period.J Dairy Sci.90(1):293-305.

Olsen,R.E. and Henderson,R.J.1989.The Rapid Analysis of Neutral and Polar Marine Lipids Using Double-Development Hptlc and Scanning Densitometry.J Exp Mar Biol Ecol.129(2):189-197.

Pappritz,J., Meyer,U., Kramer,R., Weber,E.-M., Jahreis,G., Rehage,J., Flachowsky,G. and Dänicke,S.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(2):89-107.

Perfield,J.W.I., Bernal-Santos,G., Overton,T.R. and Bauman,D.E.2002a.Effects of Dietary Supplementation of Rumen-Protected Conjugated Linoleic Acid in Dairy Cows during Established Lactation.J Dairy Sci.85(10):2609-2617.

Perfield,J.W.I., Bernal-Santos,G., Overton,T.R. and Bauman,D.E.2002b.Effects of Dietary Supplementation of Rumen-Protected Conjugated Linoleic Acid in Dairy Cows during Established Lactation.J Dairy Sci.85(10):2609-2617.

Perfield,J.W.I., Lock,A.L., Pfeiffer,A.M. and Bauman,D.E.2004a.Effects of Amide-Protected and Lipid-Encapsulated Conjugated Linoleic Acid (CLA) Supplements on Milk Fat Synthesis.J Dairy Sci.87(9):3010-3016.

Perfield,J.W.I., Lock,A.L., Pfeiffer,A.M. and Bauman,D.E.2004b.Effects of Amide-Protected and Lipid-Encapsulated Conjugated Linoleic Acid (CLA) Supplements on Milk Fat Synthesis.J Dairy Sci.87(9):3010-3016.

Perfield,J.W.I., Lock,A.L., Pfeiffer,A.M. and Bauman,D.E.2004c.Effects of Amide-Protected and Lipid-Encapsulated Conjugated Linoleic Acid (CLA) Supplements on Milk Fat Synthesis.J Dairy Sci.87(9):3010-3016.

Piamphon,N., Wachirapakorn,C., Wanapat,M. and Navanukraw,C.2009.Effects of Protected Conjugated Linoleic Acid Supplementation on Milk Fatty Acid in Dairy Cows.Asian Australas J Anim Sci.22(1):49-56.

Piperova,L.S., Sampugna,J., Teter,B.B., Kalscheur,K.F., Yurawecz,M.P., Ku,Y., Morehouse,K.M. and Erdman,R.A.2002.Duodenal and Milk Trans Octadecenoic Acid and Conjugated Linoleic Acid (CLA) Isomers Indicate that Postabsorptive Synthesis Is the Predominant Source of cis-9-Containing CLA in Lactating Dairy Cows.The Journal of Nutrition.132(6):1235-1241.

Riemeier,A., Lebzien,P. and Flachowsky,G.2004.Influence of the ruminal N-balance on rumen metabolism.J Anim Feed Sci.13:191-194.

Rohr,K., Brandt,M., Lebzien,P. and Schafft,H.1984.Measurement of Duodenal Flow in Dairy-Cows by Either Total Collection Or Spot Sampling, Using A Special Cannula.Can J Anim Sci.64:116-117.

Schafft, H.1983.Thesis/Dissertation. Untersuchungen zur Quantifizierung des Abbaus von Futterprotein in den Vormägen von Milchkühen.Universität Göttingen

Schauff,D.J., Clark,J.H. and Drackley,J.K.1992.Effects of Feeding Lactating Dairy Cows Diets Containing Extruded Soybeans and Calcium Salts of Long-chain Fatty Acids.J Dairy Sci.75(11):3003-3019.

Sippel,M.A., Spratt,R.S. and Cant,J.P.2009.Milk production responses of primiparous and multiparous dairy cows to dose of conjugated linoleic acid consumed in rumen inert form.Can J Anim Sci.89(3):393-399.

Williams,C.H., David,D.J. and Iismaa,O.1962.The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry.J Agric Sci.59:381-385.

GENERAL DISCUSSION

The *trans*-10,*cis*-12 isomer of CLA effectively reduces milk fat synthesis in the dairy cow, whereas the major CLA isomer in milk fat, *cis*-9,*trans*-11, had no effect (Baumgard et al. 2000). Since the synthesis of milk fat represents high energy expenditure in milk production, the dietary CLA supplementation could help to reduce the energy deficit at the onset of lactation. The milk fat depressing properties of CLA are also useful for farmers restricted by milk fat quotas, allowing rapid manipulation of fat production by cows. Furthermore, the addition of CLA to the diet of the dairy cow may be a strategy to increase the CLA content of milk, a natural source of CLA in the human diet. Since CLA have been reported to have a wide range of beneficial effects, increasing the CLA content of milk has the potential to raise the nutritive values of dairy products. However, results regarding the effects of the addition of CLA to the diet of dairy cows on net energy balance were inconsistent and studies on the long-term and post-treatment effects rare. Therefore, the first study performed in this thesis aimed at the examination of the effects of a dietary CLA supplementation for 26 weeks on performance, different variables of energy metabolism and fatty acid profile of milk fat. To determine possible post-supplementation effects, the animals were observed for further 12 weeks after the treatment period (**Paper I**).

In order to perform its activity, the rumen biohydrogenation of CLA should be avoided and CLA have to be intestinally absorbed. Lipid encapsulation is a commonly used protection method to prevent the process of ruminal biohydrogenation, but results in literature suggest that the protection rate is not 100%. Therefore, a further objective of this thesis was to examine the impact of the dietary addition of a lipid-encapsulated CLA preparation on parameters of ruminal metabolism and to determine the actual duodenal availability of the *trans*-10,*cis*-12 isomer (**Paper II**).

To investigate dose-dependent effects of the dietary CLA intake the lipid encapsulated CLA supplement, containing 10% *cis*-9,*trans*-11 CLA and 10% *trans*-10, *cis*-12 CLA, was fed in two different dosages: 50 g/d (CLA-1) and 100 g/d (CLA-2). The results were compared with an unsupplemented control group. The chosen doses had a high relevance to practice as 50 g of the supplement/d reflects the actual recommendation of compound feed manufacturers. The objective was an intake of 5 and 10 g of the milk fat depressing *trans*-10,*cis*-12 isomer/d for CLA-1 and CLA-2, respectively. However, the calculated intakes in both studies were lower than expected. In the fist study (**Paper I**) each animal of Group CLA-1 and CLA-2 received 4 and 8 g *trans*-10,*cis*-12 CLA/d, while in the second study

(**Paper II**) the cows had an intake of 3 and 8 g *trans*-10,*cis*-12 CLA/d, respectively. Since the analyzed proportion of *trans*-10,*cis*-12 CLA in the supplement was about 10% and corresponded to the data supplied by the manufacturer, we cannot exclude the possibility that the process of pelleting of the concentrate feed reduced the amount of this FA in feed.

1 Dry matter intake and performance

Data are inconsistent regarding CLA effects on feed intake. While most authors did not find an effect of dietary CLA supplementation on DMI of dairy cows (Perfield et al. 2002; Bernal-Santos et al. 2003; Moore et al. 2004; Piperova et al. 2004; Perfield et al. 2004a; Castañeda-Gutiérrez et al. 2005; de Veth et al. 2005a), Moallem et al. (2010) reported a decreased DMI during 11 weeks of supplementation. In the present study it was shown that the DMI of the two CLA groups was by 12 and 16% lower compared to the control in early lactation (**Paper I**). After seven weeks of supplementation the cows were apparently adapted, since from that moment on the DMI of the three groups was similar. In the second trial (**Paper II**) effects on DMI were not evaluable, as the animals were fed restrictively to avoid varying ingesta passage rates. The diet only differed in the fatty acid profile of the fat preparations and these CLA-containing supplements were consumed completely by all cows in the first and in the second trial. Therefore, the control cows consumed more saturated fatty acids and less unsaturated fatty acids compared to the CLA treated cows, as CLA was substituted by stearic acid in the fat supplement. As shown by Jenkins and Jenny (1989) and Pantoja et al. (1996) hypophagic effects of added fat increased with the proportion of unsaturated FA in the diet. Firkins and Eastridge (1994) evaluated data from 11 studies supplementing fat sources which differed primarily in the degree of saturation. They assumed that DMI was decreased because of inhibition of fiber digestion or metabolic regulation by unsaturated fatty acids. As shown in **Paper II** the actual rumen protection of CLA is less than 16%, indicating that the ruminal microbes were exposed to CLA. However, the addition of CLA to the diet did not affect the apparent ruminal digestibility of ADF and NDF in the present study (**Paper II).** Drackley et al. (1992) infused long-chain fatty acids varying in saturation and chain length into the abomasum and suggested that unsaturated long chain fatty acids reaching the small intestine of dairy cows affect gastrointestinal motility and DMI. In a study by Baumgard et al. (2000) a trend for a decreased DMI was observed for mid-lactating cows after the abomasal infusion of *trans*-10,*cis*-12 CLA, indicating that this isomer plays a major role in regulation of DMI and that the effect is independent from ruminal metabolism. In the second trial only small amounts of *trans*-10,*cis*-12 CLA reached the small intestine compared to the infusion study by Baumgard et al. (2000) but the daily flow of unsaturated fatty acids (UFA) was higher by

an average of 28% in the CLA treated groups compared to the control. Possibly the decrease of DMI after CLA supplementation is attributable to the increasing duodenal flow of UFA. It is difficult to determine the mechanisms behind diet-induced differences in feed intake, since many factors have to be taken into account. Findings by Choi et al. (2000) supported the hypothesis that endogenous cholecystokinin is a regulating factor mediating depression of feed intake in dairy cattle fed high fat diets. Furthermore, Benson and Reynolds (2001) presented evidence for a role of increased concentrations of another gut hormone, the glucagon-like peptide-1, in response to unsaturated long chain fatty acids reaching the intestine as mediator of decreased DMI.

Reviewing the literature, mostly no significant effects on milk yield were reported after dietary CLA supplementation (see Table 3, background section). However, Moallem et al. (2010) observed a by 5% significantly higher average daily milk production in the CLA group compared to the control group and assumed that the decreased milk fat synthesis after CLA treatment may shift the production towards milk yield. Furthermore, a significant increase of milk yield by 14 and 9% was reported by Kay et al. (2006) and Metzger-Petersen et al. (2009), respectively. In a study by Piamphon et al. (2009) milk yield was CLA-dose-dependently and significantly increased and they concluded that it was mainly due to the simultaneously increased DMI. Despite the lower DMI during early lactation, milk yield did not differ between the three groups in the current trial (**Paper I**). During later lactation, in the second supplementation period, we observed an apparent but not significant increase of milk yield by 13 and 9% in Group CLA-1 and CLA-2, respectively, which is in accordance with results obtained by Giesy et al. (2002), Selberg et al. (2004), Perfield et al. (2004a), Castañeda-Gutiérrez et al. (2007), Odens et al. (2007) and Sippel et al. (2009). Interestingly, the milk yield of primiparous and pluriparous cows reacted apparently different to supplemental CLA (Figure 6). For primiparous and pluriparous cows the milk yield increased by an average of 8% during the whole supplementation time, but pluriparous cows showed an increase in milk yield ten weeks earlier. After end of supplementation, milk yield of control and CLA cows was similar for pluriparous cows. However, during depletion period the CLA supplemented primiparous cows showed a by 16% higher milk yield compared to the control. Possibly, primiparous cows responded in a different way to supplemental CLA, because of their additional energy requirements for growth as well as milk production. However, available research data could not show different production responses between primiparous and pluriparous cows after CLA treatment (Sippel et al. 2009).

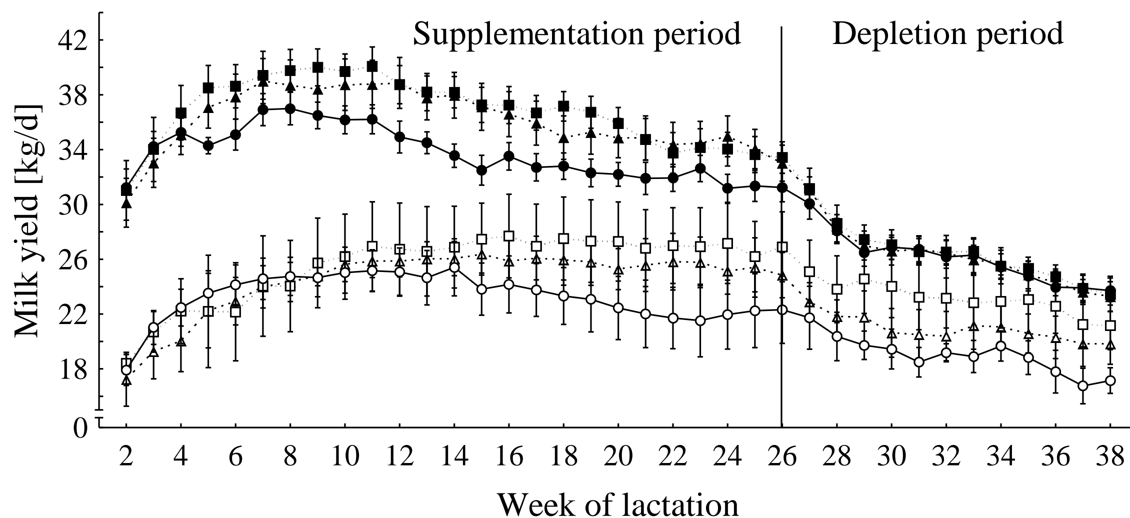

Figure 6: Milk yield (means ± SE) of primiparous (○ CON (n=5), □ CLA-1 (n=4), Δ CLA-2 (n=5)) and pluriparous (● CON (n=10), ■ CLA-1 (n=11), ▲ CLA-2 (n=11)) cows after dietary CLA treatment

The reduction of milk fat content is the most prominent effect of a CLA addition to the diet of dairy cows as shown by many authors (see Table 3, background section) and the effect has been shown to be specific for the *trans*-10,*cis*-12 isomer (Baumgard et al. 2000). Available data indicate that supplemental CLA likely decreases milk fat content through inhibition of *de novo* fatty acid synthesis in the mammary gland, as reflected by decreased short-chain fatty acid proportions in milk (see chapter 4.2, background section) and reduced expression of genes involved in milk lipid synthesis (Baumgard et al. 2002b; Peterson et al. 2003; Harvatine and Bauman 2006). In accordance with Castañeda-Gutiérrez et al. (2005) and Bernal-Santos et al. (2003) feeding moderate doses of CLA resulted in a lack of a CLA response in milk fat during the first weeks of lactation in the present study (**Paper I**), although it could be shown that the *trans*-10,*cis*-12 isomer was transferred to milk fat already at the first week of lactation. Possibly, the marked alterations of many key enzymes and biochemical pathways at the onset of lactation lead to a less CLA-sensitive and responsive mammary gland at this time, as in studies conducted in established lactation (Perfield et al. 2002; Giesy et al. 2002; Moallem et al. 2010; Medeiros et al. 2010) or with very high CLA dosages (Moore et al. 2004) an immediate reduction in milk fat content was observed. Later in lactation we observed a decrease of milk fat content by 7 and 12% in group CLA-1 and CLA-2, respectively. Apparently, the spared energy from the reduction in milk fat synthesis was spent to increase milk yield so that milk fat yield was unchanged. This finding agrees with the observations of Kay et al. (2007) who reported that cows supplemented with CLA tended to

produce more milk when MFD was moderate (less than 35%), whereas excessive MFD appears to be associated with a diminished milk yield response (de Veth et al. 2005a).

Regarding possible CLA-related effects on the milk protein content, the results of previous studies were not clear. Most studies reported no effects of CLA on protein content (Perfield et al. 2002; Giesy et al. 2002; Bernal-Santos et al. 2003; Castañeda-Gutiérrez et al. 2005; Kay et al. 2006; Castañeda-Gutiérrez et al. 2007; Odens et al. 2007; Hutchinson et al. 2011), whereas Medeiros et al. (2010) observed an increase but with relatively higher utilizable protein in the diet of the CLA supplemented animals. Corresponding to Piamphon et al. (2009), Moallem et al. (2010) and von Soosten et al. (2011) milk protein content was reduced by CLA in the present trial (**Paper I**), while no differences in protein yields were observed, although results of the investigations with fistulated dairy cows concerning nitrogen flows at the duodenum (**Paper II**) indicate that duodenal available protein, required for milk protein synthesis, is lower after CLA treatment. In literature, there is also no evidence that milk protein yield was decreased by supplemental CLA. In the present trial the reduction of milk protein content could be explained by the dilution effect of simultaneously increased milk yield.

One key objective was to assess if the dietary supplementation of a rumen protected CLA preparation to dairy cow diets improves the net energy balance by replenishing body fat reserves, especially at the onset of lactation. Although for different growing animal species a decreased body fat accretion after CLA supplementation was described (Park et al. 1997; Park et al. 1999; Delany et al. 1999; Ostrowska et al. 1999; Tsuboyama-Kasaoka et al. 2000; Stangl 2000b; José et al. 2008), the reduction in milk fat secretion after *trans*-10,*cis*-12 CLA treatment should lead to a more positive net energy balance which probably results in increased rates of adipose tissue lipogenesis. This assumption is confirmed by the results presented by Harvatine et al. (2009). After CLA treatment they observed a higher mRNA abundance of FASN, LPL, SCD, FABP4, SREBP1, thyroid hormone responsive spot 14 and PPARγ in dairy cows. Furthermore, von Soosten et al. (2011) could show that the mobilization of retroperitoneal adipose depot by CLA supplemented primiparous dairy cows was decelerated. In our own investigations (**Paper I**) we have evaluated calculated net energy balance, BCS, BFT and plasma concentrations of NEFA, BHB and glucose in order to be able to make statements about the energy status of the cow. Milk production parameters were unaffected by CLA during early lactation, but DMI was decreased by an average of 14%. Therefore the CLA supplemented cows had a more negative net energy balance compared to control in this stage of lactation. Consequently, we expected a stronger lipid mobilisation of these animals. In lactating cows, circulating concentrations of NEFA and BHB are highly

correlated with rates of lipolysis and the comparable plasma concentrations between treatments (Figure 7) suggesting that CLA have no effect on lipolysis.

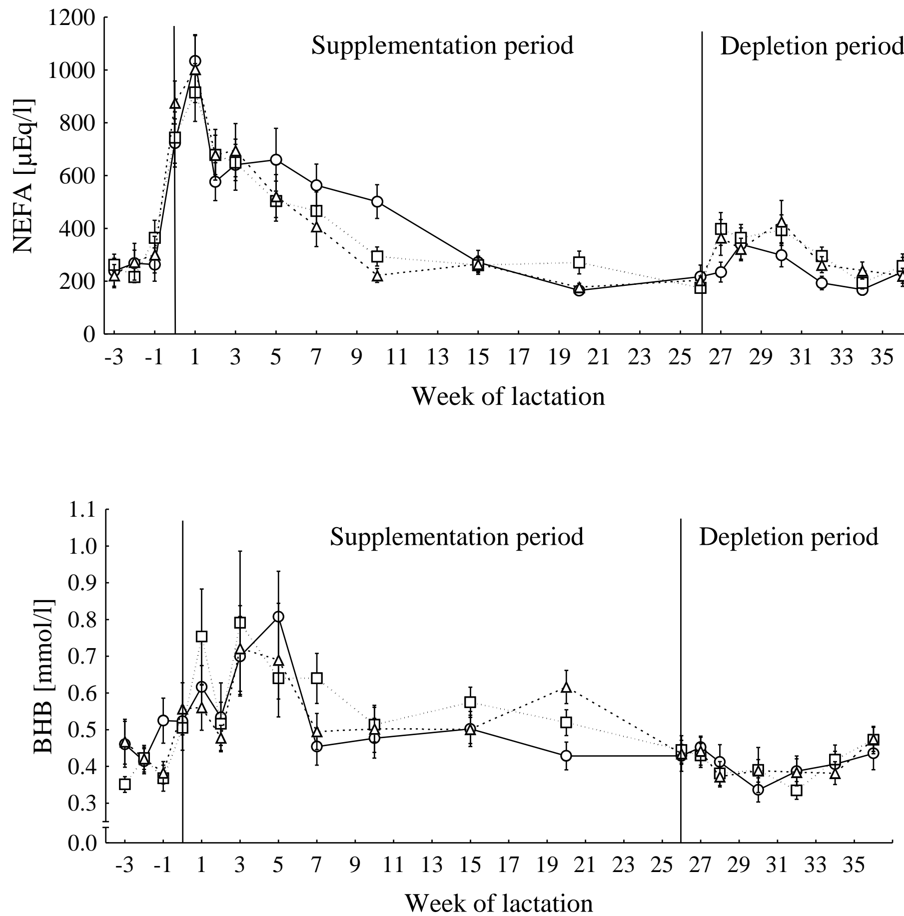

Figure 7: Effect of dietary supplemented CLA on the plasma concentrations of non esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) (means ± SE); ○ CON (n=15), □ CLA-1 (n=15), Δ CLA-2 (n=16)

Apparently, the CLA treated cows were able to utilize the energy more efficiently than the control cows in early lactation. Later in lactation the energy spared from the reduction of milk fat synthesis was spent to increase milk yield and DMI did not differ so that net energy balance remained unaffected. No changes in the plasma concentrations of NEFA and BHB were detectable in this stage of lactation as well. Additionally, BCS and BFT were not altered by CLA. Thus, there is no obvious indication from our data that CLA positively affects the energy supply of dairy cows. However, data from von Soosten et al. (2011) suggest that the retroperitoneal adipose depot was affected most by lipolysis in early lactation and they concluded that this fat depot is the most sensitive depot in dairy cows. No effects on the subcutaneous adipose depot, which could be evaluated with the measurement of BCS and BFT, were observed after CLA treatment with comparable dosages. Consequently, it is

possible that lower CLA doses cause only slight changes in lipid metabolism which were not detectable by BCS and RFT measurement. Despite of a shown CLA induced MFD in literature, all these variables often failed to show the expected decrease in mobilization of body reserves (Perfield et al. 2002; Moore et al. 2004; Castañeda-Gutiérrez et al. 2005; Medeiros et al. 2010). A comparatively high dose of CLA in the diet of the cow (30 g/d) resulted in investigations by Odens et al. (2007) in a diminished BW and BCS loss accompanied by lower NEFA plasma concentrations. Hutchinson et al. (2011) could show an improved net energy balance and BCS and a reduced peak concentration of NEFA in blood after the supplementation of a low dose of CLA (6g/d).

2 Ruminal fermentation and flow of nutrients to the duodenum

The encapsulation of fatty acids by triglycerides of hydrogenated fatty acids is a common method to protect unsaturated fatty acids from alterations in the rumen and to avoid an effect on rumen function. As described in detail in the next section (chapter 3), a large part (about 89%) of the supplemented CLA did not reach the duodenum despite the lipid-encapsulation. Accordingly, rumen microbes were exposed to the CLA and it is possible that the ruminal microbial diversity was modified. In current literature, the influence of supplemental CLA on ruminal fermentation has not been studied in detail. As there is a great interest to use fat supplements for increasing energy density of diets for lactation, a lot of studies were conducted to investigate the effects of different other fat sources on rumen fermentation. Palmquist and Jenkins (1980) summarized that the effect of supplemental fat on ruminal microbes and therefore on the digestion in rumen depends on the type of fat (saturation and esterification) and the content in the diet. A reduced digestibility of fiber in the rumen is a major consequence of fatty acids disrupting the microbial fermentation process and the effect is more severe when the fat supplement is rich in unsaturated fatty acids (Pantoja et al. 1994). However, it is commonly known that 3 to 5% fat in the ruminant diet, as applied in the current studies, appears to be tolerated by ruminal microorganisms (Palmquist and Jenkins 1980). The fat contents of the three diets in the current trial did not actually differ, only the saturation of fatty acids was different. The question arises if such special fatty acids as supplemental CLA have an impact on rumen metabolism.

After the supplementation of CLA to the diet of dairy cows the concentration of VFA and NH_3 as well as the pH-value remained unaffected (Figure 8). Supplemental CLA slightly changed the molar proportions of VFA in the rumen fluid and the effects were not dose-dependent (**Paper II**). Also for sheep Huang et al. (2009) could not observe any serious

impact of CLA on rumen metabolism. In this study supplemental CLA as free fatty acid or calcium salt did not change the concentration of total VFA, proportions of individual VFA and the NH_3 concentration in ruminal fluid.

From literature data Jenkins (1993) concluded that compared to fiber, dietary fat is less harmful to digestibility of non structural carbohydrates. The apparent digestibility of fiber remained unaffected by CLA in the present trial (**Paper II**). Thus we cannot rule out the possibility that the enhanced apparent digestibility of starch was attributed to the differences in the composition of the forage in this trial and not to a CLA effect.

If fat supplements interfere with rumen fermentation, protein metabolism is also altered. An increased efficiency of microbial protein synthesis was observed by Ikwuegbu and Sutton (1982) after dietary fat supplementation and attributed to the reduction of protozoa numbers. Oldick and Firkins (2000) investigated the effects of fat saturation on fiber digestion and microbial protein synthesis in sheep. Ruminal protozoa concentration decreased linearly as the iodine value of fat increased but there was a lack of correlation between total protozoal counts and efficiency of microbial protein synthesis, possibly explainable by the chosen high feeding frequency. The shown effects in the present study were not comparable to the results in literature and elusive, as the percentage of RDP increases, the more available NH_3 remained unused from rumen microorganisms and at the same time no significant differences could be shown for the NH_3 concentration in rumen fluid. It is inexplicable where the excess of NH_3 produced in rumen remained. Possibly a higher ruminal absorption of ammonia occurs.

Taken together, only minor changes of rumen metabolism could be observed in the second trial after CLA supplementation (**Paper II**). Furthermore, it cannot be ruled out that some of the observed effects were due to variations of the nutrient composition of the silages during the three periods, as the silos in which grass and maize silage were ensiled changed over the course of the experiment.

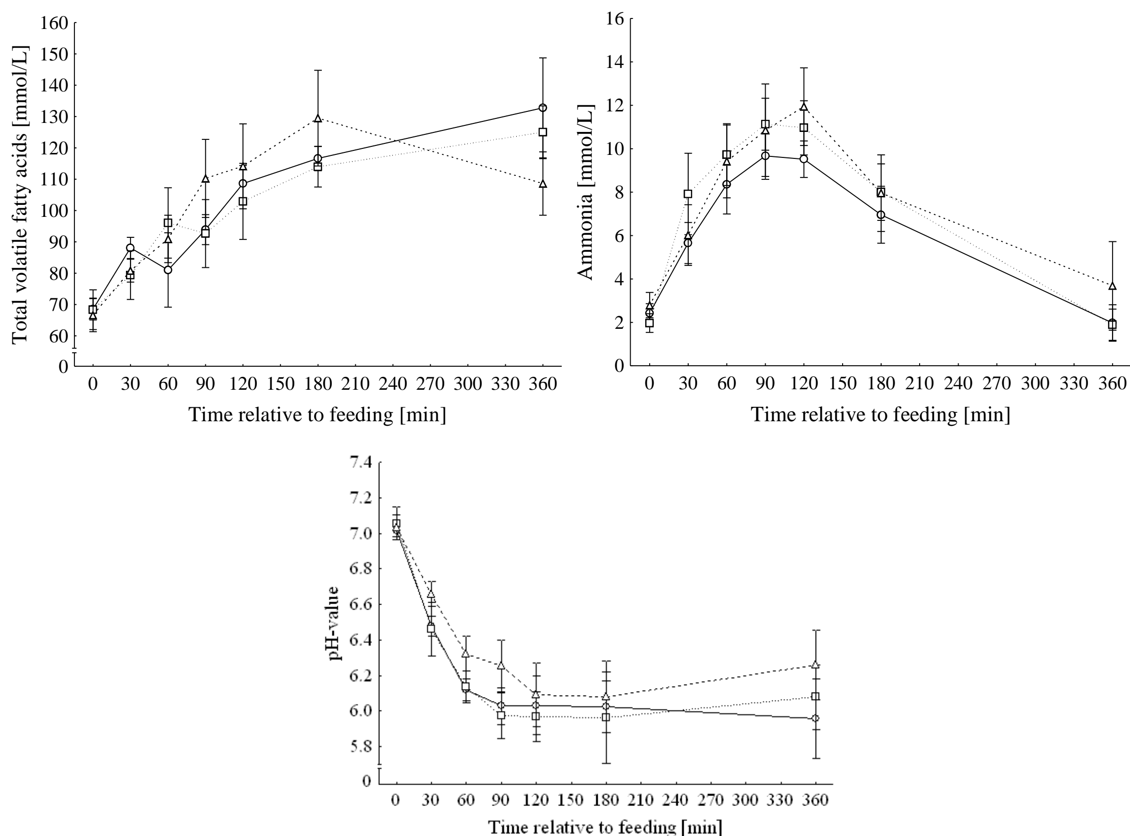

Figure 8: Effect of dietary supplemented CLA on the concentrations of volatile fatty acids and ammonia and the pH-value in ruminal fluid at different time points after feeding (means ± SE); ○ CON (n=6), □ CLA-1 (n=6), Δ CLA-2 (n=6)

3 CLA flows and *carry over* into milk

Evaluating twelve dietary CLA supplementation studies and the own results, we observed a linear relationship between fed *trans*-10,*cis*-12 CLA dose and the milk yield of *trans*-10,*cis*-12 CLA (Figure 9). Across the studies the daily intake ranged from 2.4 to 37.4 g *trans*-10,*cis*-12 CLA and the transfer efficiency (calculated as follows: CLA isomer secretion with milk [g/d] x 100 / CLA isomer intake [g/d]) from feed to milk was relatively constant at 5%. The fed CLA dose and the used method to protect the supplemented CLA had no influence on the transfer rate (see Table 2, background section). In the first study (**Paper I**), the CLA supplement provided 4 and 8 g *trans*-10,*cis*-12 CLA/d in the CLA-1 and CLA-2 diet, respectively, and on average 0.3 g/d were secreted in the milk fat in both groups. Thus, transfer efficiency to milk fat averaged 6%. The animals of the second study (**Paper II**) received 3 and 8 g *trans*-10,*cis*-12 CLA/d and transfer efficiency to milk fat averaged 5%. Results from several short-term abomasal infusion studies (see Table 2, background section) indicated that about 20% of the infused dose of *trans*-10,*cis*-12 CLA was transferred into milk. This difference suggests that a substantial portion of the dietary supplemented CLA was metabolized in the rumen.

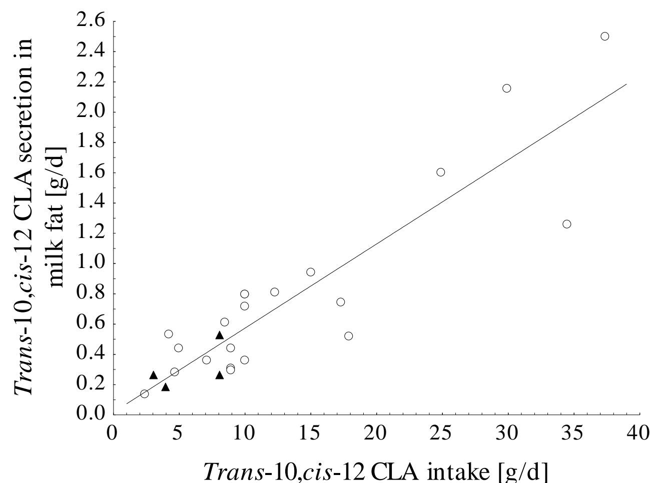

Figure 9: Relationship between *trans*-10,*cis*-12 CLA intake and *trans*-10,*cis*-12 CLA secretion in milk fat; ○ literature data, for references see Table 2 in the background section; ▲ present investigation; y=0.0175+0.0556*x (p=<0.001, r^2=0.809, RSD=0.6 g/d)

By using cows equipped with duodenal cannulas in combination with marker techniques and a controlled feeding regime, we were able to measure the post-ruminal *trans*-10,*cis*-12 CLA flow to deduce the actual rumen inertness of the lipid-encapsulated CLA supplement. To our knowledge, to date there are no comparable studies with dairy cows available. Up to now studies evaluating the degree of rumen inertness of CLA supplements were either conducted *in vitro* or the rumen inertness was estimated from the transfer efficiency of *trans*-10,*cis*-12 CLA from feed into milk. As shown in **Paper II** no *trans*-10,*cis*-12 CLA was detectable in the duodenal chyme of the control cows. Comparable amounts of 0.5 and 0.4 g *trans*-10,*cis*-12 CLA/d were detectable at the duodenum after CLA-1 and CLA-2 treatment, respectively. In consequence, the proportion of the supplemented *trans*-10,*cis*-12 isomer which reached the duodenum was lower after the CLA-2 treatment compared to the CLA-1 treatment (CLA-1: 16%, CLA-2: 5%). Possibly different conditions in the rumen were accountable for the differences in the transfer of *trans*-10,*cis*-12 CLA from feed to the duodenum in Period CLA-1 and CLA-2, since the silos in which grass and maize silage were ensiled, changed over the course of the experiment. In a study conducted with sheep, Wynn et al. (2006) demonstrated that with approximately 65% comparatively high amounts of the lipid-encapsulated CLA avoided rumen biohydrogenation. However, only a small amount of the unprotected CLA (8.5%) appeared at the duodenum, but this amount was comparable to the present results obtained with rumen-protected CLA. In accordance with the own results, Huang et al. (2009) reported for sheep fed CLA as a calcium salt that 10% bypasses ruminal biohydrogenation.

Additionally, the results shown in Figure 10 demonstrate substantial variation among individual cows within treatment groups regarding the duodenal flow of *trans*-10,*cis*-12 CLA. For three non-lactating and the cows in late lactation (cow 1, 4, 6) we observed a mean duodenal flow of 0.27 and 0.14 g/d in Period CLA-1 and CLA-2, respectively. The duodenal flow of *trans*-10,*cis*-12 CLA for three mid-lactating cows (cow 2, 3, 5) averaged 0.75 and 0.56 g/d in Period CLA-1 and CLA-2, respectively. The presumption that lower dry matter intakes and therefore lower ingesta passage rates were responsible for lower duodenal *trans*-10,*cis*-12 CLA flows could not be confirmed, as there was no correlation between organic matter intake (p=0.841, r^2=0.011) as well as organic matter flow (p=0.959, r^2=0.002) and the duodenal flow of *trans*-10,*cis*-12 CLA in Period CLA-1.

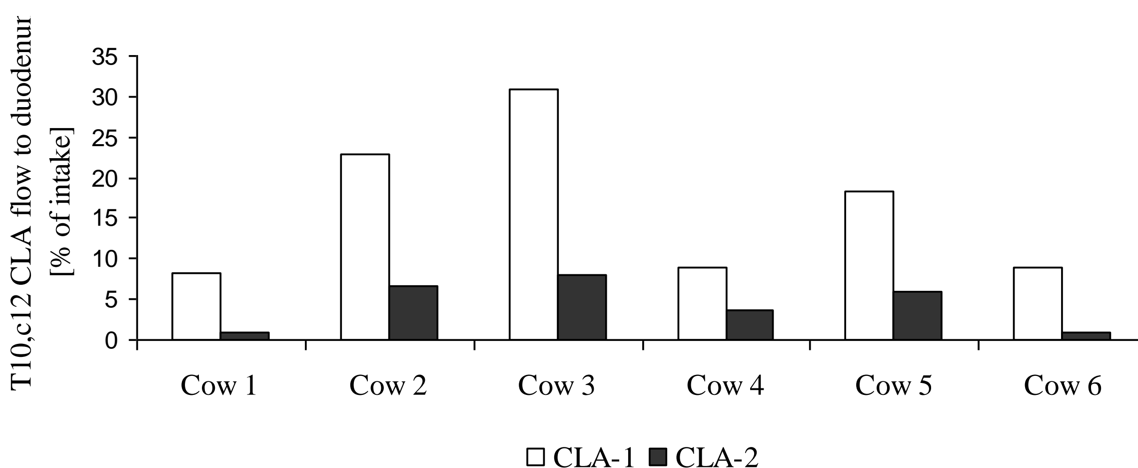

Figure 10: Individual duodenal *trans*-10,*cis*-12 CLA flow [% of intake] for each cow of the second study (means); CLA-1: white, CLA-2: dark grey

Figure 11 demonstrates the relationship between daily *trans*-10,*cis*-12 CLA duodenal flow and the excretion with milk. It can be concluded that the level of CLA in milk reflects the quantity which is available for intestinal absorption. The transfer efficiency from duodenum into milk fat averaged 34% with a high variance of the results (**Paper II**). For abomasal infusion trials a higher correlation between CLA dose and CLA milk yield was found with a relatively constant transfer efficiency of 22% over the range of doses and milk yields (de Veth et al. 2004).

Furthermore, a slight but significantly positive correlation was observed between the *trans*-10,*cis*-12 CLA flow to the duodenum and the excretion with feces (Figure 12). On average 43% of the duodenal available *trans*-10,*cis*-12 CLA were excreted with feces, assuming that no *trans*-10,*cis*-12 CLA was synthesized in the large intestine. Since 34% of duodenal available *trans*-10,*cis*-12 CLA were transferred into milk and 43% were excreted

with feces, it can be assumed that the remaining portion (23%) was accumulated in different tissues. Data evaluated by von Soosten (2011) are an argument against this assumption, as only small amounts (about 2.5%) of duodenal available *trans*-10,*cis*-12 CLA was detectable in the different body fractions of the cow.

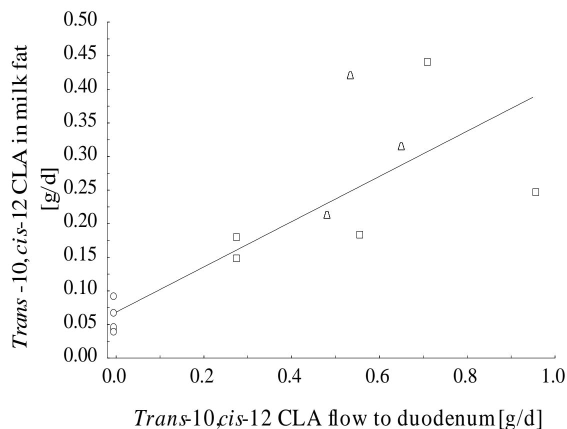

Figure 11: Relationship between *trans*-10,*cis*-12 CLA flow to duodenum and *trans*-10,*cis*-12 CLA secreted in milk fat; y=0.0684+0.3367*x (p=0.001, r^2=0.659, RSD=0.14 g/d); ○ CON (n=5), □ CLA-1 (n=5), ∆ CLA-2 (n=3)

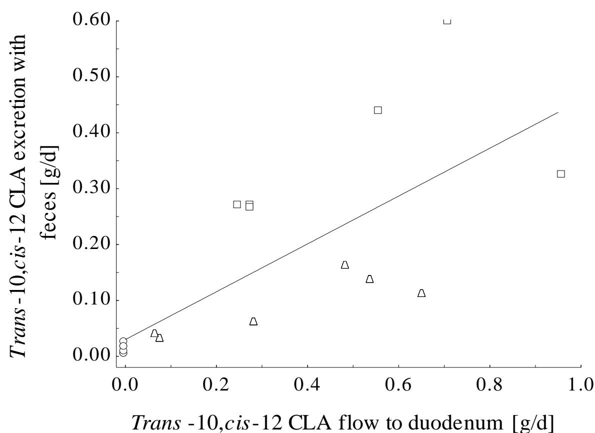

Figure 12: Relationship between *trans*-10,*cis*-12 CLA flow to duodenum and *trans*-10,*cis*-12 CLA excretion with feces; y=0.0299+0.428*x (p=<0.001, r^2=0.554, RSD=0.17 g/d); ○ CON (n=5), □ CLA-1 (n=5), ∆ CLA-2 (n=3)

Another aim of the investigations was to assess the effect of the supplementation of CLA to the diet of dairy cows on the nutritive values of milk. The dietary supplementation of various CLA supplements can lead to an increase of the total CLA proportion in milk fat (Table 5). However, a significant enhancement of total CLA (% of total FAME) by 39% and 60% in milk fat was observed only in the second trial (**Paper II**) for the CLA-1 and CLA-2 treatment, respectively. During the CLA supplementation period in the first trial (**Paper I**) the increase of milk CLA by 4% (of total FAME) on average was not significant.

The difference in the response of selected FA in milk fat between the two current studies is visualized in Figure 13. In general, the response of milk FA to the CLA treatment in the first trial was lower. A dose-dependent increase was observed for the percentages of *cis*-9,*trans*-11, *trans*-10,*cis*-12 and total CLA in milk fat in the second study which is in accordance with results obtained by Giesy et al. (2002), Castañeda-Gutiérrez et al. (2005) and Piamphon et al. (2009). In the first trial the CLA addition to the diet had no effect on the proportion of CLA in milk, except of the *trans*-10,*cis*-12 isomer, which increased dose-dependently. In both studies the proportions of this isomer ranged from 0.02 to 0.05% of total FAME after CLA supplementation which is in accordance with studies using similar amounts

of supplemental CLA (Perfield et al. 2002; Bernal-Santos et al. 2003; Castañeda-Gutiérrez et al. 2007). The *trans*-10,*cis*-12 CLA represented between 2.60 and 3.90% of total CLA.

In literature, it is reported that more than 80% of total CLA is represented by the *cis*-9,*trans*-11 isomer (Fritsche and Steinhart 1998). Results of the second trial confirm this finding, CLA was composed of about 84% *cis*-9,*trans*-11 CLA. In the first trial only 74% of total CLA in milk fat were represented by *cis*-9, *trans*-11 CLA.

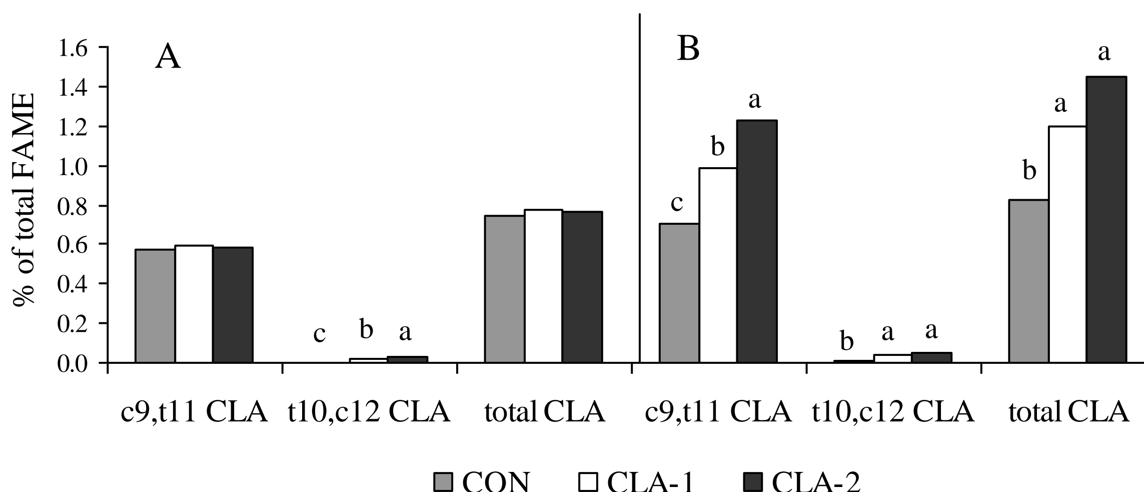

Figure 13: Effect of a dietary CLA supplementation (CON: light grey, CLA-1: white, CLA-2: black) of lactating cows on the secretion of selected milk FA (means) in the (A) first (CON (n=15), CLA-1 (n=15), CLA-2 (n=16), **Paper I**) and (B) second trial (CON (n=5), CLA-1 (n=5), CLA-2 (n=3), **Paper II**); [abc] different letters above bars represent significant treatment differences within each grouping of fatty acids

The *cis*-9,*trans*-11 CLA, as the main CLA isomer in milk fat, is assumed to be beneficial to human health due to its anticarcinogenic properties observed *in vitro* and *in vivo* in a range of human breast cells and tumors (Kelley et al. 2007). Results of epidemiological human studies reported more inconsistent effects. However, several human studies researching the association between CLA intake and breast cancer risk reported an incidence for a lower breast and colorectal cancer risk for humans consuming more natural occurring CLA (Aro et al. 2000; Voorrips et al. 2002; Larsson et al. 2005) (see chapter 5, background section).

Increasing the content of CLA in milk fat simultaneously means to enhance the CLA content of dairy products as it was shown that processing of raw milk, like homogenization (Miguel Rodriguez-Alcala et al. 2009) and pasteurization (Allocati et al. 2007) does not alter the milk fat proportion of total CLA. Furthermore, the production process of cheese or butter has no essentially influence, even when produced from CLA enriched milk, on the CLA milk fat proportion (Dhiman et al. 1999b; Jones et al. 2005). Garcia-Lopez et al. (1994) found that processing of raw milk to cheese increases the CLA proportion in fat, whereas Kim et al.

(2009) observed that ripening increases the CLA content in cheese too. The amounts of CLA in German raw milk samples analyzed by Fritsche and Steinhart (1998) were by an average of 48% higher compared to our control milk samples, and only by an average of 8% lower compared to the milk samples taken from the CLA supplemented animals of the second study. The results obtained by Fritsche and Steinhart (1998) refer to only seven raw milk samples whose source was not clearly stated. It might be that the milk originated from grazing cows. Two American studies reported the CLA content of processed milk and the values were comparable to the current results of the control group (Chin et al. 1992; Lin et al. 1995).

The comparison of the results in Table 5 leads to the assumption that the use of CLA supplements for cows is a good possibility to increase the CLA content in milk, but other methods exist also enhancing the milk fat CLA content. Concentrations of CLA are higher in milk fat from cows offered fresh forages compared to conserved forages and can also be enhanced with plant and fish oil supplements or whole oilseeds. An additional nutritional strategy is the use of ionophores like monensin in combination with plant oils. Ionophores impair ruminal biohydrogenation obviously for the benefit of CLA. Baumgard et al. (2000) speculated that endogenous production of the *trans*-10,*cis*-12 CLA isomer by rumen bacteria may be responsible for many, perhaps most, of the traditional dietary situations that cause reduced milk fat synthesis by dairy cows.

For Germany Fritsche and Steinhart (1998) calculated a mean intake of 0.35 and 0.43 g CLA/d for women and men, respectively. As mentioned above, they calculated this mean intake by assuming a CLA content of milk that is only by an average of 8% lower compared to our CLA supplemented groups. Long-term supplementation studies reported that a 10-fold higher CLA intake compared to the natural intake with food was not associated with serious adverse effects on human health (Gaullier et al. 2004; Gaullier et al. 2005). Therefore, no risk for human health could be assumed for the intake of milk and milk products obtained from dairy cows supplemented with CLA.

Table 5: Examples for methods to increase the natural CLA content of milk

Dietary method	Comments	Total CLA [% of total FAME]		References
		Control	Treatment	
Supplementation of *t*10,*c*12CLA	8 g/d	0.74	0.78	Present experiment - first study
	16 g/d		0.77	
	6 g/d	0.85	1.18	Present experiment - second study
	16 g/d		1.36	
	9.5-187g/d	0.05-0.87	0.44-2.69	Table 2 background section
Fresh forage		0.46	1.09	Kelly et al. (1998b)
		0.41	0.72	White et al. (2001)
		0.69	2.39	Mohammed et al. (2009)
Supplementation of plant oil	rapeseed oil	1.35	1.36	Rego et al. (2009)
	sunflower oil		1.82	
	linseed oil		1.92	
Supplementation of fish oil	1% of diet DM	0.71	1.71	Donovan et al. (2000)
	2% of diet DM		2.53	
	3% of diet DM		2.12	
Supplementation of fish and plant oil	45 g/kg DM	0.49	4.46	Jones et al. (2005)
	400 g/d	0.97	1.75	AbuGhazaleh (2008)
Extruded oilseeds	soybeans	0.41	0.97	Dhiman et al. (1999b)
	cottonseed		0.81	
	soybeans		1.09	Li et al. (2009)
Ionophores	Monensin + soybean oil	1.09	1.47	AlZahal et al. (2008)

Nutritional strategies for enhancing the CLA content also result in milk fat containing lower amounts of saturated fatty acids (SFA) and greater proportions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). As unsaturated fatty acids (UFA) are more susceptible to oxidation than SFA, Jones et al. (2005) investigated the chemical, physical and sensory properties of dairy products enriched with CLA (39.52 g CLA/kg milk fat) and concluded that it is feasible to produce such products with acceptable storage and sensory characteristics. Furthermore, in literature it is described that a mixture of SFA increases blood total and low-density-lipoprotein (LDL)-cholesterol concentrations relative to carbohydrates, which are considered neutral regarding their effects on LDL-cholesterol concentrations (Mensink et al. 2003). The American Heart Association recommended limiting the intake of SFA to <7% of energy to reduce the cardiovascular disease risk (Lichtenstein et al. 2006) and a scientific opinion of the European Food Safety Authority (EFSA) stated that the consumption of foods with reduced amounts of SFA may help to maintain normal blood cholesterol concentrations (EFSA 2011). Therefore the observed reduction of the proportion

of SFA in milk fat in the second trial (**Figure 14**) could be a further beneficial effect of the CLA supplementation to dairy cow diets.

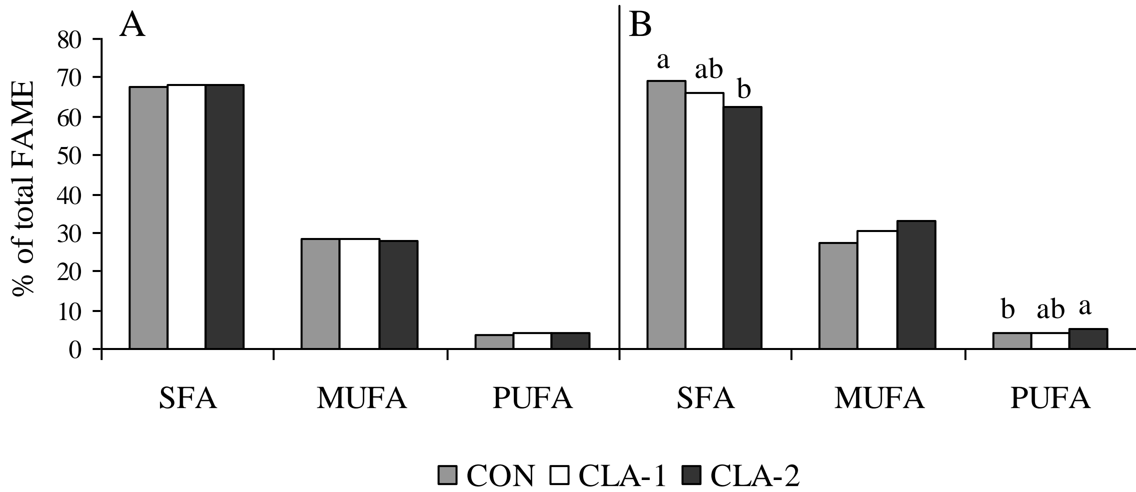

Figure 14: Effect of a dietary CLA supplementation (CON: light grey, CLA-1: white, CLA-2: black) of lactating cows on the secretion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (means) in the (A) first (CON (n=15), CLA-1 (n=15), CLA-2 (n=16), **Paper I**) and (B) second trial (CON (n=5), CLA-1 (n=5), CLA-2 (n=3), **Paper II**); [abc] different letters above bars represent significant treatment differences within each grouping of fatty acids

CONCLUSIONS

Our results are consistent with the proposed hypothesis that CLA can be used to manipulate the milk fat content. However, supplemental CLA failed to show the expected milk fat depression (MFD) as the reduction in milk fat content was accompanied by higher milk yields. Therefore milk energy output did not differ from control and this was reflected by unchanged plasma levels of non-esterified fatty acids (NEFA) and ß-hydroxynutyrate (BHB). Furthermore the use of body reserves as evaluated by changes in body condition score (BCS) and back fat thickness (BFT) remained uninfluenced by the diet.

In early lactation supplementing the diet with CLA resulted in a decreased dry matter intake (DMI) independent of CLA dose and consequently in a degraded net energy balance. Milk yield, yield of milk components, BCS, BFT and plasma concentrations of NEFA and BHB remained unaffected, indicating an increased efficiency of metabolizable energy in this stage of lactation. The available data did not allow any conclusions about the causes for these findings. Future research should be directed to clarify the partitioning of energy in CLA supplemented cows.

After CLA supplementation was terminated the milk fat content of the supplemented groups quickly reached the level of control cows. Except of little changes in circulating glucose levels no post-treatment effects were observed for the measured parameters, which is consistent with the available literature data.

The actual duodenal availability of *trans*-10,*cis*-12 CLA was very low, suggesting a protection rate of less than 16% of the lipid-encapsulated CLA supplement. Further studies are needed to improve existing or to develop novel protection methods to reduce the extent of biohydrogenation of *trans*-10,*cis*-12 CLA in the rumen and thereby to improve the efficiency of CLA supplementation.

The small proportion of *trans*-10,*cis*-12 CLA reaching the duodenum, suggested that the rumen microorganisms were partially exposed to CLA which could lead to a shift of the microbial population in the rumen. Available data indicated that the rumen functionality was only little affected with no adverse consequences for the animal and it was not excludable that the observed effects were due to variations of the nutrient composition of the silages during

the three periods. Therefore, further research is necessary to clarify the impact of CLA on the rumen microbial population.

The transfer efficiency of *trans*-10,*cis*-12 CLA from feed to milk fat was relatively constant and averaged 5%. It was shown that supplemental CLA leads to an increase of the *trans*-10,*cis*-12 isomer in milk fat but the actual increase was minimal in level. Regarding the effects of dietary CLA supplementation on total CLA content and the proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in milk fat, the two studies showed contradictory results. But it could be concluded that the supplementation of CLA preparations could lead to an increase of total CLA and PUFA in milk fat whereas the content of SFA decreases. Therefore it will be possible to influence the nutritional value of milk by supplemental CLA and no adverse effects for the consumer are expected.

SUMMARY

Investigations on the effects of graded levels of rumen protected conjugated linoleic acids on dairy cow performance, fatty acid profile of milk and rumen metabolism

Supplements with conjugated linoleic acids (CLA) are used in dairy cow diets to reduce the milk fat yield. For the cow, milk fat is the most energy consuming component to synthesize. Thus, achieving a reduction in milk fat could alleviate the negative energy balance often occurring at the onset of lactation which reduces the risk of metabolic disorders. The *trans*-10,*cis*-12 CLA was detected as the isomer that effectively reduces milk fat synthesis. Furthermore, supplemental CLA could be used as a strategy to increase the CLA proportion of milk fat and, in consequence, to enhance the nutritive values of milk, as a wide range of beneficial effects were reported for CLA. However, in order to be effective, CLA need to be intestinally absorbed. To increase the duodenal absorbable quantity of the *trans*-10,*cis*-12 isomer and to avoid an impact of supplemental fat on rumen microbes, several protection methods exist. However, results in the literature still suggest a ruminal biohydrogenation of the main part of CLA and there is a lack of information about the effects of CLA on rumen microbial diversity and the actual duodenal availability of CLA. Furthermore, results regarding the effects on energy metabolism and CLA content in milk were inconsistent. Therefore, two experiments were carried out to investigate the effects of the supplementation of graded levels rumen protected CLA to the diet of dairy cows on rumen metabolism, performance and the fatty acid (FA) profile of milk.

Firstly, a long-term study was conducted assigning 46 German Holstein cows to one of three dietary treatments. Starting on day 1 *post partum* (p.p.), cows were fed the experimental diet for a total of 26 weeks and were observed for a further 12 week depletion period to detect post-treatment effects. Each treatment group received a lipid-encapsulated fat supplement that was thoroughly mixed with the components of the concentrate feed and thereafter gently pelleted. Rumen-protected fat supplements consisted of 100 g control fat for the control treatment (CON), 50 g of a CLA fat supplement and 50 g of the control fat for the CLA-1 group, and 100 g CLA fat supplement for the CLA-2 group. The CLA supplement was composed of 10% *cis*-9,*trans*-11 CLA and 10% *trans*-10,*cis*-12 CLA. In the control fat supplement CLA was substituted by stearic acid. In addition, the cows were fed a partial mixed ration (PMR) (37% concentrate, 38% maize and 25% grass silage on dry matter (DM) basis) for *ad libitum* consumption. Milk samples were obtained in week 1, 4, 12 and 26 p.p.

and in week 2 and 10 in the depletion period. Blood samples were taken 1 d p.p., in week 1, 2, 3, 5, 7, 10, 15, 20 and 26 p.p. and in week 1, 2, 4, 6, 8 and 10 in the depletion period. The body condition score (BCS) of each animal was recorded at each blood sampling time. Back fat thickness (BFT) was measured by using ultrasound 1 d p.p., in week 3, 10, 15 and 26 p.p. and 2, 6 and 10 weeks after supplementation ended.

Based on the analyzed CLA concentrations in the concentrate we calculated a daily intake of approximately 4 and 8 g *trans*-10,*cis*-12 CLA and cow for Group CLA-1 and CLA-2, respectively. During the first seven weeks of lactation supplemental CLA diminished DMI by 2.6 – 3.3 kg DM/d. Consequently the net energy balance was degraded, whereas milk yield, yield of milk components, BCS, BFT and plasma concentrations of non-esterified fatty acids (NEFA) and ß-hydroxybutyrate (BHB) remained unaffected, indicating that CLA supplemented cows were able to utilise the energy better than the control cows. Later in lactation (week 8-26) rising CLA supplementation resulted in decreased milk fat contents (CLA-1: -7%, CLA-2: -12%). At the same time, milk yield increased numerically by 4 and 3 kg/d, respectively, resulting in unaffected milk fat yields and net energy balances. The lack of an effect on energy metabolism at this stage of lactation was confirmed by unaffected BCS, BFT values and circulating NEFA and BHB concentrations. CLA addition to the diet increased the proportion of *trans*-10,*cis*-12 CLA in milk fat dose-dependently from <0.01% to 0.02 and 0.03% of total fatty acid methyl esters (FAME), whereas the proportion of total CLA did not differ between treatments. The proportion of C16 FA in milk fat was decreased by the highest CLA dose. After the end of CLA supplementation, the observed differences were no longer evident. The plasma glucose concentration was slightly increased for Group CLA-2 during the post-treatment period, indicative of decreased insulin sensitivity.

In the second study six German Holstein cows equipped with cannulas in the dorsal sac of the rumen and the proximal duodenum were used. The diets applied differed in CLA content in the same way as already described for the first experiment. The fat supplements were offered with the concentrate feed. The amount of forage (60% maize and 40% grass silage on DM basis) that was fed was adjusted to the expected intake of each cow. Each diet was fed in one period and each cow was subjected to the three treatments in the same order: CON, CLA-1, CLA-2. A treatment period consisted of two weeks of adaption followed by two weeks of sample collection. Samples of ruminal fluid were taken before and six times after the first morning feeding once in week 3. Duodenal digesta was collected in two-hourly intervals over a five day period in week 4. For duodenal flow measurements Cr_2O_3 was used

as a marker. Once in week 4 feces samples of each cow were taken. Milk samples were taken twice in week 3 and 4.

The animals consumed 3 and 8 g *trans*-10,*cis*-12 CLA/d in a rumen-protected form in Period CLA-1 and CLA-2, respectively. Generally, minor influences of supplemental CLA on ruminal fermentation were observed and the effects were not clearly relatable to CLA, since the nutrient composition of forage slightly differed over the course of the study. The apparent ruminal digestibility of starch was increased by 6% after feeding the highest CLA dose. The N flow to the duodenum (% of intake) decreased after CLA supplementation. Moreover, the proportion of rumen-undegraddable crude protein (UDP) in relation to crude protein (CP) intake was reduced in the treatment groups. However, the increased amount of N ruminally available for microbial protein (MP) synthesis remained unused by the rumen microbes, as the proportion of MP per rumen-degradable crude protein (RDP) declined. Furthermore, it seems that microbial FA synthesis was adversely affected by supplemental CLA as the total FA flow (% of intake) decreased.

Only 16 and 5% of the *trans*-10,*cis*-12 isomer bypassed rumen biohydrogenation in Period CLA-1 and CLA-2, respectively. Looking at the individual duodenal flow of *trans*-10,*cis*-12 CLA it was noticeable that there were substantial variations among cows.

Total CLA and total polyunsaturated fatty acids (PUFA) content of milk fat was enhanced by supplemental CLA, whereby the proportion of saturated fatty acids (SFA) was diminished. In accordance with the results of the first study, the proportion of the *trans*-10,*cis*-12 isomer in milk fat increased dose-dependently to 0.03 and 0.06% of total FAME. The transfer of this isomer from feed to milk averaged 5%. About 34% of the duodenal available *trans*-10,*cis*-12 CLA were transferred into milk, while about 43% were excreted with feces.

It can be concluded that under the conditions of the present study the CLA supplementation seems to be ineffective in alleviating the negative energy balance during early lactation. The small amount of duodenal available *trans*-10,*cis*-12 CLA suggests a low protection rate of the used lipid-encapsulated CLA supplement and therefore a low effectivity. However, dietary CLA supplementation has the potential to raise the nutritive values of milk by increasing total CLA and PUFA proportion in milk fat without any health implications for milk consumers.

ZUSAMMENFASSUNG

Untersuchungen zur Wirkung einer gestaffelten Supplementation von pansengeschützten konjugierten Linolsäuren auf die Leistungsparameter, das Fettsäuremuster der Milch und den Pansenmetabolismus der Milchkuh

Konjugierte Linolsäuren (CLA) werden in der Milchkuhfütterung zur Senkung der Milchfettsynthese eingesetzt. Bei der Milchbildung stellt das Milchfett die energieaufwendigste Komponente dar. Somit ist es denkbar, dass eine erfolgreiche Senkung der Milchfettsynthese zu einer Abschwächung der oft in der Frühlaktation auftretenden negativen Energiebilanz der Milchkuh beiträgt und so das Risiko für Stoffwechselstörungen senkt. Die milchfettsenkenden Eigenschaften werden vor allem dem *trans*-10,*cis*-12-Isomer zugeschrieben. Weiterhin stellt die Supplementation von CLA eine Möglichkeit dar, den Anteil dieser Fettsäuren (FS) im Milchfett zu erhöhen und so den Nährwert der Milch zu verbessern, da den CLA eine Vielzahl von positiven Wirkungen nachgesagt werden. Damit CLA ihre Wirksamkeit entfalten können müssen sie im Darm absorbiert werden. Um die am Darm ankommende Menge des *trans*-10,cis-12-Isomers zu erhöhen und eine Beeinflussung der Mikroben im Pansen durch das supplementierte Fett zu vermeiden wurden verschiedene Methoden zum Schutz der Fette entwickelt. Jedoch weisen Ergebnisse aus der Literatur darauf hin, dass der Hauptteil der eingesetzten CLA im Pansen biohydrogeniert wird. Es fehlen Daten, die Auskunft über mögliche Effekte von CLA auf die mikrobielle Diversität und die tatsächliche duodenale Verfügbarkeit von CLA geben. Zudem sind die Ergebnisse zu den Wirkungen der CLA auf den Energiemetabolismus und bezüglich der CLA-Gehalte in der Milch widersprüchlich. Daher wurden zwei Studien durchgeführt, welche die Wirkung einer gestaffelten Supplementation mit pansenstabilen CLA zur Ration der Milchkuh auf den Pansenmetabolismus, die Leistungsparameter und die FS-Verteilung in der Milch untersuchen.

Zunächst wurden für eine Langzeitstudie 46 Milchkühe der Rasse Deutsche Holstein in drei Gruppen aufgeteilt. Die Tiere erhielten das Versuchsfutter vom 1. Tag *post partum* (p.p.) über 26 Wochen und verblieben noch weitere 12 Wochen im Versuch, um Effekte nach der Behandlung erfassen zu können. Jede der drei Gruppen erhielt ein Fettsupplement in pansenstabiler Form welches in das Kraftfutter eingemischt war. Es ergaben sich folgende Versuchsgruppen: KON: 100 g Kontrollfettsupplement; CLA-1: 50 g Kontrollfett und 50 g CLA-Supplement; CLA-2: 100 g CLA-Supplement. Das CLA-Supplement enthielt 10% *trans*-10,*cis*-12 CLA und 10% *cis*-9,*trans*-11 CLA. Im Kontrollfettsupplement wurden die CLA durch Stearinsäure ersetzt. Zusätzlich erhielten die Tiere eine partielle Mischration

(PMR) (37% Kraftfutter, 38% Mais- und 25% Grassilage auf Trockenmasse (T)-Basis), die ihnen *ad libitum* zur Verfügung stand. Die Milchprobenahme erfolgte in den Wochen 1, 4, 12 und 26 p.p. sowie in Woche 2 und 10 während der Depletionszeit. Blutproben wurden am 1. Tag p.p., in den Wochen 1, 2, 3, 5, 7, 10, 15, 20 und 26 p.p. und in den Wochen 1, 2, 4, 6, 8 und 10 während der Depletionszeit genommen. Der Body Condition Score (BCS) eines jeden Tieres wurde an den genannten Blutentnahmeterminen ebenfalls erfasst. Die Rückenfettdicke (RFD) wurde mit Hilfe von Ultraschall am 1. Tag p.p., in den Wochen 3, 10, 15 und 26 p.p. und in der 2., 6. und 10. Woche nach Absetzen der Fettsupplemente gemessen.

Basierend auf der im Kraftfutter gemessenen CLA-Konzentration nahmen die Tiere in Gruppe CLA-1 und CLA-2 täglich etwa 4 bzw. 8 g *trans*-10,*cis*-12 CLA auf. Die Trockenmasseaufnahme war in den ersten sieben Wochen der Laktation bei den CLA-supplmentierten Tieren um 2,6 – 3,3 kg T/d gesenkt. Infolgedessen trat eine verminderte Energiebilanz bei den CLA-supplementierten Tieren auf, wobei kein negativer Einfluss auf tägliche Milchmenge, Milchinhaltsstoffe, BCS, RFD und die Plasmakonzentrationen an unveresterten Fettsäuren (NEFA) und ß-Hydroxybutyrat (BHB) zu beobachten war, was für eine bessere Nutzung der zur Verfügung stehenden Energie spricht. Im späteren Laktationsverlauf (8.-26. Woche) führte CLA in der Ration zu einer dosisabhängigen Reduktion des Milchfettgehaltes (CLA-1: -7%, CLA-2: -12%). Gleichzeitig stieg die täglich Milchmenge numerisch um 4 bzw. 3 kg/d wodurch die Milchfettsynthese und die kalkulierte Energiebilanz von der CLA-Zulage nicht beeinflusst wurden. Auch die unveränderten BCS-, RFD-Werte und Plasmakonzentrationen an NEFA und BHB sprechen gegen eine CLA-Wirkung auf den Energiemetabolismus der Milchkuh. Die CLA-Zulage bewirkte einen dosisabhängigen Anstieg des Anteils des *trans*-10,*cis*-12-Isomers in der Milch von <0,01% auf 0,02 und 0,03% der Fettsäuremethylester (FSME), der Gesamtanteil an CLA blieb jedoch unbeeinflusst. Der Anteil an C16-FS in der Milch wurde durch die höchste CLA-Dosis gesenkt. Mit Ausnahme der Glucosekonzentration im Plasma konnten nach Absetzen des CLA-Supplements keine Unterschiede hinsichtlich der untersuchten Parameter mehr festgestellt werden. Die Glukosekonzentration der CLA-2-Gruppe war zu diesem Zeitpunkt leicht erhöht was auf eine geringere Insulinsensitivität hinweist.

In einer weiteren Studie wurden 6 Milchkühe der Rasse Deutsche Holstein mit je einer Fistel am dorsalen Pansensack und proximalen Duodenum eingesetzt. Die Rationen unterschieden sich in ihrem CLA-Gehalt, wie schon für den ersten Versuch beschrieben. Die Fettsupplemente wurden mit dem Kraftfutter verabreicht. Die gefütterte Menge an Grundfutter (60% Mais-, 40% Grassilage auf T-Basis) wurde an die erwarteten Aufnahmen der Tiere angepasst. Jede Ration wurde in einer Periode gefüttert und jede Kuh durchlief jede Periode in der gleichen Reihenfolge: Kontrolle, CLA-1, CLA-2. Eine Versuchsperiode erstreckte sich über zwei Adaptationswochen auf die zwei Wochen der Probenentnahme

folgten. Die Pansensaftentnahme erfolgte einmalig in Woche 3 direkt vor der ersten Morgenfütterung sowie zu sechs späteren Zeitpunkten. Duodenalchymus wurde in zweistündigen Intervallen über fünf Tage in Woche vier gesammelt. Zur Bestimmung des duodenalen Nährstoffflusses wurde Cr_2O_3 als Marker verwendet. Kotproben wurden einmal in Woche 4 jeder Periode genommen. Die Milchprobenentnahme erfolgte an zwei Tagen in den Wochen 3 und 4.
Die tägliche Aufnahme der Tiere an pansengeschütztem CLA lag bei 3 bzw. 8 g in Periode CLA-1 und CLA-2. Die im Folgenden gezeigten Effekte auf die Fermentationsvorgänge im Pansen sind sehr gering und nicht eindeutig auf CLA zurückführbar, da sich die Nährstoffzusammensetzung über den Versuchszeitraum leicht veränderte. Die scheinbare ruminale Verdaulichkeit der Stärke war in der CLA-2-Gruppe um 6% erhöht. Der N-Fluss am Darm (% der Aufnahme) ging in den CLA-supplementierten Gruppen zurück. Außerdem kam es in den CLA-Gruppen zu einer Reduktion des im Pansen unabgebauten Proteins relativ zur Rohproteinaufnahme. Die höhere Menge an N die im Pansen zur Mikrobenproteinsynthese zur Verfügung stand, blieb von den Mikroben ungenutzt, was sich in einem Rückgang des Verhältnisses von Mikrobenprotein zu ruminal abbaubaren Protein äußerte. Weiterhin schien es, dass die mikrobielle FS-Synthese nachteilig durch CLA beeinflusst wurde, da sich der duodenale Fluss der Gesamt-FS in Relation zur Aufnahme verringerte. Nur 16 und 5% des aufgenommenen *trans*-10,*cis*-12 CLA-Isomers umgingen in Periode CLA-1 bzw. CLA-2 die ruminale Biohydrogenierung. Betrachtet man den tierindividuellen *trans*-10,*cis*-12 CLA-Fluss fällt auf dass es große Unterschiede zwischen den einzelnen Tieren gab. In der Milch erhöhte sich der Anteil an Gesamt-CLA und den mehrfach ungesättigten FS (PUFA) infolge der CLA-Zulage, wobei sich der Anteil an gesättigten FS (SFA) verringerte. In Übereinstimmung mit den Ergebnissen der ersten Untersuchung erhöhte sich der Anteil des *trans*-10,*cis*-12-Isomers in der Milch dosisabhängig auf 0,03 und 0,06% der FSME. Der Transfer dieses Isomers in die Milch betrug im Mittel 5%. Etwa 34% der im Darm ankommenden Menge *trans*-10,*cis*-12 CLA wurde in die Milch transferiert während ca. 43% mit dem Kot ausgeschieden wurden.

Aus den vorliegenden Untersuchungen kann geschlossen werden, dass eine Supplementation mit CLA scheinbar keine Verbesserung der negativen Energiebilanz in der Frühlaktation bewirkt. Die geringe Menge des im Darm ankommenden *trans*-10,*cis*-12-Isomers sprechen für einen geringen Pansenschutz des eingesetzten CLA-Präparates. Weiterhin kann die Supplementation von CLA zur Ration der Milchkuh zu einer Verbesserung des Nährwertes der Milch durch die Erhöhung des Anteils an CLA und PUFA beitragen, wobei keine negativen Effekte auf die Gesundheit des Verbrauchers zu erwarten sind.

REFERENCES

(cited in General Introduction, Background and General Discussion)

AbuGhazaleh AA.2008.Effect of fish oil and sunflower oil supplementation on milk conjugated linoleic acid content for grazing dairy cows.Anim Feed Sci Tech.141:220-232.

Akter SH, Haussler S, Dänicke S, Muller U, von Soosten D, Rehage J and Sauerwein H.2011.Physiological and conjugated linoleic acid-induced changes of adipocyte size in different fat depots of dairy cows during early lactation.J Dairy Sci.94:2871-2882.

Allocati P, Cabona E, Puhl L and Gonzalez J.2007.Impact of the pasteurization process on the conjugated linoleic acid (CLA) content in bovine raw milk (communication).Revista Argentina de Produccion Animal.27:189-195.

AlZahal O, Odongo NE, Mutsvangwa T, Or-Rashid MM, Duffield TF, Bagg R, Dick P, Vessie G and McBride BW.2008.Effects of monensin and dietary soybean oil on milk fat percentage and milk fatty acid profile in lactating dairy cows.J Dairy Sci.91:1166-1174.

Aro A, Männistö S, Salminen I, Ovaskainen ML, Kataja V and Uusitupa M.2000.Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women.Nutr Cancer.38:151-157.

Azain MJ, Hausman DB, Sisk MB, Flatt WP and Jewell DE.2000.Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number.J Nutr.130:1548-1554.

Banni S, Carta G, Angioni E, Murru E, Scanu P, Melis MP, Bauman DE, Fischer SM and Ip C.2001.Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver.J Lipid Res.42:1056-1061.

Banni S, Petroni A, Blasevich M, Carta G, Angioni E, Murru E, Day BW, Melis MP, Spada S and Ip C.2004.Detection of conjugated C16 PUFAs in rat tissues as possible partial beta-oxidation products of naturally occurring conjugated linoleic acid and its metabolites.BBA-Mol Cell Biol L.1682:120-127.

Bauman DE, Peel CJ, Steinhour WD, Reynolds PJ, Tyrrell HF, Brown ACG and Haaland GL.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 LH, Corl BA, Dwyer DA and Bauman DE.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 Anim Sci.80:1285-1293.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA and Bauman DE.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 LH, Sangster JK and Bauman DE.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 LH, Corl BA, Dwyer DA, Saebo A and Bauman DE.2000.Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis.Am J Physiol Regul Integr Comp Physiol.278:R179-R184.

Beam SW and Butler WR.1999.Effects of energy balance on follicular development and first ovulation in postpartum dairy cows.J Reprod Fert.Supplement 54:411-424.

Bell AW and Bauman DE.1997.Adaptations of glucose metabolism during pregnancy and lactation.J Mammary Gland Biol.2:265-278.

Belury MA, Nickel KP, Bird CE and Wu YM.1996.Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion.Nutr Cancer.26:149-157.

Benson JA and Reynolds CK.2001.Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows.J Dairy Sci.84:1488-1500.

Berdeaux O, Christie W, Gunstone F and Sebedio J.1997.Large-scale synthesis of methyl *cis*-9, *trans*-11-octadecadienoate from methyl ricinoleate.J Am Oil Chem Soc.74:1011-1015.

Bernal-Santos G, Perfield JW, Barbano DM, Bauman DE and Overton TR.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, Benefield BC, de Veth MJ, Santos NR, Gilbert RO, Butler WR and Bauman DE.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, Overton TR, Butler WR and Bauman DE.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.

Castro N, Capote J, Martín D and Argüello A.2006.The influence of dietary conjugated linoleic acid on blood serum and colostrum immunoglobulin G concentration in female goats before and after parturition.J Anim Physiol An N.90:429-431.

Chajès V, Lavillonnière F, Ferrari P, Jourdan ML, Pinault M, Maillard V, Sèbèdio JL and Bougnoux P.2002.Conjugated linoleic acid content in breast adipose tissue is not associated with the relative risk of breast cancer in a population of french patients.Cancer Epidem Biomar.11:672-673.

Chen CA and Sih CJ.1998.Chemoenzymatic synthesis of conjugated linoleic acid.J Org Chem.63:9620-9621.

Chin SF, Liu W, Storkson JM, Ha YL and Pariza MW.1992.Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens.J Food Compos Anal.5:185-197.

Choi BR, Palmquist DL and Allen MS.2000.Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets.Domest Anim Endocrin.19:159-175.

Choi JS, Jung MH, Park HS and Song J.2004.Effect of conjugated linoleic acid isomers on insulin resistance and mRNA levels of genes regulating energy metabolism in high-fat-fed rats.Nutrition.20:1008-1017.

Chouinard PY, Corneau L, Barbano DM, Metzger LE and Bauman DE.1999.Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows.J Nutr.129:1579-1584.

Collard BL, Boettcher PJ, Dekkers JCM, Petitclerc D and Schaeffer LR.2000.Relationships between energy balance and health traits of dairy cattle in early lactation.J Dairy Sci.83:2683-2690.

Cook ME, Miller CC, Park Y and Parizaa M.1993.Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression.Poultry Sci.72:1301-1305.

Corino C, Pastorelli G, Rosi F, Bontempo V and Rossi R.2009.Effect of dietary conjugated linoleic acid supplementation in sows on performance and immunoglobulin concentration in piglets.J Anim Sci.87:2299-2305.

de Deckere EAM, van Amelsvoort JMM, McNeill GP and Jones P.1999.Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster.Br J Nutr.82:309-317.

de Veth MJ, Bauman DE, Koch W, Mann GE, Pfeiffer AM and Butler WR.2009.Efficacy of conjugated linoleic acid for improving reproduction: A multi-study analysis in early-lactation dairy cows.J Dairy Sci.92:2662-2669.

de Veth MJ, Griinari JM, Pfeiffer AM and Bauman DE.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.39:365-372.

de Veth MJ, Gulati SK, Luchini ND and Bauman DE.2005a.Comparison of calcium salts and formaldehyde-protected conjugated linoleic acid in inducing milk fat depression.J Dairy Sci.88:1685-1693.

de Veth MJ, van Straalen WM, Koch W, Keller T, Hayler R and Pfeiffer AM.2005b.Effect of CLA dose on milk production in early lactation cows.J Dairy Sci.88:220.

Dehkordi SK, Vlaeminck B, Hostens M, Opsomer G and Fievez V.2008.In vitro rumen biohydrogenation of trans-10, cis-12 conjugated linoleic acid in a lipid-encapsulated (LE-CLA) supplement incorporated or not in a processing pellet.Comm Appl Biol Sci.73:119-122.

Delany JP, Blohm F, Truett AA, Scimeca JA and West DB.1999.Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake.Am J Physiol-Reg I.276:R1172-R1179.

Dhiman TR, Anand GR, Satter LD and Pariza MW.1999a.Conjugated linoleic acid content of milk from cows fed different diets.J Dairy Sci.82:2146-2156.

Dhiman TR, Helmink ED, McMahon DJ, Fife RL and Pariza MW.1999b.Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds.J Dairy Sci.82:412-419.

Donovan DC, Schingoethe DJ, Baer RJ, Ryali J, Hippen AR and Franklin ST.2000.Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows.J Dairy Sci.83:2620-2628.

Drackley JK.1999.Biology of dairy cows during the transition period: the final frontier.J Dairy Sci.82:2259-2273.

Drackley JK, Klusmeyer TH, Trusk AM and Clark JH.1992.Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows.J Dairy Sci.75:1517-1526.

Duckett SK, Andrae JG and Owens FN.2002.Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets.J Anim Sci.80:3353-3360.

Dulloo AG and Samec S.2001.Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered.Brit J Nutr.86:123-139.

Ealey K, El-Sohemy A and Archer M.2002.Effects of dietary conjugated linoleic acid on the expression of uncoupling proteins in mice and rats.Lipids.37:853-861.

EFSA.2010.Scientific Opinion on the substantiation of health claims related to conjugated linoleic acid (CLA) isomers and contribution to the maintenance or achievement of a normal body weight (ID 686, 726, 1516, 1518, 2892, 3165), increase in lean body mass (ID 498, 731), increase in insulin sensitivity (ID 1517), protection of DNA, proteins and lipids from oxidative damage (ID 564, 1937), and contribution to immune defences by stimulation of production of protective antibodies in response to vaccination (ID 687,1519) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.EFSA Journal.8

EFSA.2011.Scientific Opinion on the substantiation of health claims related to foods with reduced amounts of saturated fatty acids (SFAs) and maintenance of normal blood LDL cholesterol concentrations (ID 620, 671, 4332) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.EFSA Journal.9

Erlanson-Albertsson C.2003.The role of uncoupling proteins in the regulation of metabolism.Acta Physiol Scand.178:405-412.

Evans M, Lin X, Odle J and McIntosh M.2002.Trans-10, cis-12 conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes.J Nutr.132:450-455.

Firkins JL and Eastridge ML.1994.Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production.J Dairy Sci.77:2357-2366.

Fritsche J and Steinhart H.1998.Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake.Z Lebensm Unters F A.206:77-82.

Garcia-Lopez S, Echeverria E, Tsui I and Balch B.1994.Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing.Food Res Int.27:61-64.

Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H and Gudmundsen O.2004.Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans.Am J Clin Nutr.79:1118-1125.

Gaullier JM, Halse J, Høye K, Kristiansen K, Fagertun H, Vik H and Gudmundsen O.2005.Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans.J Nutr.135:778-784.

Gavino VC, Gavino G, Leblanc MJ and Tuchweber B.2000.An isomeric mixture of conjugated linoleic acids but not pure cis-9,trans-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters.J Nutr.130:27-29.

GfE.2001.Empfehlungen zur Energie- und Nährstoffaufnahme der Milchkühe und Aufzuchtrinder.DLG Verlag,Frankfurt am Main.

Giesy JG, McGuire MA, Shafii B and Hanson TW.2002.Effect of dose of calcium salts of conjugated linoleic acid (CLA) on percentage and fatty acid content of milk fat in midlactation Holstein cows.J Dairy Sci.85:2023-2029.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV and Bauman DE.2000.Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase.J Nutr.130:2285-2291.

Gutgesell A, Ringseis R and Eder K.2009.Short communication: Dietary conjugated linoleic acid down-regulates fatty acid transporters in the mammary glands of lactating rats.J Dairy Sci.92:1169-1173.

Ha YL, Grimm NK and Pariza MW.1987.Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid.Carcinogenesis.8:1881-1887.

Haarstrich D.2011.Thesis/Dissertation.Insulinsensitivität und Insulinresponse nach einer Langzeit-Supplementation von konjugierten Linolsäuren bei laktierenden Milchkühen.Tierärztliche Hochschule Hannover (Germany)

Halade G, Rahman M and Fernandes G.2009.Effect of CLA isomers and their mixture on aging C57Bl/6J mice.Eur J Nutr.48:409-418.

Harfoot CG.1978.Lipid metabolism in the rumen.Prog Lipid Res.17:21-54.

Harvatine KJ and Bauman DE.2006.SREBP1 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.

Harvatine KJ, Perfield JW, II and Bauman DE.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.139:849-854.

Hayek MG, Han SN, Wu D, Watkins BA, Meydani M, Dorsey JL, Smith DE and Meydani SN.1999.Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice.J Nutr.129:32-38.

Hayirli A.2006.The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle.Vet Res Commun.30:749-774.

Houseknecht KL, Heuvel JPV, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP and Belury MA.1998.Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker Diabetic Fattyfa/faRat.Biochem Bioph Res Co.244:678-682.

Huang Y, Schoonmaker JP, Bradford BJ and Beitz DC.2008.Response of milk fatty acid composition to dietary supplementation of soy oil, conjugated linoleic acid, or both.J Dairy Sci.91:260-270.

Huang Y, Schoonmaker JP, Oren SL, Trenkle A and Beitz DC.2009.Calcium salts of CLA improve availability of dietary CLA.Livest Sci.122:1-7.

Hussen J, Dänicke S, and Schuberth H-J.2011.The effect of a long term dietary suppplementation with conjugated linoleic acid (CLA) on the composition of bovine peripheral blood mononuclear cells (PBMC) and the concentration of IgG isotypes in blood and milk.Proc Soc Nutr Physiol.20:85.

Hussen J and Schuberth H-J.2011.The effect of 9-cis:11-trans conjugated linoleic acid (CLA) , 15d-*PGJ2* and GW9662 on the proloferation of bovine peripheral blood mononuclear cells in vitro.Proc Soc Nutr Physiol.20:97.

Hutchinson I, de Veth MJ, Stanton C, Dewhurst RJ, Lonergan P, Evans ACO and Butler ST.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.

Ikwuegbu OA and Sutton JD.1982.The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep.Brit J Nutr.48:365-375.

Ip C, Chin SF, Scimeca JA and Pariza MW.1991.Mammary-cancer prevention by conjugated dienoic derivative of linoleic-acid.Cancer Res.51:6118-6124.

Jahreis G, Fritsche J, Möckel P, Schöne F, Müller U and Steinhart H.1999.The potential anticarcinogenic conjugated linoleic acid, cis-9,trans-11 C18:2, in milk of different species: Cow, goat, ewe, sow, mare, woman.Nutr Res.19:1541-1549.

Jahreis G, Fritsche J and Steinhart H.1997.Conjugated linoleic acid in milk fat: High variation depending on production system.Nutr Res.17:1479-1484.

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

Jenkins TC and Jenny BF.1989.Effect of hydrogenated fat on feed-intake, nutrient digestion, and lactation performance of dairy-cows.J Dairy Sci.72:2316-2324.

Jones DF, Weiss WP and Palmquist DL.2000.Short communication: influence of dietary tallow and fish oil on milk fat composition.J Dairy Sci.83:2024-2026.

Jones EL, Shingfield KJ, Kohen C, Jones AK, Lupoli B, Grandison AS, Beever DE, Williams CM, Calder PC and Yaqoob P.2005.Chemical, physical, and sensory properties of dairy products enriched with conjugated linoleic acid.J Dairy Sci.88:2923-2937.

José AAFBV, Gama MAS and Lanna DDP.2008.Effects of *trans*-10,*cis*-12 conjugated linoleic acid on gene expression and lipid metabolism of adipose tissue of growing pigs.Genet Mol Res.7:284-294.

Joseph SV, Liu X, Wakefield A, Chouinard PY, Aukema H, Jones PJ and Jacques H.2010.Trans-8, cis-10+ cis-9, trans-11-conjugated linoleic acid mixture alters body composition in Syrian golden hamsters fed a hypercholesterolaemic diet.Br J Nutr.104:1443-1449.

Kahn RC.1978.Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction.Metabolism.27:1893-1902.

Kang JH, Lee GS, Jeung EB and Yang MP.2007.*Trans*-10, *cis*-12-conjugated linoleic acid increases phagocytosis of porcine peripheral blood polymorphonuclear cells *in vitro*.Brit J Nutr.97:117-125.

Kang K, Liu W, Albright KJ, Park Y and Pariza MW.2003.Trans-10,cis-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPARγ expression.Biochem Bioph Res Co.303:795-799.

Kay JK, Mackle TR, Bauman DE, Thomson NA and Baumgard LH.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 JK, Roche JR, Moore CE and Baumgard LH.2006.Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture.J Dairy Res.73:367-377.

Kelley D, Warren J, Simon V, Bartolini G, Mackey B and Erickson K.2002.Similar effects of *c*9, *t*11-CLA and *t*10, *c*12-CLA on immune cell functions in mice.Lipids.37:725-728.

Kelley NS, Hubbard NE and Erickson KL.2007.Conjugated linoleic acid isomers and cancer.J Nutr.137:2599-2607.

Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, Van Amburgh ME and Bauman DE.1998a.Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows.J Nutr.128:881-885.

Kelly ML, Kolver ES, Bauman DE, Van Amburgh ME and Muller LD.1998b.Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows.J Dairy Sci.81:1630-1636.

Kim JH, Kwon OJ, Choi NJ, Oh SJ, Jeong HY, Song MK, Jeong I and Kim YJ.2009.Variations in conjugated linoleic acid (CLA) content of processed cheese by lactation time, feeding regimen, and ripening.J Agr Food Chem.57:3235-3239.

Kostogrys RB, Maslak E, Franczyk-Zarow M, Gajda M and Chlopicki S.2011.Effects of trans-10,cis-12 and cis-9,trans-11 CLA on atherosclerosis in apoE/LDLR(-/-) mice.Eur J Lipid Sci Technol.113:572-583.

Kraft J.2003.Thesis/Dissertation.Inkorporation von konjugierten Linolsäuren in Körperlipide unter besonderer Berücksichtigung der Isomerenverteilung.Friedrich-Schiller-Universität Jena.Jena (Germany).

Kritchevsky D, Tepper SA, Wright S, Tso P and Czarnecki SK.2000.Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits.J Am Coll Nutr.19:472S-477S.

Larsson SC, Bergkvist L and Wolk A.2005.High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort.Am J Clin Nutr.82:894-900.

Larsson SC, Bergkvist L and Wolk A.2009.Conjugated linoleic acid intake and breast cancer risk in a prospective cohort of Swedish women.Am J Clin Nutr.90:556-560.

Lee KN, Kritchevsky D and Pariza MW.1994.Conjugated linoleic acid and atherosclerosis in rabbits.Atherosclerosis.108:19-25.

Li Y, Diao Q and Meng Q.2009.The effect of steam-flaked and extruded full-fat soybeans on the concentration of conjugated linoleic acid in the milk fat of dairy cows.Arch Anim Nutr.63:243-253.

Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M and Wylie-Rosett J.2006.Diet and lifestyle recommendations revision 2006.Circulation.114:82-96.

Lie Ken Jie M, Pasha M and Alam M.1997.Synthesis and nuclear magnetic resonance properties of all geometrical isomers of conjugated linoleic acids.Lipids.32:1041-1044.

Lin H, Boylston D, Chang MJ, Luedecke LO and Shultz TD.1995.Survey of the conjugated linoleic acid contents of dairy products.J Dairy Sci.78:2358-2365.

Lin X, Liang G, Su P and Wang Z.2010.Effects of supplemental energy sources and trans-10, cis-12 conjugated linoleic acids (CLA) on milk yield and composition in lactating Holstein cows.Front Agr China.4:101-108.

Liu SJ, Bu DP, Wang JQ, Sun P, Wei HY, Zhou LY and Yu ZT.2011.Effect of ruminal pulse dose of polyunsaturated fatty acids on ruminal microbial populations and duodenal flow and milk profiles of fatty acids.J Dairy Sci.94:2977-2985.

Lock AL and Bauman DE.2004.Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health.Lipids.39:1197-1206.

Lock AL and Garnsworthy PC.2002.Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk.Anim Sci.74:163-176.

Lock AL, Perfield JW, Putnam DE and Bauman DE.2004.Evaluation of the degree of rumen inertness and bioavailability of trans-10, cis-12 CLA in a lipid encapsulated supplement.J Dairy Sci.87:335.

Loor JJ, Lin XB and Herbein JH.2002.Dietary trans-vaccenic acid (trans11-18 : 1) increases concentration of cis9, trans11-conjugated linoleic acid (rumenic acid) in tissues of lactating mice and suckling pups.Reprod Nutr Dev.42:85-99.

Loor JJ and Herbein JH.1998.Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis.J Nutr.128:2411-2419.

Lucy MC, Staples CR, Thatcher WW, Erickson PS, Cleale RM, Firkins JL, Clark JH, Murphy MR and Brodie BO.1992.Influence of diet composition, dry-matter intake, milk-production and energy-balance on time of postpartum ovulation and fertility in dairy-cows.Anim Prod.54:323-331.

Maia MRG, Chaudhary LC, Figueres L and Wallace RJ.2007.Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen.Anton Leeuw Int J G.91:303-314.

Mann GE, Lock AL, Bauman DE and Kendall NR.2007.Reproductive function in dairy cows fed a lipid encapsulated conjugated linoleic acid supplement.J Dairy Sci.90:401.

Martin JC, Grégoire S, Siess MH, Genty M, Chardigny JM, Berdeaux O, Juaneda P and Sébédio JL.2000.Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats.Lipids.35:91-98.

Mattos R, Staples CR and Thatcher WW.2000.Effects of dietary fatty acids on reproduction in ruminants.Rev Reprod.5:38-45.

May KCP, Bobe G, Mueller CJ and Cannon MJ.2011.Conjugated linoleic acid decreases prostaglandin synthesis in bovine luteal cells in vitro.Mol Reprod Dev.78:328-336.

McCann SE, Ip C, Ip MM, McGuire MK, Muti P, Edge SB, Trevisan M and Freudenheim JL.2004.Dietary intake of conjugated linoleic acids and risk of premenopausal and postmenopausal breast cancer, Western New York exposures and breast cancer study (WEB Study).Cancer Epidem Biomar.13:1480-1484.

Medeiros SR, Oliveira DE, Aroeira LJM, McGuire MA, Bauman DE and Lanna DPD.2010.Effects of dietary supplementation of rumen-protected conjugated linoleic acid to grazing cows in early lactation.J Dairy Sci.93:1126-1137.

Mensink RP, Zock PL, Kester ADM and Katan MB.2003.Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials.Am J Clin Nutr.77:1146-1155.

Metzger-Petersen K, Pries M, van de Sand H, and Südekum K.2009.Does a supplementation of conjugated linoleic acids during early lactation affect milk and milk component yields beyond the cessation of supplementation.Proc Soc Nutr Physiol.18:81.

Meyer U, Brömmel C, Flachowsky G and Jahreis G.2007.Effect of dietary conjugated linoleic acid (CLA) on milk composition of dairy cows.Proceedings of the 2nd EAAP International Symposium on Energy and Protein Metabolism and Nutrition:157-158.

Miguel Rodriguez-Alcala L, Harte F and Fontecha J.2009.Fatty acid profile and CLA isomers content of cow, ewe and goat milks processed by high pressure homogenization.Innov Food Sci Emerg.10:32-36.

Miller CC, Park Y, Pariza MW and Cook ME.1994.Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection.Biochem Bioph Res Co.198:1107-1112.

Mir PS, Ivan M, He ML, Pink B, Okine E, Goonewardene L, McAllister TA, Weselake R and Mir Z.2003.Dietary manipulation to increase conjugated linoleic acids and other desirable fatty acids in beef: A review.Can J Anim Sci.83:673-685.

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

Mohammed R, Stanton CS, Kennelly JJ, Kramer JKG, Mee JF, Glimm DR, O'Donovan M and Murphy JJ.2009.Grazing cows are more efficient than zero-grazed and grass silage-fed cows in milk rumenic acid production.J Dairy Sci.92:3874-3893.

Moore CE, Hafliger HC, III, Mendivil OB, Sanders SR, Bauman DE and Baumgard LH.2004.Increasing amounts of conjugated linoleic acid (CLA) progressively reduces milk fat synthesis immediately postpartum.J Dairy Sci.87:1886-1895.

Mosley EE, McGuire MK, Williams JE and McGuire MA.2006.Cis-9, trans-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women.J Nutr.136:2297-2301.

Moya-Camarena SY, Heuvel JPV, Blanchard SG, Leesnitzer LA and Belury MA.1999.Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα.J Lipid Res.40:1426-1433.

Munday JS, Thompson KG and James KAC.1999.Dietary conjugated linoleic acids promote fatty streak formation in the C57BL/6 mouse atherosclerosis model.Br J Nutr.81:251-255.

Neville MC and Picciano MF.1997.Regulation of milk lipid secretion and composition.Annu Rev Nutr.17:159-183.

Nichols PL, Herb SF and Riemenschneider RW.1951.Isomers of Conjugated Fatty Acids. I. Alkali-isomerized Linoleic Acid.J Am Chem Soc.73:247-252.

Odens LJ, Burgos R, Innocenti M, VanBaale MJ and Baumgard LH.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.

Oikawa S and Oetzel GR.2006.Decreased insulin response in dairy cows following a four-day fast to induce hepatic lipidosis.J Dairy Sci.89:2999-3005.

Oldick BS and Firkins JL.2000.Effects of degree of fat saturation on fiber digestion and microbial protein synthesis when diets are fed twelve times daily.J Anim Sci.78:2412-2420.

Onnen-Lübben EF.2009.Thesis/Dissertation.Einfluss einer gestaffelten CLA-Supplementation auf die Gelbkörperfunktion während des Zyklus und der frühen Gravidität bei Hochleistungsmilchkühen.Tierärzliche Hochschule Hannover.Hannover (Germany).

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

O`Shea M, Bassaganya-Riera J and Mohede IC.2004.Immunomodulatory properties of conjugated linoleic acid.Am J Clin Nutr.79:1199S-1206S.

Palmquist DL and Jenkins TC.1980.Fat in lactation rations: Review.J Dairy Sci.63:1-14.

Pantoja J, Firkins JL and Eastridge ML.1996.Fatty acid digestibility and lactation performance by dairy cows fed fats varying in degree of saturation.J Dairy Sci.79:429-437.

Pantoja J, Firkins JL, Eastridge ML and Hull BL.1994.Effects of fat saturation and source of fiber on site of nutrient digestion and milk production by lactating dairy cows.J Dairy Sci.77:2341-2356.

Pariza MW.2004.Perspective on the safety and effectiveness of conjugated linoleic acid.Am J Clin Nutr.79:1132S-1136S.
Park Y, Albright KJ, Liu W, Storkson JM, Cook ME and Pariza MW.1997.Effect of conjugated linoleic acid on body composition in mice.Lipids.32:853-858.
Park Y, Storkson JM, Albright KJ, Liu W and Pariza MW.1999.Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice.Lipids.34:235-241.
Park Y and Pariza MW.2007.Mechanisms of body fat modulation by conjugated linoleic acid (CLA).Food Res Int.40:311-323.
Perfield JWI, Bernal-Santos G, Overton TR and Bauman DE.2002.Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation.J Dairy Sci.85:2609-2617.
Perfield JWI, Delmonte P, Lock AL, Yurawecz MP and Bauman DE.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 JWI, Lock AL, Griinari JM, Saebo A, Delmonte P, Dwyer DA and Bauman DE.2007.Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows.J Dairy Sci.90:2211-2218.
Perfield JWI, Lock AL, Pfeiffer AM and Bauman DE.2004a.Effects of amide-protected and lipid-encapsulated conjugated linoleic acid (CLA) supplements on milk fat synthesis.J Dairy Sci.87:3010-3016.
Perfield JWI, Saebo A and Bauman DE.2004b.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.
Peterson DG, Kelsey JA and Bauman DE.2002.Analysis of variation in cis-9, trans-11 conjugated linoleic acid (CLA) in milk fat of dairy cows.J Dairy Sci.85:2164-2172.
Peterson DG, Matitashvili EA and Bauman DE.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 DG, Matitashvili EA and Bauman DE.2004.The inhibitory effect of trans-10, cis-12 CLA on lipid synthesis in bovine mammary epithelial cells involves reduced proteolytic activation of the transcription factor SREBP-1.J Nutr.134:2523-2527.
Piamphon N, Wachirapakorn C, Wanapat M and Navanukraw C.2009.Effects of protected conjugated linoleic acid supplementation on milk fatty acid in dairy cows.Asian Australas J Anim Sci.22:49-56.
Piperova LS, Moallem U, Teter BB, Sampugna J, Yurawecz MP, Morehouse KM, Luchini D and Erdman RA.2004.Changes in milk fat in response to dietary supplementation with calcium salts of trans-18:1 or conjugated linoleic fatty acids in lactating dairy cows.J Dairy Sci.87:3836-3844.
Ramírez-Santana C, Castellote C, Castell M, Rivero M, Rodríguez-Palmero M, Franch À and Pérez-Cano FJ.2009.Long-term feeding of the cis-9,trans-11 isomer of conjugated linoleic acid reinforces the specific immune response in rats.J Nutr.139:76-81.
Rego OA, Alves SP, Antunes LMS, Rosa HJD, Alfaia CFM, Prates JAM, Cabrita ARJ, Fonseca AJM and Bessa RJB.2009.Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils.J Dairy Sci.92:4530-4540.
Renner L, von Soosten D, Sipka A, Döll S, Schuberth H-J, and Dänicke S.2011.Effects of rumen-protected conjugated linoleic acids (CLA) on prolferation and cytokine expression of bovine blood mononuclear cells (PBMC) and splenocytes ex vivo.Proc Soc Nutr Physiol.20:84.

Ringseis R and Eder K.2009.Influence of conjugated linoleic acids on functional properties of vascular cells.British Journal of Nutrition.102:1099-1116.

Risérus U, Arner P, Brismar K and Vessby B.2002.Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome.Diabetes Care.25:1516-1521.

Salas-Salvadó J, Márquez-Sandoval F and Bulló M.2006.Conjugated linoleic acid intake in humans: A systematic review focusing on its effect on body composition, glucose, and lipid metabolism.Crit Rev Food Sci.46:479-488.

Schwarz F, Lierman T and Pfeiffer A.2007.Effect of rumen-protected conjugated linoleic acid in combination with propylene glycol or rumen protected fat on performance and metabolic parameters of early lactation dairy cows.Proceedings of the 2nd EAAP International Symposium on Energy and Protein Metabolism and Nutrition:155-156.

Seifert MF and Watkins BA.1997.Role of dietary lipid and antioxidants in bone metabolism.Nutr Res.17:1209-1228.

Selberg KT, Lowe AC, Staples CR, Luchini ND and Badinga L.2004.Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids.J Dairy Sci.87:158-168.

Shingfield KJ, Ahvenjärvi S, Toivonen V, Vanhatalo A, Huhtanen P and Griinari JM. 2008.Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows.Brit J Nutr.99:971-983.

Shultz TD, Chew BP, Seaman WR and Luedecke LO.1992.Inhibitory effect of conjugated dienoic derivatives of linoleic acid and ß-carotene on the in vitro growth of human cancer cells.Cancer Lett.63:125-133.

Sieber R and Collomb M.2004.Bildung von konjugierten Linolsäuren durch Starterkulturen.AGRARForschung.11:60-65.

Sigl T, Schlamberger G, Kienberger H, Wiedemann S, Meyer H and Kaske M.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

Sippel MA, Spratt RS and Cant JP.2009.Milk production responses of primiparous and multiparous dairy cows to dose of conjugated linoleic acid consumed in rumen inert form.Can J Anim Sci.89:393-399.

Stachowska E, Dolegowska B, Dziedziejko V, Rybicka M, Kaczmarczyk M, Bober J, Rac M, Machalinski B and Chlubek D.2009.Prostaglandin E-2 (Pge(2)) and thromboxane A(2) (Txa(2)) synthesis is regulated by conjugated linoleic acids (Cla) in human macrophages.J Physiol Pharmacol.60:77-85.

Stangl GI.2000a.High dietary levels of a conjugated linoleic acid mixture alter hepatic glycerophospholipid class profile and cholesterol-carrying serum lipoproteins of rats.Journal of Nutritional Biochemistry.11:184-191.

Stangl GI.2000b.Conjugated linoleic acids exhibit a strong fat-to-lean partitioning effect, reduce serum VLDL lipids and redistribute tissue lipids in food-restricted rats.J Nutr.130:1140-1146.

Stangl GI, Müller H and Kirchgessner M.1999.Conjugated linoleic acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine.European Journal of Nutrition.38:271-277.

Sugano M, Tsujita A, Yamasaki M, Noguchi M and Yamada K.1998.Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats.Lipids.33:521-527.

Sugano M, Tsujita A, Yamasaki M, Yamada K, Ikeda I and Kritchevsky D.1997.Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats.J Nutr Biochem.8:38-43.

Suksombat W and Chullanandana K.2008.Effects of soyean oil or rumen protected conjugated linoleic acid supplementation on accumulation of conjugated linoleic acid in dairy cows` milk.Asien-Aust J Anim Sci.21:1271-1277.
Terré M, Nudda A, Boe F, Gaias G and Bach A.2011.Performance, immune response and fatty acid profile in lambs supplemented with a CLA-mixture.Anim Feed Sci Tech.165:1-7.
Truitt A, McNeill G and Vanderhoek JY.1999.Antiplatelet effects of conjugated linoleic acid isomers.BBA -Mol Cell Biol L.1438:239-246.
Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S and Ezaki O.2000.Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice.Diabetes.49:1534-1542.
Tyrrell HF and Reid JT.1965.Prediction of the Energy Value of Cow's Milk.J Dairy Sci.48:1215-1223.
von Soosten D.2011.personal communication.Braunschweig (Germany)
von Soosten D, Meyer U, Weber EM, Rehage J, Flachowsky G and Dänicke S.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.94:2859-2870.
Voorrips LE, Brants HA, Kardinaal AF, Hiddink GJ, van den Brandt PA and Goldbohm RA.2002.Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer.Am J Clin Nutr.76:873-882.
White SL, Bertrand JA, Wade MR, Washburn SP, Green J and Jenkins TC.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.
Wlodarek J, Jaskowski JM, Nowak W, Mikula J and Olechnowicz J.2011.Development of ovarian follicles, quality of oocytes and fertility of cows in view of a negative energy balance in the transition period.Med Weter.67:219-223.
Wynn RJ, Daniel ZCTR, Flux CL, Craigon J, Salter AM and Buttery PJ.2006.Effect of feeding rumen-protected conjugated linoleic acid on carcass characteristics and fatty acid composition of sheep tissues.J Anim Sci.84:3440-3450.
Yamasaki M, Mansho K, Mishima H, Kasai M, Sugano MTH and Yamada K.1999.Dietary effect of conjugated linoleic acid on lipid levels in white adipose tissue of Sprague-Dawley rats.Biosci Biotech Bioch.63:1104-1106.
Yamasaki M, Chujo H, Hirao A, Koyanagi N, Okamoto T, Tojo N, Oishi A, Iwata T, Yamauchi-Sato Y, Yamamoto T, Tsutsumi K, Tachibana H and Yamada K.2003a.Immunoglobulin and cytokine production from spleen lymphocytes is modulated in C57BL/6J mice by dietary cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid.J Nutr.133:784-788.
Yamasaki M, Ikeda A, Oji M, Tanaka Y, Hirao A, Kasai M, Iwata T, Tachibana H and Yamada K.2003b.Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets.Nutrition.19:30-35.
Zheng HC, Liu JX, Yao JH, Yuan Q, Ye HW, Ye JA and Wu YM.2005.Effects of dietary sources of vegetable oils on performance of high-yielding lactating cows and conjugated linoleic acids in milk.J Dairy Sci.88:2037-2042.

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EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich Eides statt, dass die vorliegende Dissertation „Investigations on the effects of graded levels of rumen protected conjugated linoleic acids on dairy cow performance, fatty acid profile of milk and rumen metabolism" selbständig und nur unter der Verwendung der angegebenen Literatur und Hilfsmittel angefertigt wurde. Die Arbeit lag bisher in gleicher oder ähnlicher Form keiner Prüfungsbehörde vor.

Halle/Saale, den 02.12.2011 ………………………………

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