Institut für Tierernährung
des Bundesforschungsinstitutes für Tiergesundheit
des Friedrich-Loeffler-Institutes
&
Institut für Agrar- und Ernährungswissenschaften
der Naturwissenschaftlichen Fakultät III
der
Martin-Luther-Universität Halle-Wittenberg

Investigations on the effects of conjugated linoleic acids on performance, body composition, body mass mobilization, energy utilization and fatty acid composition of different tissues in early lactation dairy cows

Dissertation

zur Erlangung des akademischen Grades
doctor agriculturarum (Dr. agr.)

vorgelegt von
Master of Science (agr.) **Dirk von Soosten**
geb. am 13.04.1983 in Bremerhaven

Gutachter: Prof. Dr. Dr. Sven Dänicke
Prof. Dr. Dr. Helga Sauerwein
PD Dr. Holger Kluth

Verteidigung am: 29.10.2012

Halle/Saale 2012

gewidmet

Meiner Frau(ke)

Abbreviations

ACC	acetyl CoA carboxylase
ADF	acid detergent fiber
AIA	acid insoluble ash
ATP	adenosine triphosphate
AUC	Area under the curve
BCS	body condition score
BFT	back fat thickness
BF_3	boron trifluoride
BHBA	β-hydroxybutyrate
BW	body weight
C	carbon
CLA	conjugated linoleic acids
CON	control
CP	crude protein
*c*9,*t*11	*cis*-9,*trans*-11
DIM	days in milk
DM	dry matter
DMI	dry matter intake
EBM	empty body mass
EBW	empty body weight
EE	energetic efficiency
E_G	energy in body mass
E_L	milk energy
E_M	maintenance energy
FAME	fatty acid methyl ester
FAS	fatty acid synthase
FFDM	fat free dry matter
FLI	Friedrich-Loeffler-Institute
GC	gas chromatography
GfE	Gesellschaft für Ernährungsphysiologie
HP	heat production
IG	initial group

IGF-I	insulin-like-growth factor I
k_l	efficiency of utilisation of metabolizable energy for lactation
$kg^{0.75}$	metabolic body mass
LAVES	Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit
LPL	lipoprotein lipase
LSmeans	least square means
ME	metabolizable energy
MEI	metabolizable energy intake
ME_M	metabolizable energy requirement for maintenance
MFD	milk fat depression
mRNA	messenger ribonucleic acid
MS	measurement site
MUFA	monounsaturated fatty acids
N	nitrogen
NDF	neutral detergent fiber
NEB	negative energy balance
NEFA	nonesterified fatty acids
NE_L	net energy for lactation
NE_M	net energy for maintenance
PEBE	proportion of empty body energy
PMR	partial mixed ration
PPAR γ	peroxisome proliferator-activated receptor γ
PUFA	polyunsaturated fatty acids
RE	retained energy
RSD	residual standard deviation
SFA	saturated fatty acids
s.c.	subcutaneous
SCC	somatic cell count
SE	standard error
SEM	standard error of the means
SREBP	sterol regulatory element binding protein
TG	triglyceride
*t*10,*c*12	*trans*-10,*cis*-12

UCP2	uncoupling protein 2
VDLUFA	Verband Deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten
VLDL	very low density lipoproteins
wk	week/ weeks

Tables

Paper III

Gerneral discussion

Figures

Background

Paper I

Paper II

Gerneral discussion

Contents

1. Introduction

The average annual milk yield of German dairy cows is growing steadily and was 7050 kg in 2010 (ADR, 2011). Nevertheless, milk yields higher than 10,000 kg per year are becoming the rule rather than the exception. The steadily increasing milk yields of dairy cows result in the intensification of periods of negative energy balance (**NEB**) after calving. The slow rise of energy intake from dry matter in relation to the rapidly increasing energy demands for milk yield are responsible for this critical period of NEB. The dairy cow counters this NEB with a mobilization of body reserves, mainly in the form of body fat (Flachowsky et al., 2009).

Excessive fat mobilization in periods of NEB is the reason for metabolic disorders caused by triglyceride (**TG**) accumulation in the liver. This process preceded ketosis and is associated with health problems and a decreased reproductive performance of dairy cows (Drackley, 1999). The economic losses for dairy farmers due to these health problems could be within a considerable range. Therefore, in the last two decades dairy research focused on the critical period of early lactation and on the development of nutritional strategies to reduce the NEB and body fat mobilization in early lactation. Approaches for those nutritional strategies are, for example, restricted feeding during the dry period to improve dry matter intake *post partum*. Another approach is the addition of dietary fat to increase the energy density of dairy cow rations and achieve an increased energy intake during the period of NEB. Furthermore, the glycogenic precursor propylene glycol is recommended to improve the glucose and thereby the energy supply of the dairy cow (Overton and Waldron, 2004).

A new approach to counteract the NEB emerges from the finding that conjugated linoleic acids (**CLA**) induce milk fat depression (**MFD**). The energy demands for milk synthesis decrease due to a reduced energy requirement for daily milk fat synthesis and milk fat excretion. Currently it is not clear whether a relief of the dairy cows' energy metabolism occurs as a consequence of the spared energy. No experiments were carried out investigating the effects of CLA induced MFD on the changes in body composition of dairy cows during early lactation. Especially the consequences of the CLA induced MFD, which is paralleled by secretion of milk with lower energy content, on the entire energy metabolism of the dairy cows and on depot-specific energy-partitioning processes have not been completely understood thus far. An understanding of these processes could more specifically help to influence the energy metabolism of the high yielding dairy cow in the period of NEB.

2. Background

2.1 Conjugated linoleic acids and fat metabolism

The discovery that CLA reduced milk fat synthesis leads to great attention for research on CLA effects in dairy cows. Conjugated linoleic acids are derived from linoleic acid and are a group of octadecadienoic fatty acids containing two conjugated double bonds. These conjugated double bonds could be located along the carbon chain (e.g. at the carbon atoms 9 and 11 or 10 and 12). In conformity with the geometric position of the hydrogen atom at the carbon atoms connected with double bonds, configurations of *cis*/*cis*, *trans*/*trans*, *cis*/*trans* and *trans*/*cis* isomers are possible (Bauman et al., 1999). For ruminants the *cis*-9,*trans*-11 (***c*9,*t*11**) and *trans*-10,*cis*-12 (***t*10,*c*12**) CLA isomers are most important (Figure 1).

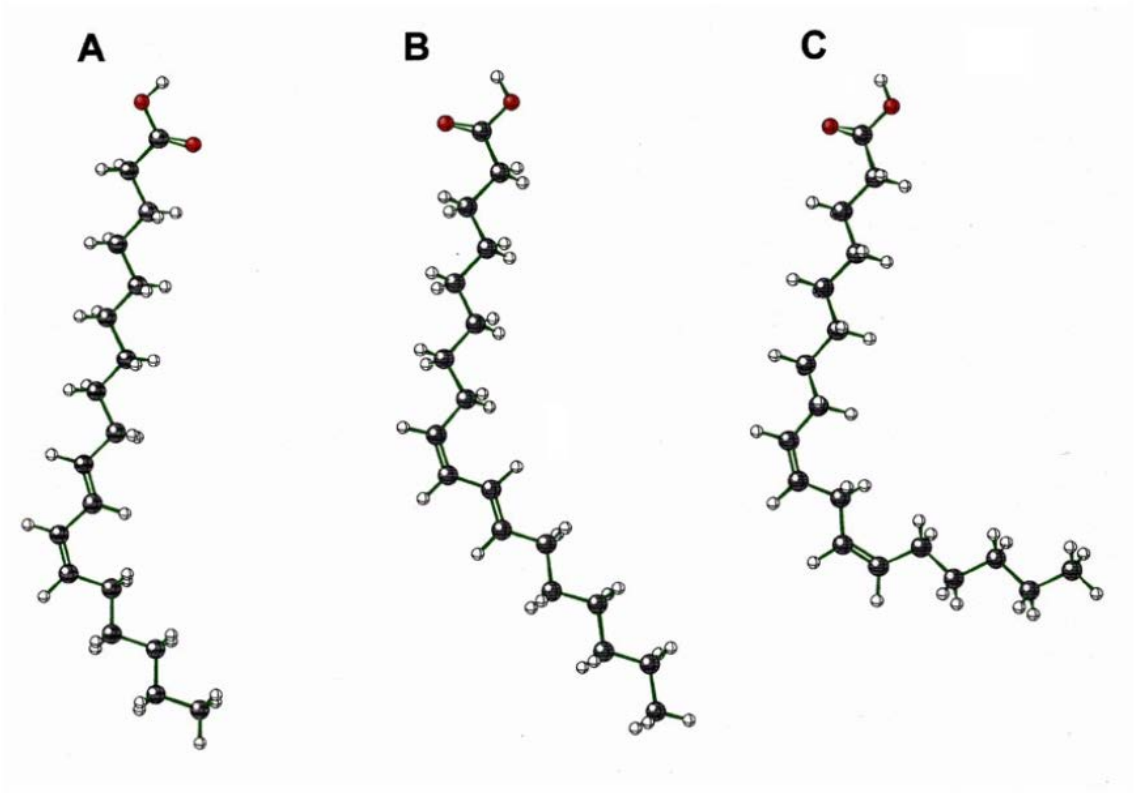

Figure 1. Chemical structure of conjugated linoleic acid isomers and linoleic acid. Fatty acids are *trans*-10,*cis*-12 octadecadienoic acid (A), *cis*-9,*trans*-11 octadecadienoic acid (B) and *cis*-9,*cis*-12 octadecadienoic acid (linoleic acid) (C) (Bauman et al., 1999).

In ruminant meat the CLA content ranges from 0.3-0.5% up to 1 % CLA of total fatty acids (Griinari and Bauman, 1999). In milk fat of dairy cows in bulk raw milk samples the CLA content varied from 0.26 to 1.14% and was influenced by the production system (Jahreis et al., 1997). In ruminant milk fat and meat the *c*9,*t*11 CLA isomer accounted for 70% and 90%

of total CLA, respectively. Therefore *c*9,*t*11 CLA is the major CLA isomer in ruminant products (Chin et al., 1992).

The high *c*9,*t*11 CLA amounts in ruminant fat originates from ruminal biohydrogenation of linoleic acid or from endogenous synthesis in different tissues of the animal (Bauman et al., 1999). During ruminal biohydrogenation of linoleic acid (*c*9,*c*12 C18:2) to stearic acid (C18:0) the *c*9,*t*11 CLA isomer is generated as an intermediate of this process. The enzyme linoleate isomerase associated to different bacterial species like *Butyrivibrio fibrisolvens* (Kepler and Tove, 1967) or other rumen bacteria (Hartfoot and Hazlewood, 1988) is responsible for transformation of the *c*12 double bond into the *t*11 double bond, which is conjugated to the *c*9 double bond. The next step of biohydrogenation is the hydrogenation of *c*9,*t*11 CLA to *t*11 C18:1 (vaccenic acid). Finally vaccenic acid is hydrogenated to stearic acid (Bauman et al., 1999). For endogenous synthesis of *c*9,*t*11 CLA the enzyme Δ^9-deaturase uses vaccenic acid as a precursor. Corl et al. (2001) demonstrated that 78% of *c*9,*t*11 CLA in milk fat originated from endogenous synthesis in the mammary gland.

The second important CLA isomer for ruminants is the *t*10,*c*12 CLA isomer, which could be also generated as an intermediate during the ruminal biohydrogenation of linoleic acid to stearic acid (Lee and Jenkins, 2011). The amount of synthesis of this isomer is dependent on feeding of the dairy cow. Higher content of linoleic acid in combination with a high proportion of concentrate in the ration resulted in a higher production of *t*10,*c*12 CLA isomer from biohydrogenation in the rumen (Flachowsky et al., 2006).

In lactating ruminats postruminal infusion studies demonstrated the capability of CLA mixtures containing the *t*10,*c*12 CLA isomer to reduce milk fat synthesis (Loor and Herbein, 1998, Chouinard et al., 1999). The postruminal infusion of only *t*10,*c*12 CLA provides the evidence that this isomer is responsible for MFD, whereas the infusion of *c*9,*t*11 CLA did not affect milk fat synthesis (Baumgard et al., 2000). Furthermore, the *t*9,*c*11 CLA isomer (Perfield et al., 2007) and the *c*10,*t*12 CLA isomer (Saebo et al., 2005) reduced milk fat synthesis. A mixture of abomasally infused C18:1 fatty acids (*c*9, *c*12, *t*10, *t*11 and *t*12) reduced milk fat content but only half as much as the *t*10,*c*12 CLA isomer (Shingfield et al., 2009). However, Lock et al. (2007) observed no effect of *t*10 C18:1 on milk fat synthesis. De Veth et al. (2004) demonstrated by using data of seven abomasal *t*10,*c*12 CLA infusion experiments a dose dependent milk fat reduction of *t*10,*c*12 CLA supplementation and the nadir of this reduction in milk fat synthesis ranging from 40 to 50% at maximal abomasally infused dosage of 6g/d. In their review Bauman and Griinari (2003) described the mechanisms involved in CLA induced MFD. The activity of key enzymes necessary for

mammary lipid synthesis is reduced. For fatty acid uptake and the transport of fatty acids the enzyme lipoprotein lipase (**LPL**) and fatty acid binding proteins are responsible. For de novo fatty acid synthesis acetyl CoA carboxylase (**ACC**) and fatty acid synthase (**FAS**) are the important enzymes. The *t*10,*c*12 CLA does not affect these enzymes directly, rather a CLA influence on the transcription factors for these enzymes is supposed. These transcription factors are the peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element binding protein (**SREBP**).

Furthermore, in experimental animal models CLA showed anticarcinogenic, antiatherosclerotic, antidiabetogenic, immunomodulatory and antiadipogenic effects (Belury, 2002). In addition to this and besides to the MFD, further CLA effects on fat metabolism are known. In growing mice the body fat content was reduced after CLA supplementation (Park et al., 1997, West et al., 1998) and studies feeding pure *t*10,*c*12 CLA to growing and lactating mice demonstrated, that this body fat lowering ability is related to the *t*10,*c*12 CLA isomer (Park et al., 1999, Clément et al., 2002, Loor et al., 2003). Especially the fat depots are affected by CLA supplementation. West et al. (2000) observed in mice a reduction of the retroperitoneal, epididymal and inguinal fat depot. For these fat depots and the mesenteric fat depot Delany et al. (1999) showed a similar reduction. In both studies supplemented CLA preparations contained the *t*10,*c*12 and *c*9,*t*11 CLA isomer in equal amounts. In studies supplementing only the *t*10,*c*12 isomer it was shown that the reductions of fat depots was caused by the *t*10,*c*12 CLA isomer (Tsuboyama-Kasaoka et al., 2000, Jaudszus et al., 2010). In these studies high experimental dosages in the range of 0.5 up to 1.0% CLA in the diet were used and the reduction of the fat depots was accompanied by the development of liver steatosis. The body fat reducing ability of CLA was also observed in pigs (Ostrowska et al., 1999) and men (Smedman and Vessby, 2001). The mechanisms how CLA reduces body fat content are not fully understood. In their review Wang and Jones (2004) proposed several possible mechanisms. The uncoupling protein 2 (**UCP2**) uncouples the oxidative phosphorylation from adenosine triphosphate (**ATP**) synthesis and resulted in a higher production of heat. In CLA fed mice the messenger ribonucleic acid (**mRNA**) amounts of UCP2 in fat tissue were increased and a higher heat production (**HP**) accounted for the decreased adipose deposition. Therefore the reduced adipose deposition could be associated with an increased energy expenditure or heat production. In line with the reduction of milk fat synthesis, a further mechanism could be the lower activity of enzymes involved in fatty acid synthesis. Analogical to the CLA effect on milk fat synthesis the activity of the enzymes LPL, FAS and ACC could be influenced by CLA in a similar way.

2.2 Fat and energy metabolism in early lactation

The dairy cow experiences massive metabolic changes during the transition period. In this time the physiological stage of the cow changes from pregnancy to lactation and the mammary gland, liver and adipose tissue experience metabolic changes particularly with regard to the lipid and energy metabolism (Table 1). These adaptations in metabolism serve to supply the tremendous required amounts of nutrients and energy for milk production. In late pregnancy energy is prevailing partitioned to the conceptus and for replenishment of body reserves. With onset of lactation the nutrients are partitioned to the mammary gland for milk synthesis. This adaptation of nutrient partitioning during the transition period is named as homeorhesis (Bauman and Currie, 1980).

Table 1. Selected physiological adaptations which occur in lactating dairy cows (Bauman, 2000)

Tissue	Metabolic change
Mammary tissue	Increased number of secretory cells
	Increased nutrient utilization
	Increased supply of blood
Liver	Increased size
	Increased rates of gluconeogenesis
	Increased glycogen mobilization
	Increased protein synthesis
Adipose tissue	Decreased de novo fat synthesis
	Decreased uptake of preformed fatty acids
	Decreased re-esterification of fatty acids
	Increased lipolysis

In early lactation the energy demands of the mammary gland for milk synthesis and the requirements for maintenance are markedly higher than the available energy from dry matter intake (**DMI**). At 4 d *post partum* the net energy for lactation ($\mathbf{NE_L}$) of the dairy cow exceeded intakes by 26%. The energy amount used for milk production at this time is conforming to 97% of the energy intake. These enormous energy costs for milk production resulted in a NEB (Drackley, 1999). The *post partum* energy deficit in high yielding Holstein-Friesian cows measured with indirect calorimetry at 42 days in milk (**DIM**) and 84 DIM was -21 MJ/d and -6 MJ/d, respectively. The energy balance turned into the positive range between

84 and 126 DIM (Hattan et al., 2001). In their review Bauman and Currie (1980) showed that cows did not reach a positive energy balance before day 112 of lactation.

For compensation of the energy deficit in early lactation the dairy cow mobilizes body reserves to provide additional energy for milk production (Moe et al., 1971). The body fat mobilization in this time determined in different studies with different methods ranged from 16 kg to 54 kg (Table 2). Neglecting the different observation periods the data showed an average body fat mobilization of 38 kg. This amount is one-third of the whole body fat of the animals at start of the experiments.

Table 2. Body fat mobilization in early lactation dairy cows

Author	Animals[1]	Method[2]	Time[3]	Body fat[4]	Body fat mobilization	
				(kg)	(kg)	(%)[5]
(Andrew et al., 1994)	pp (n = 17)	cs	-7 to 63	90	42	47
(Chilliard et al., 1991)	pp (n = 23)	D_2O	1 to 56	100	30	30
(Komaragiri and Erdman 1997)	pp (n = 20)	D_2O	-14 to 35	143	54	38
(Komaragiri et al., 1998)	pp (n = 22)	D_2O	-14 to 35	159	46	29
(Bath et al., 1965)	p (n = 12)	cs	1 to 49	65	16	25

[1] pp = pluriparous; p = primiparous

[2] cs = comparative slaughter; D_2O = D_2O dilution technique

[3] Days relative to parturition

[4] Body fat content at start of the experiment

[5] Proportion of mobilized body fat from body fat at start of the experiment.

The variation of body fat mobilization between the studies could be explained by the different performance level of the animals used in the experiments. The average daily milk yield in the studies by Komaragiri and Erdman (1997) and Komaragiri et al. (1998) was 41 kg. This is markedly lower as in the studies by Andrew et al. (1994) and Chillard et al. (1991) with a performance level of 32 kg and 30 kg milk per day. Additionally, the different methods used and the variation in time points estimating the body composition have to be considered by interpretation of body fat mobilization data.

This occurrence of massive lipid mobilization in periods of NEB in dairy cows is the result of an increased lipolysis in the adipose tissue. The stored triglycerides in the adipose tissue are hydrolysed to glycerine and fatty acids and were released to blood circulation from the adipose tissue. Besides the increased lipolysis the body fat mobilization also resulted from

decreased lipid synthesis in the adipose tissue. The de novo fatty acid synthesis from acetate, the uptake of fatty acids from circulating lipoproteins and the esterification of these fatty acids into triglycerides is reduced in periods of NEB (Bauman and Currie, 1980).

Principal characteristics of fat metabolism in adipose tissue, liver and mammary gland is shown in Figure 2. The consequence of the increase in lipolysis and reduction of lipid synthesis in adipose tissue are elevated blood plasma nonesterified fatty acids (**NEFA**) concentrations around parturition (Adewuyi et al., 2005). The NEFA are taken up by the liver, but uptake of NEFA exceeded the oxidation and release of lipids from liver as very low density lipoproteins (**VLDL**). Therefore the NEFA were esterified as TG and were accumulated in the liver (Grummer, 1993).

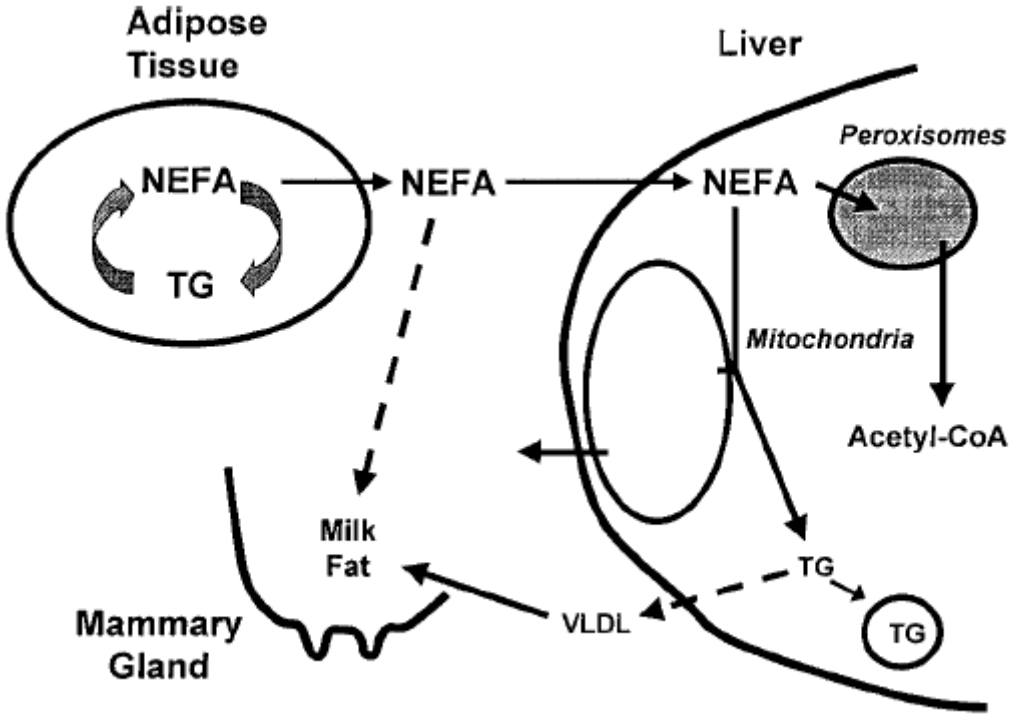

Figure 2: Relationships of lipid metabolism between adipose tissue, liver and mammary gland (Drackley, 1999). TG = triglyceride, VLDL = very low density lipoproteins, NEFA = nonesterified fatty acids

To a small extent the lipid accumulation in liver hepatocytes is not detrimental to the dairy cow's health. However, is the lipid accumulation, as a consequence of severe NEB, massive mobilization of body fat, and high NEFA uptake in the liver, reaching the limit of NEFA oxidation and release, an increased TG accumulation and the synthesis of ketone bodies is the consequence. This metabolic disorder is responsible for health problems in the dairy cow (Goff and Horst, 1997). In his review Grummer (1993) concluded that hepatic lipidosis preceded ketosis and is related to decreased health status and reproductive performance.

Furthermore, the gluconeogenic capacity is reduced indirectly by decreased ureagenesis via increased hepatic lipid accumulation (Drackley et al., 2001).

2.3 CLA induced milk fat depression in early lactation dairy cows

In the mammary gland the lipid synthesis is on a high level in early lactation and requires the greatest proportion of energy. According to Tyrrell and Reid (1965) the energy in milk fat accounted for 50% of milk energy. Therefore milk fat synthesis is the main factor for the higher energy demands in the early lactation dairy cow. Knowing about the milk fat reduction capability of *t*10,*c*12 CLA, the idea grows to feed this isomer to induce milk fat depression for reduction of milk energy output. The assumption was that the spared energy expense for milk fat synthesis resulted in a less negative energy balance and an earlier change into a positive energy balance during early lactation. In trials using commercial CLA preparations containing equal amounts of *t*10,*c*12 and *c*9,*t*11 CLA milk fat content was reduced in early lactation (Bernal-Santos et al., 2003, Pappritz et al., 2011b). Additionally, the milk fat yield was decreased in studies by Castaneda-Gutierrez et al. (2005) and Odens et al. (2007). However, the calculated energy balance was not improved in the CLA groups. As described above in the first weeks of lactation the cows are in a NEB and mobilize the equivalent amount of the energy deficit from body mass (mainly from body fat) to balance the energy deficit. The absence of improvement for the calculated energy balance suggested that CLA does not influence the mobilization of body fat in early lactation. With the commonly used indicators for body mass mobilization body weight (**BW**), body condition score (**BCS**) and back fat thickness (**BFT**) an assessment for the real mobilized fat and protein mass for calculation of the actually mobilized energy from body reserves is inexact. The data for body fat mobilization estimated in different studies, which are presented above (Table 2), showed that measuring the mobilization of body reserves in early lactation still is a difficult ascertainable field in research with early-lactation dairy cows.

3. Scope of the thesis

The background shows that an enormous gap of knowledge exists, how the CLA induced milk fat depression effects body mass mobilization in early lactation. The partitioning of energy spared from milk fat synthesis and excretion towards body reserves is unclear. Additionally the effect of CLA feeding on body composition, body fat depots and liver is less examined in dairy cows. Furthermore the transfer of the supplemented CLA into the dairy cows' body is not clarified. Therefore the aims of this thesis were to investigate the following topics:

1. The effects of CLA supplementation on milk energy concentration, milk energy output and calculated energy balance. *(Paper I)*

2. The influence of CLA supplementation on the weights of liver and fat depots in early-lactation dairy cows. *(Paper I)*

3. The effect of CLA supplementation on body composition with special regard to body fat and protein mobilization or accretion during early lactation. *(Paper II)*

4. The impact of CLA supplementation on energy metabolism and energy utilization in early lactation. *(Paper II)*

5. The influence of CLA supplementation on fatty acid composition of the dairy cows tissues and the transfer of *t*10,*c*12 CLA into different fat depots and body tissue fractions of the dairy cow. *(Paper III)*

For investigation of these topics a slaughter experiment with 25 primiparous German Holstein heifers was conducted by using the principle of the comparative slaughter technique. The experiment started at 1 DIM with the slaughter of five animals of an initial group (**IG**) receiving no CLA supplementation. These animals were slaughtered to obtain baseline values for calculation of body composition at the start of the experiment, of the actually later slaughtered animals, in the different treatment groups. After 42 DIM on a CLA-supplemented (CLA) or control diet (CON) five more cows were slaughtered from each feeding treatment (42/CLA and 42/CON). The remaining five cows on each feeding treatment were slaughtered after 105 DIM (105/CLA and 105/CON). The animals of the CLA groups consumed 6.0 g/d

of the *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA. In the CON diet a control fat supplement was included and the CLA isomers were substituted by stearic acid. With the body composition of the animals at the end of the treatment period and the calculated start body composition, a calculation of body mass mobilization during the different treatment periods was possible. In the following three publications the results of the experiment with regard to the above mentioned topics are presented. Subsequently, in the General discussion these results are subjected to an overlapping discussion.

4. Paper I

Effect of *trans*-10,*cis*-12 conjugated linoleic acid on performance, adipose depot weights and liver weight in early-lactation dairy cows.

D. von Soosten*, U. Meyer*, E. M. Weber†, J. Rehage†, G. Flachowsky* and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Clinic for Cattle, School of Veterinary Medicine Hanover, 30173 Hanover; Germany

Journal of Dairy Science
(2011)
94 :2859–2870

ABSTRACT

In feeding practice conjugated linoleic acid (CLA) supplements are used to decrease milk fat excretion in early lactation dairy cows to save energy to counteract the physiological negative energy balance. The present study was conducted to examine the effects of CLA on energy metabolism, changes in liver weight and the weight of different adipose depots during early-lactation. Primiparous lactating German Holstein cows (n = 25) were divided into 5 groups and each group contained 5 animals. The experiment started 21 d prepartum and continued until 105 d in milk (DIM). Cows were slaughtered at 1, 42 and 105 DIM. The experiment was divided into a prepartum period (21 d prepartum until calving), period 1 (1 until 42 DIM) and period 2 (> 42 until 105 DIM). In the prepartum period all animals were housed together and fed the same diet with no CLA supplementation. At 1 DIM an initial group, with no CLA supplementation, was slaughtered. The 20 remaining cows were assigned to 2 diets. One group received 100 g/d of a control fat supplement (CON; n = 10) and the other group 100 g/d of a CLA supplement (CLA; n =10) from 1 DIM until slaughter. Five cows of each feeding group were slaughtered after 42 DIM and the remaining animals after 105 DIM. The CLA supplement contained approximately 10% each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA. During the slaughter process the empty body weight was recorded and the omental, mesenteric, retroperitoneal and subcutaneous (s.c.) adipose depots, as well as the liver, were dissected and weighed. The CLA treatment decreased milk fat content in period 1 (14.1%). In period 2, milk fat content (25.4%) and yield (17.1%) were lower in the CLA group. No effect of CLA on milk yield. The net energy intake, milk energy output, and the calculated energy balance remained unchanged by CLA supplementation. No effect of CLA on the weights of liver, omental, mesenteric or s.c. adipose depots when related to empty body weight. Liver weight increased with DIM, whereas the retroperitoneal adipose depot weight decreased at the same time. Compared with the initial group, the retroperitoneal adipose depot weight for control animals slaughtered after 42 DIM was decreased (47.7%), however, for the CLA group slaughtered after 42 DIM a trend to a lower retroperitoneal adipose depot weight (34.0%) was observed. This suggests a CLA-induced deceleration of mobilization of the retroperitoneal adipose depot during the first 42 DIM.

Key words: conjugated linoleic acid, dairy cow, adipose depot, energy balance

INTRODUCTION

High energy demands of the mammary gland in early lactation of dairy cows with concomitant inadequate DMI lead to a negative energy balance (Bell, 1995). Milk fat represents 50 % of the total milk energy and is the greatest proportion of milk energy output (Tyrrell and Reid, 1965). Thus, a decrease in milk fat excretion provides the opportunity to save energy. The *trans*-10, *cis*-12 conjugated linoleic acid (**CLA**) isomer induces milk fat depression (**MFD**) by inhibiting the synthesis of milk fat (Baumgard et al., 2000). During MFD, milk fat content and yield are decreased and CLA is used with the intention to save energy and improve the energy balance. Several studies demonstrated that in early lactation, milk fat is reduced, but milk energy output is not affected due to the propensity to increase milk yield. Energy is repartitioned to milk yield and CLA has mostly no effect on calculated energy balance (Bernal-Santos et al., 2003; Bauman et al., 2008). For the duration of a negative energy balance the mobilization of body reserves from adipose tissue is needed to cover the energy requirements (Bauman and Currie, 1980). The effects of the *trans*-10, *cis*-12 CLA isomer on adipose depot weights in dairy cows are not clear. Current studies on cows during MFD showed that the *trans*-10 *cis*-12 isomer increases abundance of mRNA for the expression of lipid synthesis genes of key enzymes involved in lipid synthesis in adipose depots (Harvatine et al., 2009). Then again, studies on mice indicated that the *trans*-10, *cis*-12 isomer decreases adipose depots and enlarges the liver (Delany et al., 1999; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002; Degrace et al., 2003; Wang and Jones, 2004; Jaudszus et al., 2010). These different results could be linked with the different contribution of the liver and adipose depots to fatty acid synthesis in ruminants and rodents. Furthermore the ruminant has other major sources of carbon (**C**) for fatty acid synthesis, like acetate from ruminal fermentation (Pearce, 1983). These known differences in fat metabolism of ruminants and rodents make it necessary to investigate the development of adipose depots and liver weights under CLA-induced MFD during early lactation in dairy cows. Therefore, the present study was conducted to examine the effects of CLA supplementation on milk production, energy partitioning and the weights of liver and adipose depots in early-lactation dairy cows.

MATERIALS AND METHODS

Animals, Experimental Design and Feeding

In compliance with the European Union Guidelines concerning the protection of experimental animals, the study was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (**FLI**), Brunswick, Germany, and was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany (File Number 33.11.42502-04-071/07). To exclude possible effects from preceding lactations on adipose depots and the liver, only primiparous cows were used in the present trial. Twenty-five late pregnant heifers of the German Holstein breed were assigned to five groups with different slaughter times and feeding treatments. Four sires were involved in breeding of the animals used. Twenty animals originated from the same father and were evenly distributed over the 5 groups. All cows were born at the experimental station of the Institute of Animal Nutrition (FLI) and reared under the same conditions (equal housing and feeding). The first parturition was at an average age of 23 ± 0.2 months. The experiment started 21 d prepartum and continued until 105 DIM. The animals were assigned to the 5 groups by considering the BW measured three weeks (**wk**) before expected parturition. The experiment was divided into 3 periods according to the slaughter times at 1, 42 and 105 DIM. The prepartum period (21 d prepartum until calving), period 1 (1 DIM until 42 DIM) and period 2 (> 42 DIM until 105 DIM). At the first slaughter time, 5 animals of an initial group (IG) were slaughtered, whereas 20 animals were assigned to a control (CON) or CLA-supplemented (CLA) diet. The CLA supplementation started at 1 DIM and continued until the slaughter. After 42 DIM, 5 animals of the control (42/CON) and CLA (42/CLA) group were slaughtered. The remaining five animals of the control (105/CON) and CLA (105/CLA) group were slaughtered after 105 DIM. For financial reasons and the experimental capacity the number of animals was restricted to 5 cows in each group due to the expensive and labour intensive slaughter.

All animals were housed in group pens in a free stall barn equipped with slatted floors and stalls covered with rubber mattresses. The possibility to feed concentrate over a computerized concentrate feeding station existed in each group pen (type RIC, Insentec, B.V., Marknesse, The Netherlands). The diets were formulated to meet the nutritional requirements of dairy cows stated by the German Society of Nutrition Physiology (GfE, 2001). In the prepartum period all animals were housed together and received a diet consisting of a partial mixed

ration (**PMR**) for ad libitum consumption and 2 kg concentrate/d in pelleted form by a computerized concentrate feeding station (Table 1).

Table 1. Ingredients and chemical composition of the prepartum diet

Variable	Concentrate	PMR[1]
Ingredient, %		
Wheat grain	25	
Barley grain	25	
Soybean meal	20	
Sugar beet pulp, dried	11	
Zeolithe[2]	12.5	
Vitamin/mineral premix[3]	6.5	
Chemical composition		
DM, (g/kg)	894	445
Nutrients, (g/kg of DM)		
Crude ash	155	56
Crude protein	173	89
Ether extract	22	29
ADF	68	256
NDF	157	469
Energy[4]; (MJ/kg DM)		
ME	10.6	11.0
NE_L	6.7	6.7

[1] Partial mixed ration on DM basis (60% corn silage, 40% grass silage on DM basis).
[2] According to Grabherr et al. (2009) cows received 250 g Zeolithe (IMPAG Gmbh, Offenbach, Germany) per d for prevention of hypocalcaemia.
[3] Per kg mineral feed: 105 g of Na; 80 g of P; 60 g of Ca; 50 g of Mg; 7 g of Zn; 5.4 g of Mn; 1,25 g of Cu; 100 mg of I; 40 mg of Se; 30 mg of Co; 800 000 IU of vitamin A; 100 000 IU of vitamin D_3; 1500 mg of vitamin E.
[4] Calculation based on nutrient digestibilities measured with wethers (GfE, 1991) and tabulated values (Universität Hohenheim - Dokumentationsstelle, 1997); Abbreviations: ME = Metabolizable energy, NE_L = Net energy lactation**.**

The 20 animals who received the control or CLA diet after calving were housed in 2 group pens according to the diet. Each pen was equipped with ten self-feeding stations to provide the PMR (type RIC, Insentec, B.V., Marknesse, The Netherlands). In addition, all animals were marked with an ear transponder to record the daily individual feed intake at the feeding stations. The diet fed in period 1 and 2, respectively (Table 2), consisted of a PMR comprising of 25% grass silage (35% *Lolium perenne*, 32% *Festuca pratensis*, 18% *Phleum pratense*, 10% *Poa pratensis*, 5% *Festuca rubra*), 38% corn silage (*Zea mays*) and 37% concentrate (on a DM basis) and was provided for ad libitum intake.

Table 2. Ingredients and chemical composition of the postpartum concentrates and partial mixed ration (PMR[1])

	Concentrate			PMR
Variable	CON	CLA	PMR	
Ingredient, %				
Wheat grain	39.5	39.5	41	
Sugar beet pulp, dried	29	29	30	
Rapeseed meal	20	20	20	
Soybean meal	6.5	6.5	6.5	
Vitamin/mineral premix[2]	2	2	2	
Control fat supplement	2.5			
CLA supplement		2.5		
Calcium carbonate	0.5	0.5	0.5	
Analyzed chemical profile				
DM, (g/kg)	873	871	870	445
Nutrients, (g/kg of DM)				
Crude ash	65	69	64	62
Crude protein	182	180	182	124
Ether extract	50	44	20	28
ADF	134	133	134	208
NDF	259	260	265	405
Energy[3], (MJ/kg of DM)				
ME	13.9	13.7	13.5	11.9
NE_L	8.9	8.7	8.7	7.5
CLA[4]; (g/kg of DM)				
C18:2 *trans*-10, *cis*-12	0.0	1.7	0.0	0.0
C18:2 *cis*-9; *trans*-11	0.0	1.6	0.0	0.0

[1] Partial mixed ration (25% grass silage, 38% corn silage, 37% PMR-concentrate on DM basis).
[2] 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.
[3] Calculation based on nutrient digestibilities measured with wethers (GfE, 1991); Abbreviations: ME = Metabolizable energy, NE_L = Net energy lactation.
[4] Conjugated linoleic acid.

Additionally, 3.5 kg concentrate (on a dry matter (**DM**) basis), which contained the control fat supplement or the CLA supplement, was provided by the computerized concentrate feeding stations in pelleted form and water was available ad libitum. The cows fed the CLA-supplemented diet obtained 100 g/d of a rumen-protected (lipid encapsulation technique) CLA supplement (Lutrell Pure, BASF SE, Ludwigshafen, Germany) and the animals of the control group received 100 g/d of a control fat supplement (Silafat, BASF SE, Ludwigshafen, Germany). The CLA supplement contained 78 % fatty acids with a proportion of approximately 12 % each of the *trans*-10, *cis*-12 CLA isomer and *cis*-9, *trans*-11 CLA isomer. The fatty acid profile provided by the CLA supplement and the control fat supplement are shown in Table 3.

Table 3. Fatty acid profile of fat supplements[1]

Fatty acid, (% of total fatty acids)	CON	CLA
C16:0	10.89	10.89
C18:0	87.30	50.31
C18:1 c9	<0.01	10.66
CLA		
C18:2 c9, t11	0.06	11.99
C18:2 t10, c12	0.02	11.88
Other CLA	0.15	0.95
Other	1.58	3.32

[1] Supplemental CLA was included in the additional concentrate portion (fed by the computerized concentrate feeding station) as a rumen-protected CLA supplement, for the control group conjugated linoleic acids were substituted by stearic acid

The pelleted CLA-concentrate contained 1.7 % of the *trans*-10, *cis*-12 CLA isomer and 1.6 % of the *cis*-9, *trans*-11 CLA isomer (Table 2). That implies animals of the CLA group consumed (calculated based on the analyzed proportion in concentrates) 6.0 g/d of the *trans*-10, *cis*-12 CLA-isomer and 5.7 g/d *cis*-9, *trans*-11 CLA isomer. In the control fat supplement these isomers were substituted by stearic acid.

Measurements and Sample Collection

In the prepartum period pooled samples of grass silage, corn silage, and the concentrate were taken over 4 weeks. Partial mixed ration samples in the postpartum period were collected 4 times per wk directly after feeding from each trough and pooled over approximately 4 wk. Samples of the concentrates were taken once per wk and composited monthly. The composition of the PMR was adjusted daily based on changes in DM content of grass and maize silage. In the prepartum period the daily DMI could not be recorded for technical reasons. In accordance with the different slaughter times (postpartum), in period 1 data are available for 10 animals each in the CLA and CON group. In period 2, data from 5 animals are available for each treatment. Milking took place twice a day at 0530 and 1530 h. Meanwhile, milk yield was recorded with automatic milk counters and BW was automatically recorded when leaving the milking parlor. Milk samples were taken twice per wk and stored at 4°C until analysis. From 21 d prepartum until the slaughter after 42 DIM and immediately before the slaughter after 105 DIM, blood samples were drawn from the vena jugularis once per wk after the morning milking. Contemporaneously, BCS was recorded using a 5-point scale (Edmonson et al., 1989). Back fat thickness (**BFT**) was determined 21 d before expected parturition, at 1 DIM, after 21 DIM, and before slaughter, applying ultrasound (Staufenbiel, 1997). Mastitis was documented and 3 animals of the control group and 4 of the CLA group

were diseased in the first wk of lactation. The weak alignment and recovery within a few days of the health complications did not make it necessary to eliminate data from analysis.

The slaughter took place in the slaughter facilities at the experimental station of the Institute of Animal Nutrition, FLI, next to the animals' freestall barn. The slaughter process was carried out in accordance with the methods described by Janssen (2006). After the morning milking, the cow was transported to the slaughter facilities, weighed, stunned with a captive bolt gun and exsanguinated. Blood was collected and weighed. Body parts were weighed as they were dissected from the animal. The head, udder, tail and feet (below carpal joint or hock) were detached first. Claws were sawed off, weighed and removed. The full gastrointestinal tract, urinary and gall bladder were removed. The hide, heart, lung, liver, kidneys, uterus, spleen, thyroid gland, thymus and retroperitoneal adipose depot were dissected and weighed during the slaughter process. After separation of the gastrointestinal tract into reticulo-rumen, omasum/ abomasum and intestine, these parts were weighed full and after opening and cleaning of ingesta. Adherent mesenteric fat at the intestine and the omental adipose depot, which included fat from the reticulo-rumen, omasum and abomasum, were cut off and weighed. The gastrointestinal tract fill was calculated as the difference in weights of the full and cleaned gastrointestinal tract. The carcass was divided longitudinally in 2 symmetric parts, weighed, and stored at 4 °C. The next day carcass parts were weighed once again to determine water loss, and s.c. adipose tissue was dissected manually from the left carcass with a knife and weighed. The whole s.c. adipose tissue weight was calculated by multiplying the weight of the s.c. adipose tissue of the left carcass with the quotient resulting from the weight of both carcass parts and the weight of the left carcass. The empty body weight (**EBW**) is defined as the whole body of the cow without claws off cut, ingesta and content of urinary and gall bladder. It was calculated as the sum of all body parts weights, which were recorded during the slaughter process.

Analyses

The composition of the feedstuffs (DM, crude ash, crude protein (**CP**), ether extract, neutral detergent fibre (**NDF**) and acid detergent fibre (**ADF**)) was determined according to the suggestions of the Association of German Agricultural Analysis and Research Centres (VDLUFA, 1993). The fatty acid profile of fat supplements and feedstuffs was analyzed by extracting the lipid content according to Folch et al. (1957). The lipid extract was incubated with Boron trifluoride (**BF_3**) to produce fatty acid methyl esters (**FAME**), which were purified by thin-layer chromatography (SIL G-25 UV_{254}, MACHERY-NAGEL, Germany)

and quantified by gas chromatography (**GC**) (GC-17A Version 3, Schimadzu, Japan) equipped with an auto sampler and flame ionisation detector. The applied GC procedures were as previously described by Flachowsky et al. (2006).

Milk samples were analyzed for fat, protein, lactose, and urea concentration, and somatic cell count (**SCC**) using an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark).

Blood samples were analyzed for plasma concentrations of glucose, NEFA and BHBA by enzymatic analysis. For glucose, a commercial kit (Glucose Hexokinase Fluid 5+1; MTI Diagnostics, Idstein) containing the enzyme glucose hexokinase was used. NEFA and BHBA were analyzed with commercial kits (NEFA C; WAKO Chemicals GmbH, Neuss and RANBUT, Randox Laboratories GmbH, Wülfrath).

Calculations

Based on the equations published by the German Society of Nutrition Physiology (GfE, 2001) the net energy requirements for maintenance (**NE_M**) and lactation (**NE_L**) as well as milk energy concentration and output were calculated as follows:

$$NE_M \text{ (MJ } NE_L\text{/d)} = 0.293 \times BW^{0.75}$$

$$\text{Milk energy concentration (MJ } NE_L\text{/kg)} = 0.38 \times \text{milk fat (\%)} + 0.21 \times \text{milk protein (\%)} + 0.95$$

$$NE_L \text{ (MJ } NE_L\text{/d)} = [\text{milk energy concentration (MJ } NE_L\text{/kg)} + 0.086] \times \text{milk yield (kg/d)}$$

Fat corrected milk (FCM) was calculated based on the equation of Gaines (1928):

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

The net energy balance was calculated with the following equation:

$$\text{Net energy balance (MJ } NE_L\text{/d)} = \text{energy intake (MJ } NE_L\text{/d)} - [NE_M \text{ (MJ } NE_L\text{/d)} + NE_L \text{ (MJ } NE_L\text{/d)}]$$

The milk energy concentration (MJ NE_L/kg) multiplied with daily milk yield (kg/d) results in daily milk energy output (MJ NE_L/d). Energy intake was calculated by multiplying the daily DMI with the overall energy content of the PMR and the concentrate. The liver and adipose tissue weights were referred to EBW and additionally presented as proportion of EBW in percent for consideration of different EBW of the animals.

Statistical Analyses

All statistical analyses were carried out using the software SAS version 9.1 (SAS Institute Inc., 2004). Data for DMI, milk yield, BW and milk components recorded during the two periods of the trial were reduced to weekly means. These variables plus data for BCS, BFT, NEFA, glucose and BHBA were analyzed as repeated measures using the MIXED procedure of SAS. An autoregressive covariance structure was modelled using week of lactation as the repeated effect. The model contained treatment, week of lactation, and interactions of treatment × week of lactation as the fixed effects. Cows were treated as random effect. Weights of empty body, liver and adipose depots were analyzed using the GLM procedure of SAS. The CLA and control groups at the 42 and 105 DIM slaughter were compared to the initial group with the Dunnett-test. For all analyses, significance was declared when *P*-values were < 0.05 and a tendency was noted when $0.05 < P < 0.10$.

RESULTS

The initial BW recorded 21 d before parturition was 583 kg (± 6) for the IG, 571 kg (± 13) for the 42/CON group, 577 kg (± 27) for the 42/CLA group, 589 kg (± 12) for the 105/CON group and 573 kg (± 22) for the 105/CLA group. The values for BCS, BFT, BHBA, and glucose were similar between the groups in the prepartum period. The NEFA concentration in blood plasma showed no differences in the prepartum period between the groups (Figure 1).

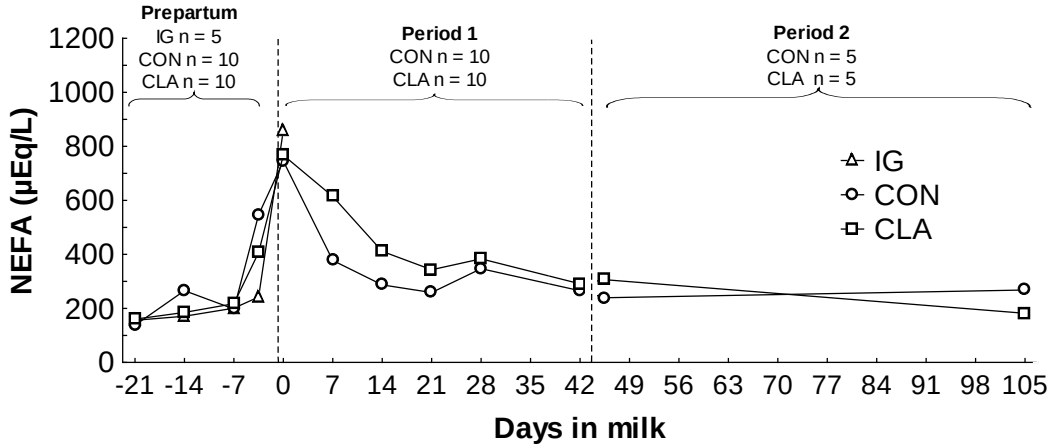

Figure 1. Development of NEFA plasma concentrations (means) during the trial. Prepartum, all animals received the same diet and the curve for the initial group (IG) ended after parturition due to slaughter. Groups did not differ in this period (P = 0.279) and the SEM averaged 21 and ranged from 9 to 23 µEq/L. In period 1, each group consisted of 10 animals and in period 2 are samples of 5 animals available in each group, because of the slaughter after 42 DIM. Cows received from 1 DIM until slaughter 100 g/d of a control fat supplement (CON) or 100 g/d of a CLA supplement (CLA). Animals of the CLA group consumed approximately 6 g *trans*-10, *cis*-12 CLA. In period 1, no differences between the groups were observed (P = 0.171) and the SEM averaged 53 and ranged from 39 to 90 µEq/L. In period 2, no differences between the groups were observed (P = 0.871) and the SEM averaged 41 and ranged from 40 to 43 µEq/L.

In period 1, milk fat content was 14.1% lower for cows receiving CLA (Table 4). Milk fat content was decreased first after 28 DIM and reached a plateau after 49 DIM (Figure 2A). Milk fat yield and milk protein yield were not affected by CLA supplementation in period 1, but a trend for decreased milk protein content in CLA fed cows was observed. Conditional to the mastitis occurrence, the SCC tended to be higher in period 1 in the CLA group (Table 4).

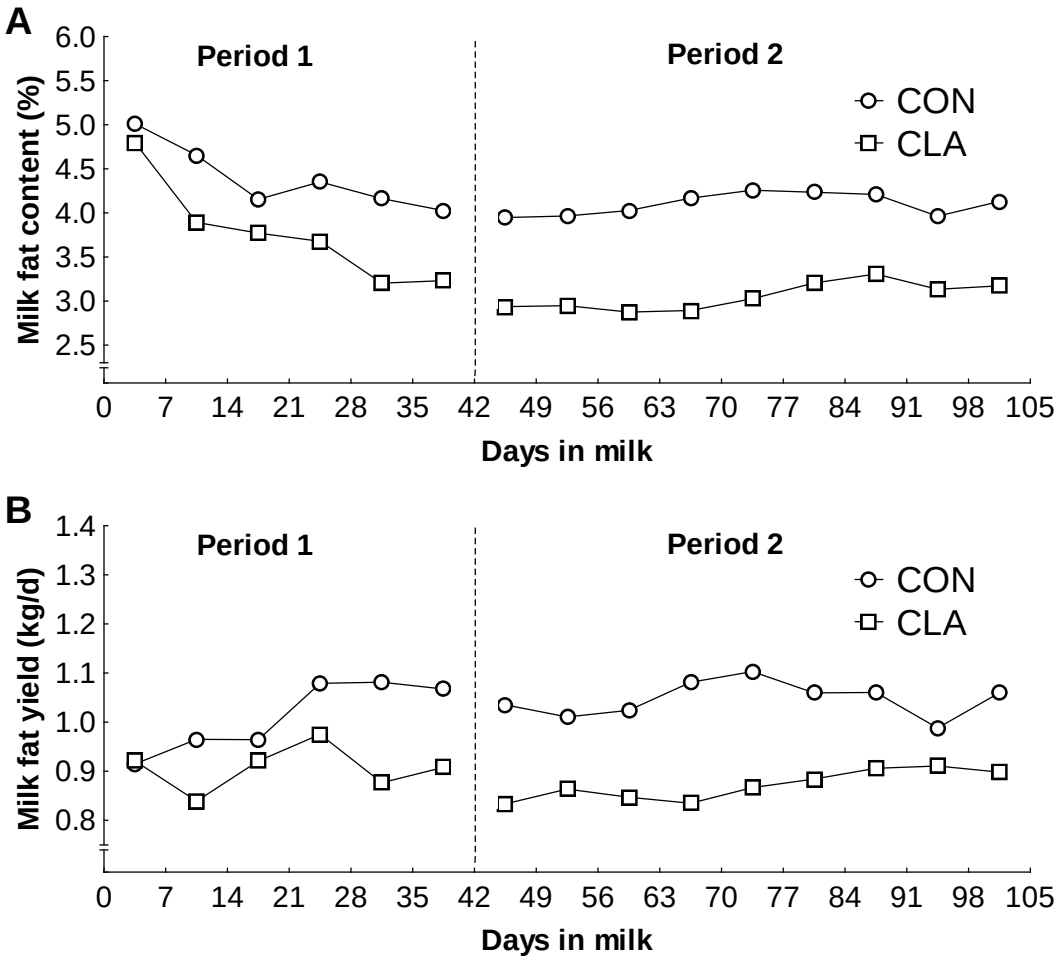

Figure 2. Development of milk fat content (A) and milk fat yield (B) during the two periods of the trial. Cows received 100 g/d of a control fat supplement (CON) or 100 g/d of a CLA supplement (CLA). Animals of the CLA group consumed approximately 6 g/d *trans*-10, *cis*-12 CLA. In period 1 each group consisted of 10 animals and in period 2 of 5 animals, because of the slaughter after 42 DIM. Values represent weekly means. The weekly SEM for milk fat content averaged 0.18 and ranged from 0.11 to 0.42% in period 1 and averaged 0.24 and ranged from 0.21 to 0.28% in period 2. The weekly SEM for milk fat yield averaged 0.05 and ranged from 0.03 to 0.07 kg/d in period 1 and averaged 0.05 and ranged from 0.03 to 0.06 kg/d in period 2.

Table 4. Performance, milk composition and energetic variables (LSmeans) during period 1 (1 until 42 DIM)

	Treatment[1]			*P*	
Variable	CON	CLA	SEM[2]	Trt	Trt x WL[3]
BW, kg	497.7	487.6	12.5	0.576	0.386
DMI, kg/d	14.8	14.1	0.4	0.233	0.520
Net energy intake, MJ NE_L/d	112.1	106.7	3.5	0.281	0.741
Milk yield, kg/d	23.4	24.5	0.9	0.396	0.801
FCM, kg/d	24.6	23.7	1.0	0.545	0.404
Milk fat					
Content, %	4.40[a]	3.78[b]	0.15	0.010	0.214
Yield, kg/d	1.02	0.92	0.05	0.164	0.057
Milk protein					
Content, %	3.42	3.32	0.05	0.198	0.081
Yield, kg/d	0.79	0.80	0.02	0.816	0.423
Milk lactose					
Content, %	4.88	4.87	0.03	0.771	0.178
Yield, kg/d	1.14	1.20	0.04	0.379	0.701
Milk urea, ppm	139[a]	113[b]	4	<0.001	0.195
SCC x 1000/ml	254	553	113	0.082	0.121
Energetic variables					
Milk energy concentration, MJ/kg	3.34[a]	3.08[b]	0.06	0.007	0.156
Milk energy output, MJ/d	77.7	74.9	3.1	0.523	0.184
Net energy balance, MJ NE_L/d	1.8	-0.7	3.6	0.623	0.646
BCS	3.0	3.0	0.03	0.865	0.521
BFT[4], cm	2.5	2.6	0.07	0.921	0.520
BHBA, mmol/L	0.54	0.58	0.04	0.460	0.609
Glucose, mmol/L	3.76	3.65	0.06	0.194	0.282

[a, b] LSmeans within a row with different superscripts differ ($P < 0.05$).
[1] Treatment (Trt): Cows of the CLA group (n = 10) consumed approximately 6 g/d *trans*-10, *cis*-12 CLA and approximately 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group (n = 10) received a control fat supplement, in which the CLA's were substituted with stearic acid.
[2] Standard error of the LSmean.
[3] Week of lactation.
[4] Back fat thickness.

In period 2, CLA supplementation decreased milk fat content (25.4%), milk fat yield (17.1%) and milk protein content (10.4 %) (Table 5). Overall the milk protein yield was unchanged. In both periods, the milk lactose content and yield were similar. Milk energy concentration was lower in periods 1 and 2 for the CLA group, however milk energy output was equal for the CLA and CON group. Milk yield was not affected by CLA supplementation. The DMI between control and CLA groups did not differ in period 1 but tended to be decreased in period 2 by CLA feeding (Table 4 and 5). The calculated energy balance was negative until 14 DIM (Figure 3B). Differences between CLA treated animals and controls were not observed in period 1, although a trend for a decreased energy balance in period 2 was seen (Table 5).

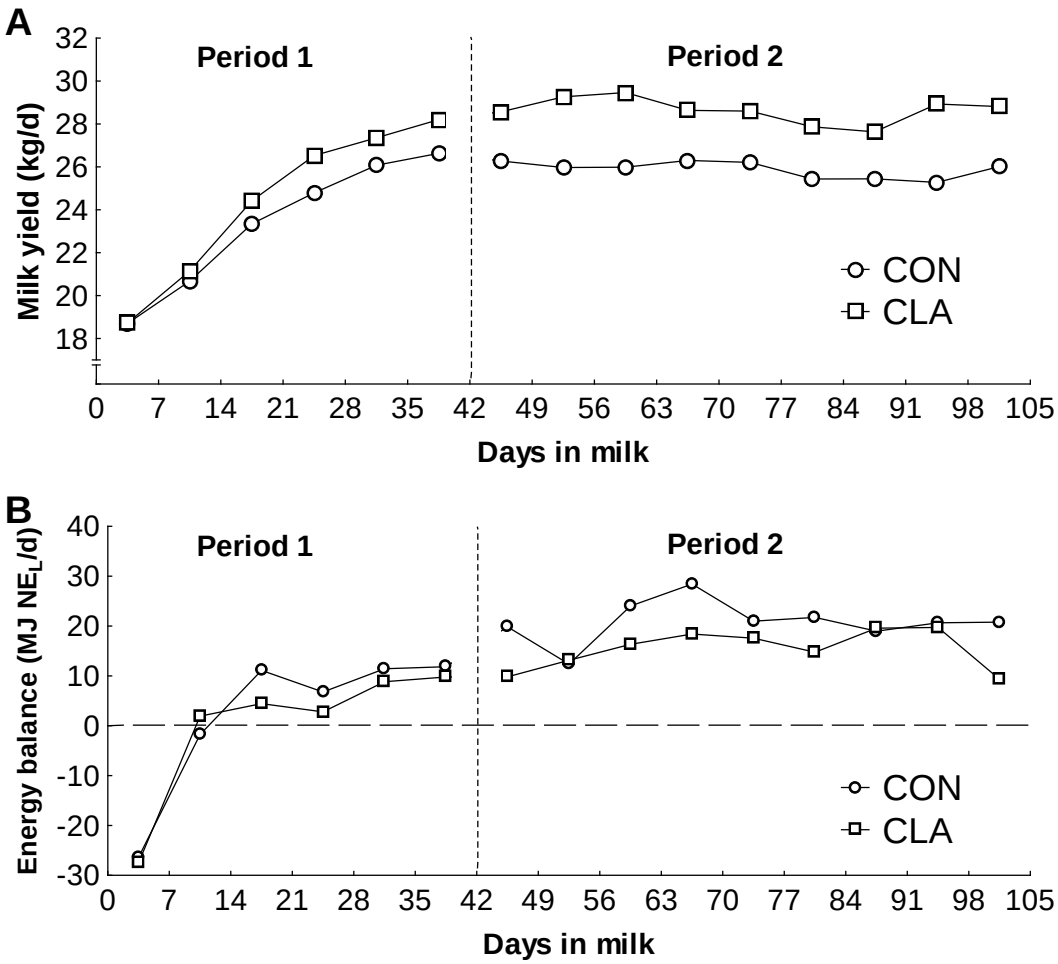

Figure 3. Development of milk yield (A) and energy balance (B) during the 2 periods of the trial. Cows received 100 g/d of a control fat supplement (CON) or 100 g/d of a CLA supplement (CLA). Animals of the CLA group consumed approximately 6 g/d *trans*-10, *cis*-12 CLA. In period 1 each group consists of 10 animals and in period 2 of 5 animals, because of the slaughter after 42 DIM. Values represent weekly means. The weekly SEM for milk yield averaged 0.75 and ranged from 0.71 to 0.85 kg/d in period 1 and averaged 1.14 and ranged from 0.84 to 1.28 kg/d in period 2. The weekly SEM for net energy balance averaged 3.55 and ranged from 2.47 to 5.68 MJ Ne_L/d in period 1 and averaged 3.28 and ranged from 2.31 to 5.19 MJ Ne_L/d in period 2.

Table 5. Performance, milk composition and energetic variables (LSmeans) during period 2 (> 42 DIM until 105 DIM)

	Treatment[1]			*P*	
Variable	CON	CLA	SEM[2]	Trt	Trt x WL[3]
BW, kg	524.5	491.3	19.8	0.268	0.958
DMI, kg/d	18.1	16.6	0.6	0.075	0.034
Net energy intake, MJ NE_L/d	137.6	126.8	4.2	0.094	0.176
Milk yield, kg/d	25.9	28.6	1.3	0.176	0.428
FCM, kg/d	26.2	24.5	0.7	0.118	0.825
Milk fat					
Content, %	4.10[a]	3.06[b]	0.16	0.001	0.993
Yield, kg/d	1.05[a]	0.87[b]	0.03	0.001	0.858
Milk protein					
Content, %	3.35[a]	3.00[b]	0.09	0.025	0.058
Yield, kg/d	0.86	0.86	0.03	0.981	0.289
Milk lactose					
Content, %	4.90	4.85	0.02	0.110	0.760
Yield, kg/d	1.27	1.39	0.06	0.199	0.275
Milk urea, ppm	145[a]	95[b]	8	<0.001	0.426
SCC x 1000/ml	34	275	101	0.108	0.564
Energetic variables					
Milk energy concentration, MJ/kg	3.21[a]	2.74[b]	0.08	0.002	0.996
Milk energy output, MJ/d	82.4	78.3	2.5	0.264	0.694
Net energy balance; MJ NE_L/d	20.9	15.4	2.1	0.082	0.710
BCS	3.0	2.9	0.06	0.105	0.478
BFT[4], cm	2.63[a]	2.32[b]	0.08	0.021	0.777
BHBA, mmol/L	0.59	0.57	0.05	0.857	0.868
Glucose, mmol/L	3.80	3.78	0.09	0.902	0.068

[a, b] LSmeans within a row with different superscripts differ ($P < 0.05$)

[1] Treatment (Trt): Cows of the CLA group (n = 10) consumed approximately 6 g/d *trans*-10, *cis*-12 CLA and approximately 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group (n = 10) received a control fat supplement, in which the CLA's were substituted with stearic acid.

[2] Standard error of the LSmean.

[3] Week of lactation.

[4] Back fat thickness.

In periods 1 and 2, BW and BCS were not changed by CLA supplementation, although BFT showed a decrease in period 2 in the CLA group. Overall plasma NEFA (Figure 3), BHBA and glucose were not affected in any period of the trial (Table 4 and 5). Performance and energetic variables during period 1 were similar for the 42/CON compared to the 105/CON and the 42/CLA compared with the 105/CLA group.

Compared with the EBW of the IG, the EBW was not influenced by CLA supplementation. The liver weight and the liver weight as a proportion of EBW was lower in the IG compared to all other groups (Table 6). The retroperitoneal fat depot reacted most sensitively to lactation progression. Its weight and the weight as a proportion of EBW was decreased for the 42/CON, 105/CON and 105/CLA group compared to the IG, whereas the 42/CLA group tended to be decreased. For the omental, mesenteric and s.c. adipose depot no differences with regard to CLA supplementation were found (Table 6).

Table 6. Empty body weight (EBW), liver weight, and adipose depot weights and the proportion of liver weight and adipose depots weights of EBW (means)

Variable	Treatment[1]					SEM[2]
	IG (n = 5)	42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)	
EBW; kg	447	397	402	434	413	15
Liver						
Weight; kg	6.58	8.06*	8.14*	9.34***	9.16***	0.39
Proportion of EBW; %	1.48	2.04***	2.02***	2.16***	2.22***	0.09
Adipose depots[3]						
Weight; kg	30.03	20.42	25.62	22.29	19.12	3.03
Proportion of EBW; %	6.69	5.13	6.29	5.08	4.56	0.58
Retroperitoneal						
Weight; kg	8.76	4.58*	5.78†	5.56*	4.27**	0.97
Proportion of EBW; %	1.95	1.14*	1.42†	1.26*	1.00**	0.19
Omental						
Weight; kg	11.02	7.58	9.11	8.53	7.40	1.14
Proportion of EBW; %	2.45	1.91	2.24	1.94	1.76	0.22
Mesenteric						
Weight; kg	5.93	4.97	5.87	4.44	4.48	0.54
Proportion of EBW; %	1.33	1.24	1.44	1.02	1.08	0.11
Subcutaneous						
Weight; kg	4.32	3.29	4.86	3.75	2.98	0.66
Proportion of EBW; %	0.97	0.83	1.19	0.86	0.71	0.14

† Indicates means are tended to be significantly different ($P < 0.10$) from those of the IG (Dunnett test).

* Indicates means are significantly different ($P < 0.05$) from those of the IG (Dunnett test).

** Indicates means are highly significantly different ($P < 0.01$) from those of the IG (Dunnett test).

*** Indicates means are very highly significantly different ($P < 0.001$) from those of the IG (Dunnett test).

[1] Treatment: Cows of the IG received no fat supplement and no CLA. Cows of the CLA groups consumed approximately 6 g/d *trans*-10, *cis*-12 CLA and approximately 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON groups received a control fat supplement, in which the CLA's were substituted with stearic acid.

[2] Pooled standard error of the mean.

[3] Sum of the retorperitioneal, omental, mesenteric and s.c. adipose depots.

DISCUSSION

In the present investigation the decrease in milk fat content and yield was consistent with results from other studies using similar *trans*-10, *cis*-12 CLA doses in the range of 3.4 to 9 g/d during early or established lactation (Perfield et al., 2002; Castaneda-Gutierrez et al., 2005; Brömmel et al., 2007; Odens et al., 2007). The animals in this trial consumed 6 g/d *trans*-10, *cis*-12 CLA. In a study by Castaneda-Gutierrez et al. (2007), it was calculated, based on equations of de Veth et al. (2004), that 18% of the *trans*-10, *cis*-12 CLA escaped from rumen biohydrogenation. Assuming the same conditions in our trial, the implemented dose provided 1.1 g *trans*-10, *cis*-12 CLA at the duodenum. This dose could lead to a reduction in milk fat yield of approximately 12% (de Veth et al., 2004). In periods 1 and 2 of the current study, we observed a decrease in milk fat yield of 10% and 17% for CLA-treated animals, respectively. Furthermore, a lack of reduction in milk fat until 28 DIM occurred and is responsible for an unchanged milk fat yield in period 1. This gradual response of milk fat yield to CLA is also reported in other studies (Perfield et al., 2002; Bernal-Santos et al., 2003; Moore et al., 2004; Castaneda-Gutierrez et al., 2005; Metzger-Petersen et al., 2008; Sigl et al., 2010). Open to question is, if the start of CLA supplementation before calving could be a reason for this observation. However, a start of supplementation 2 weeks before expected parturition showed the same lack of response (Castaneda-Gutierrez et al., 2005; Metzger-Petersen et al., 2008).

A decrease in milk protein content in period 2 was observed, whereas milk protein yield was unaffected for CLA-treated animals in the present study. This could be explained as an effect of diluting. The equal milk protein yield in both periods is dispensed on a numerically higher level of milk yield (Figure 3A). Additionally, the decreased milk urea content in the CLA group in both periods pointed to a protein deficit, which is potentially caused by the trend for a decreased DMI in the CLA group and maybe fortified the reduction in milk protein content. Similar results for decreased DMI and milk protein content for CLA-treated cows, but no change in milk protein yield, were reported by Moallem et al. (2010). Moreover, several studies observed no effect of CLA on milk protein content and yield (Bernal-Santos et al., 2003; Moore et al., 2004; Perfield et al., 2004; Castaneda-Gutierrez et al., 2005). For DMI, a recent study showed a reduction for CLA-fed cows (Pappritz, 2011); concededly reasons for this effect are not known. In several other trials DMI was not influenced by CLA administration, no matter whether abomasaly infused or mixed into the feed (Bernal-Santos et al., 2003; Shingfield et al., 2004; Odens et al., 2007; Liermann and Schwarz, 2007; Liermann

et al., 2008; Harvatine et al., 2009). However, in a review, Bauman et al. (2008) mentioned that in periods of inadequate energy intake CLA-induced MFD coincides with marginal, not statistically detectable, changes in DMI.

In the present study, the trend for a decreased DMI for the CLA group in period 2 contributed to a lower energy balance. Interactions of treatment and week of lactation in period 2 occurred for DMI (Table 4), but the reasons for this interaction are unclear. The effect of DMI in combination with a moderate increase in milk yield of 4.7% in period 1 and 10.4% in period 2 (Figure 3A) lead to no positive CLA effects on the calculated energy balance. The lack of MFD response in period 1 (Figure 2A and B) and the moderate increase in milk yield with continuous CLA feeding during both periods are responsible for an unchanged milk energy output despite a decreased milk energy concentration in both periods. These results indicate that energy spared by CLA-induced MFD is repartitioned to an increased milk yield and explain why the calculated energy balance was not improved in this trial. The obvious but not statistically detectable, CLA-induced milk yield response during period 1 and 2 (Figure 3A) could led back to the moderate slope of milk yield in period 1 and the low animal number in period 2. Bernal-Santos et al. (2003) observed similar circumstances in early lactation and reasoned a milk yield response by CLA administration during periods of inadequate energy intake. In our study, only primiparous cows were used and energy supply was balanced after 14 DIM, whereby the increasing milk yield became obvious after 21 DIM (Figure 3A). This is in contrast to the duration of negative energy balance in pluriparous cows (Bauman and Currie, 1980) or a trial with primi- and pluriparous cows (Pappritz, 2011) in which the negative energy balance lasted until week 8 to 12 of lactation. With regard to pluriparous cows, it is questionable if the milk yield response only occurred in times of negative energy balance, because milk yield response continued till the end of the present trial and energy was still repartitioned to milk yield despite a positive calculated energy balance.

Milk yield, and consequentially the energy demand, in primiparous cows ranges in a smaller magnitude than in pluriparous cows. Nevertheless, mobilization of body reserves is still needed to cover energy requirements. Moreover, growth is not completed and requires additional energy (Coffey et al., 2006). Neither alleviation nor a reinforcement of mobilization of body reserves induced by CLA supplementation is detectable by the measured BW and BCS in the current trial, however, BFT was decreased in period 2 for the CLA group. Similar energy balances and no differences in plasma NEFA concentrations between the CLA and the CON group were consistent with the development of BW and BCS in both groups. This is in line with other trials, which observed no differences in BW and BCS (Bernal-Santos

et al., 2003; Moore et al., 2004; Castaneda-Gutierrez et al., 2005; Castaneda-Gutierrez et al., 2007). However, in the study of Odens et al. (2007), CLA-supplementation diminishes the BW and BCS loss with an improved energy balance at the same time.

In contrast to BW, the EBW is a more appropriate variable to illustrate the mobilization of body reserves, because the ingesta part of BW increases with progressive lactation, conditional to the increase in DMI in early lactation (Flachowsky et al., 2004). The decrease of EBW at the 42 DIM slaughter time is obvious, but not significant, in both groups compared with the IG, and conforms with results for BCS and BFT in period 1. This suggests no influence of CLA on the mobilization of body reserves and no effects on body condition. The animals slaughtered after 105 DIM showed small increases in EBW, with regard to the 42 DIM slaughter time, and indicate that mobilization of body reserves occurs mainly during the first 42 DIM.

The development of plasma NEFA concentrations indicate that the highest mobilization of body tissue occurred within 7 d before parturition up to 21 DIM (Figure 1). This is concurrent with the cessation of a negative energy balance in the present trial, but no differences were found between the CLA and control treatment. A trial by Sigl et al. (2010) with primiparous cows and other studies (Bernal-Santos et al., 2003; Moore et al., 2004; Castaneda-Gutierrez et al., 2005) also showed no effect of CLA supplementation on plasma NEFA concentrations.

The mobilization of body reserves increased plasma NEFA concentrations and this resulted in an increased fatty acid accumulation in the liver (Grummer, 1993). Dramatic decreases of adipose depots in mice were paralleled by a marked hepatomegalia (Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002; Jaudszus et al., 2010). It is not clear, if CLA amplifies the naturally occurring mobilization of body reserves and fatty acid accumulation when fed to dairy cows. Therefore, we investigated liver weight and adipose depot distribution in the present trial. Liver weight as a proportion of EBW was lower in the IG compared with all other groups. The liver weight increased until 42 DIM and continued to increase until 105 DIM. However, no differences between the control and CLA group were detected (Table 5). Andrew et al. (1994) slaughtered dairy cows at different physiological stages and measured liver weights of 6.08 kg 7 days before parturition and 10.34 kg after 63 DIM. The measured liver weights in the present trial were in the same range and support the view that the increase in liver weight is a normal physiological effect during early lactation.

The adipose depot distribution in the dairy cow is not well investigated. Due to CLA-induced reductions of adipose depots, which were observed in mice (Delany et al., 1999; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002), we investigated how the adipose depots react

under conditions of CLA feeding. For the 42/CLA and 42/CON group the retroperitoneal adipose depot was decreased compared with the IG, but the decrease in the 42/CLA group only tended to be significant and the decrease was 14.3% lower in comparison with the decrease of the 42/CON group. After 105 DIM in the experiment, the retroperitoneal fat depot was decreased in the CLA and control group. This suggests a lipomobilization protective effect of CLA in the first 42 DIM. The retroperitoneal fat depot is the most sensitive depot in mice (Delany et al., 1999) and the results of the present study indicate that this is the same in dairy cows. Effects of CLA on the weight of the omental, mesenteric and s.c. fat depot were not observed. Changes on the s.c. adipose depot over time were very small and no effect of CLA on the weight could be detected. Tsuboyama-Kasaoka et al. (2000) showed that s.c. fat in mice was decreased. In contrast, Harvatine et al. (2009) proved an increase in the expression of lipid synthesis enzymes in subcutaneous adipose tissue of cows during MFD.
The converse effects of CLA on dairy cows compared with mice might be due to 2 different reasons. The first reason could be the differences in fat metabolism of mice and ruminants (Pearce, 1983) and the second could be the administrated doses. Doses from 0.01 to 0.05% CLA in the diet are sufficient for MFD. Negative effects on the liver weights and adipose depots as demonstrated in mice occurred at doses as high as 0.5 – 1.0% CLA of the diet (Bauman et al., 2008). The dose in the present trial was 0.04 % *trans*-10, *cis*-12 CLA isomer in the diet relative to an average DMI of 15.3 kg in both periods. Furthermore, Bauman et al. (2008) concluded in their review that lower doses, as required for MFD, lead to different effects on the fat metabolism of adipose tissue in dairy cows than higher doses and the results of our trial support this statement.

CONCLUSIONS

Dietary CLA supplementation in dairy cows decreased milk fat content and yield but showed no effect on daily milk energy output and calculated energy balance due to slightly increased milk yield and marginal decrease in DMI. The energy was repartitioned to higher milk synthesis. The decrease of EBW, adipose depots, and the increase in liver weight were interpreted to be typical for the early-lactating cows, whereas CLA effects were only marginal (retroperitoneal fat) or not detectable. The retroperitoneal adipose depot underwent the most pronounced alteration in early lactation and CLA decelerated this mobilization when compared with the unsupplemented control group.

ACKNOWLEDGEMENTS

The authors thank the Deutsche Forschungsgemeinschaft (DFG, Bonn, Germany) for financial support (PAK 286/1, DA 558/5-1). Furthermore, the assistance of the coworkers of the Institute of Animal Nutrition and the experimental station of the Friedrich-Loeffler-Institute (FLI) in Brunswick Germany, in performing the experiment and analyses, is gratefully acknowledged.

REFERENCES

Andrew, S. M., D. R. Waldo, and R. A. Erdman. 1994. Direct analysis of body composition of dairy cows at three physiological stages. J. Dairy Sci. 77:3022-3033.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E., J. W. Perfield, K. J. Harvatine, and L. H. Baumgard. 2008. Regulation of fat synthesis by conjugated linoleic acid: Lactation and the ruminant model. J. Nutr. 138:403-409.

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

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition form late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

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

Brömmel, C., U. Meyer, M. Wahner, and G. Flachowsky. 2007. Effect of conjugated linoleic acid (CLA) supplementation on milk yield and composition of dairy cows. Proc. Soc. Nutr. Physiol. 16:48. (Abstr.)

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

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

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

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

de Veth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of CLA on milk fat synthesis in dairy cows: Comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-372.

Degrace, P., L. Demizieux, J. Gresti, J. M. Chardigny, J. L. Sebedio, and P. Clouet. 2003. Association of liver steatosis with lipid oversecretion and hypotriglyceridaemia in C57BL/6j mice fed *trans*-10,*cis*-12-linoleic acid. FEBS Lett. 546:335-339.

Delany, J. P., F. Blohm, A. A. Truett, J. A. Scimeca, and D. B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 276:R1172-R1179.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.

Flachowsky, G., K. Erdmann, L. Hüther, G. Jahreis, P. Möckel, and P. Lebzien. 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. Arch. Anim. Nutr. 60:501-511.

Flachowsky, G., P. Lebzien, and U. Meyer. 2004. Zur energetischen Futterbewertung bei Hochleistungskühen (Contribution to the energetic feed evaluation for high performing dairy cows). Übersichten zur Tierernährung 32:23-56.

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

Gaines, W. L. 1928. The energy basis of measuring milk yield in dairy cows. IL Agric. Exp. Sta. Bull. 308. Page 401-438.

GfE, 1991. Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern (Guidelines for determining the digestibility of crude nutrients in ruminants). J. Anim. Physiol. Anim. Nutr. 65:229–234.

GfE, 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder (Recommendations of energy and nutrient supply for dairy cows and breeding cattle). DLG Verlag, Frankfurt am Main, Germany.

Grabherr, H., M. Spolders, M. Fürll, and G. Flachowsky. 2009. Effect of several doses of zeolite A on feed intake, energy metabolism and on mineral metabolism in dairy cows around calving. J. Anim. Physiol. Anim. Nutr. 93:221-236.

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

Harvatine, K. J., J. W. Perfield, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-854.

Janssen, H. 2006. Untersuchungen zum Einfluß unterschiedlicher Fütterungsintensitäten auf das Körperwachstum, die Futteraufnahme, die Körperzusammensetzung sowie den Stoff- und Energieansatz von weiblichen Kälbern und Aufzuchtrindern (Studies on the influence of different feeding intensities on growth, feed intake, body composition as well as substance and energy retention of female calves and breeding cattle).University of Göttingen. Cuvillier Verlag, Göttingen, Germany. (Dissertation)

Jaudszus, A., P. Möckel, E. Hamelmann, and G. Jahreis. 2010. *Trans*-10, *cis*-12-CLA-caused lipodystrophy is associated with profound changes of fatty acid profiles of liver, white adipose tissue and erythrocytes in mice: Possible link to tissue-specific alterations of fatty acid desaturation. Ann. Nutr. Metab. 57:103-111.

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

Liermann, T. and F. J. Schwarz. 2007. Effects on performance and metabolic parameters of early lactation dairy cows to dietary rumen-protected conjugated linoleic acid and propylene glycol. Proc. Soc. Nutr. Physiol.16:18. (Abstr.)

Metzger-Petersen, K., K.-H. Südekum, M. Pries, and H. van de Sand. 2008. Effects of feeding conjugated linoleic acids to dairy cows during the transition period and early lactation upon milk and milk component yields. Proc. Soc. Nutr. Physiol.17:64. (Abstr.)

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

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

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

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

Pearce, J. 1983. Fatty acid synthesis in liver and adipose tissue. Proc. Nutr. Soc. 42:263-271.

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

Perfield, J. W., A. L. Lock, A. M. Pfeiffer, and D. E. Bauman. 2004. Effects of amide-protected and lipid-encapsulated conjugated linoleic acid (CLA) supplements on milk fat synthesis. J. Dairy Sci. 87:3010-3016.

SAS Institute Inc. 2004. SAS/STAT 9.1 User's Guide. SAS Inst., Inc., Cary, NC.

Shingfield, K. J., D. E. Beever, C. K. Reynolds, S. K. Gulati, D. J. Humphries, B. Lupoli, G. Hervas, and M. J. Griinari. 2004. Effect of rumen protected conjugated linoleic acid on energy metabolism of dairy cows during early to mid-lactation. J. Dairy Sci. 87(Suppl. 1):307. (Abstr.)

Sigl, T., G. Schlamberger, H. Kienberger, S. Wiedemann, H. H. D. Meyer, and M. Kaske. 2010. Rumen-protected conjugated linoleic acid supplementation to dairy cows in late pregnancy and early lactation: effects on milk composition, milk yield, blood metabolites and gene expression in liver. Acta Vet. Scand. 52:16. 1-8.

Staufenbiel, R. 1997. Konditionsbeurteilung von Milchkühen mit Hilfe der sonographischen Rückenfettdickenmessung (Evaluation of body condition in dairy cows by ultrasonographic measurement of back fat thickness). Prakt. Tierarzt 78:87-92.

Tsuboyama-Kasaoka, N., M. Takahashi, K. Tanemura, H. J. Kim, T. Tange, H. Okuyama, M. Kasai, S. Ikemoto, and O. Ezaki. 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 49:1534-1542.

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of energy value of cows milk. J. Dairy Sci. 48:1215-1223.

Universität Hohenheim – Dokumentationsstelle. 1997. DLG Futterwerttabellen Wiederkäuer (Feed value tables for ruminants). 7th ed. DLG-Verlag, Frankfurt am Main, Germany.

VDLUFA. 1993. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch). Vol. III. die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Wang, Y. W. and P. J. H. Jones. 2004. Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr. 79:1153-1158.

5. Paper II

Effect of conjugated linoleic acid supplementation on body composition, body fat mobilization, protein accretion and energy utilization in early-lactation dairy cows.

D. von Soosten*, U. Meyer*, M. Piechotta†, G. Flachowsky* and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Clinic for Cattle, School of Veterinary Medicine Hanover, 30173 Hanover; Germany

Journal of Dairy Science
(2012)
95:1222-1239

ABSTRACT

The objective of the study was to investigate the effects of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) supplementation on body composition, mobilization or accretion of body fat and protein mass, as well as the energy metabolism of dairy cows during the first 105 d in milk (DIM). For this purpose a comparative slaughter experiment was conducted with 25 primiparous German Holstein cows. The experiment started at 1 d in milk (DIM) with the slaughter of 5 animals of an initial group (IG) receiving no CLA supplement. The remaining animals were fed a CLA supplement (CLA; n = 10) or a stearic acid-based control fat supplement (CON; n = 10) from 1 DIM up to slaughter. After 42 DIM, 5 more cows from each treatment (42/CLA and 42/CON) were slaughtered. The remaining 5 cows in each treatment were slaughtered after 105 DIM (105/CLA and 105/CON). The animals of the CLA groups consumed 6.0 g/d *trans*-10, *cis*-12 CLA and 5.7 g/d *cis*-9, *trans*-11 CLA. During the slaughter process, the empty body mass was recorded and partitioned to nine fractions (meat, bone, offal, hide, mammary gland, retroperitoneal fat, omental fat, mesenteric fat and s.c. fat). The fractions were analyzed for dry matter, ether extract, crude protein and ash to calculate the body composition of the empty body mass at the different slaughter times. The principle of the comparative slaughter technique was applied to estimate body fat or protein mobilization and accretion in the viewed periods from 1 DIM until 42 and 105 DIM. The heat production (HP) was calculated by subtracting the energy in milk and energy changes in body mass from the metabolizable energy intake. The body composition was not affected by CLA supplementation. However, the mobilization of body fat mass from 1 until 42 DIM was 24.1 kg in the 42/CON group and 14.3 kg in the 42/CLA group. This resulted in a trend to lower body mass (fat and protein) mobilization of 10.5 kg in the 42/CLA group. Energy mobilization from body mass was 21.2 MJ/d in the 42/CON and 11.5 MJ/d in the 42/CLA group. The HP was unchanged for the 42/CON and 42/CLA group with 123.0 MJ/d and 116.9 MJ/d, respectively. From 1 until 105 DIM the protein accretion was 4.3 kg and the daily energy retention in body protein 1.0 MJ higher for CLA-supplemented cows. The HP was decreased in this period for the 105/CLA group (115.5 MJ/d) as compared to the 105/CON group (125.9 MJ/d). Overall, the trend for a decreased body mass mobilization suggested a protective effect of CLA supplementation against excessive use of body reserves within 42 DIM. Continuous CLA supplementation until 105 DIM increased protein accretion. The effects on body mass mobilization and protein accretion in combination with the decreased HP in the CLA-fed cows suggested a more efficient utilization of metabolizable energy in CLA-supplemented early lactation dairy cows.

Key words: conjugated linoleic acid, body composition, dairy cow, energy metabolism

INTRODUCTION

In the physiological stage of early lactation, the available metabolizable energy (**ME**) from DMI is not sufficient to satisfy the energy requirements of the dairy cow. The demands for milk energy ($\mathbf{E_L}$) and maintenance ($\mathbf{E_M}$) exceed the available ME from feed intake. Therefore, the dairy cow mobilizes energy from body mass ($\mathbf{E_G}$) to provide the required energy for E_L (Bauman and Currie, 1980; Wenk et al., 2001). The synthesised milk fat represents about 50% of the E_L (Tyrrell and Reid, 1965) and is the major reason for the high energy demands in early lactation and, consequently for mobilization of E_G, mainly in form of body fat. Conjugated linoleic acid (**CLA**), especially the *trans*-10, *cis*-12 isomer, affected the energy metabolism of the lactating dairy cow by inducing milk fat depression (**MFD)** manifested as inhibition of milk fat synthesis (Baumgard et al., 2000). However, in energy limitation situations it appears that the dairy cow expends the spared milk fat energy for higher milk yield (Bernal-Santos et al., 2003; de Veth et al., 2006). This effect was also observed for animals used in the present study and published in our previous work (von Soosten et al., 2011). Furthermore, CLA supplementation, influences fat metabolism in ruminants, not only in the mammary gland, by inhibiting milk fat synthesis. For the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA-supplemented dairy cows of the present study a trend was observed to a decelerated reduction of the retroperitoneal adipose depot mass within 42 DIM (von Soosten et al., 2011). Conversely, in the same animals, Akter et al. (2011) measured adipose cell size of different adipose tissues and concluded lipolytic or antilipogenic effects in CLA-fed animals. In general, performance studies with lactating dairy cows investigate the effect of combined *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA supplementation on fat and energy metabolisms by using changes of BW, BCS or back fat thickness (**BFT**) and the blood plasma NEFA concentration or the calculated energy balance as indicators for mobilization of body reserves. Odens et al. (2007) showed that CLA-fed cows lost less body condition in early lactation and a decreased BW loss was observed for one CLA group. Blood plasma NEFA concentration was decreased and an improved energy balance was observed. However, often no effect of CLA was observed on BW, BCS, plasma NEFA concentration and energy balance (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2007) or BFT (Pappritz et al., 2011). In lactating ewes, Sinclair et al. (2010) found that BFT determined between the 10^{th} and 11^{th} thoracic vertebrae was reduced, between the 5^{th} and 6^{th} lumbar vertebrae was not changed, and variables of body composition (body fat, body protein, and body energy

content) were not altered after CLA supplementation for 10 wk. Overall, none of the studies quantified the changes in body fat over the CLA treatment period. Also, adequate evaluation for the mobilization of body reserves in early lactation using the change of BW is confounded by variation in DMI. The overlap of these processes conceals the mobilization of body reserves, and additionally, water deposition could replace the mobilized body fat (Moe et al., 1971). Furthermore, with the change of BW, BCS and BFT, it is not possible to differentiate into fat and protein mobilization or accretion. Using this general approach, performance trials with CLA supplementation of dairy cow rations in early lactation pointed out that further research in this field of energy utilisation and repartitioning of body reserves is needed if CLA is fed in early lactation (Bernal-Santos et al., 2003; Odens et al., 2007; Sinclair et al., 2010). For this purpose the comparative slaughter technique offers the opportunity to determine the mobilization/accretion of body fat and protein mass over a longer period (Lofgreen and Otagaki, 1960; Bath et al., 1965).

Besides CLA effects on fat metabolism, less is known about CLA effects on protein metabolism in the dairy cow. The CLA effects on daily milk protein synthesis in dairy cows are inconsistent, most studies observed no effect on milk protein yield (e.g., Odens et al., 2006; Pappritz et al., 2011; von Soosten et al., 2011), whereby in studies by de Veth et al. (2006) and Medeiros et al. (2010) CLA fed cows with restricted energy supply showed an increased milk protein yield. Additionally, for nutrient partitioning and growth in dairy cows the somatotrophic axis, with insulin-like-growth factor I (**IGF-I**) as a key mediator, plays an important roll (Lucy et al., 2001) and Baumgard et al. (2000) showed that after abomasal infusion of the *cis*-9, *trans*-11 isomer IGF-I blood plasma concentrations were slightly higher, whereby the *trans*-10, *cis*-12 isomer had no effect.

Overall, these aspects of CLA effects on energy, fat and protein metabolism are not well investigated in dairy cows. The objective of the present study was to investigate the effect of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA supplementation on energy metabolism and energy partitioning with special regard to body fat and protein mobilization or accretion from calving up to 105 DIM by using the comparative slaughter technique.

MATERIALS AND METHODS

Animals, Experimental Design and Feeding

The study was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (**FLI**), Brunswick, Germany, in compliance with the European Union Guidelines concerning the protection of experimental animals. Authorization for this experiment was given by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany (File Number 33.11.42502-04-071/07). The whole experiment is described in full detail by von Soosten et al. (2011). In brief, primiparous lactating German Holstein cows were slaughtered after 1, 42 and 105 DIM. Twenty-five animals were assigned to 5 groups with 5 animals in each group. First, an initial group (**IG**), receiving no CLA supplement, was slaughtered at 1 DIM. The remaining 20 animals were allocated to 1 of 2 feeding treatments (10 animals in each treatment) receiving a control (**CON** treatment) or CLA-supplemented (CLA treatment) diet, beginning at calving and ceasing at experimental slaughter. After 42 DIM, 5 animals of the CON treatment (42/CON), and 5 animals of the CLA treatment (42/CLA), were slaughtered. The remaining 5 animals of the CON (105/CON) and CLA (105/CLA) treatment were slaughtered after 105 DIM. The control fat supplement (Silafat, BASF SE, Ludwigshafen, Germany) or the rumen-protected CLA supplement (Lutrell Pure, BASF SE, Ludwigshafen, Germany) were mixed into the concentrate which was provided by a computerized concentrate feeding station (3.5 kg/d on a DM basis). The animals of the CLA groups consumed 6.0 g/d of the *trans*-10, *cis*-12 CLA-isomer and 5.7 g/d *cis*-9, *trans*-11 CLA isomer (calculated based on the analyzed proportion in concentrates). In the control fat supplement, these isomers were substituted by stearic acid. Together with the feeding of the different supplemented concentrates, all animals received a partial mixed ration (**PMR**) comprising of 38% corn silage (*Zea mays*), 25% grass silage and 37% concentrate (on a DM basis) for ad libitum intake from calving until slaughter (Table 1). The average milk yield (mean ± SE) for the 42/CON and 42/CLA cows from 1 until 42 DIM was 24.5 ± 0.8 kg/d and 24.7 ± 0.9 kg/d, respectively. Milk fat content averaged 4.4 ± 0.1% for the 42/CON and 3.6% ± 0.2 for the 42/CLA group. From 1 to 105 DIM for the 105/CON and 105/CLA group the milk yield averaged 24.4 ± 0.5 kg/d and 27.2 ± 0.5 kg/d, respectively. The milk fat content was 4.2 ± 0.1% for the 105/CON group and 3.3 ± 0.1% for the 105/CLA group.

Table 1. Ingredients and chemical composition of the concentrates and partial mixed ration (PMR[1])

	Concentrate			PMR
Variable	CON	CLA	PMR	
Ingredient (%)				
Wheat grain	39.5	39.5	41	
Sugar beet pulp, dried	29	29	30	
Rapeseed meal	20	20	20	
Soybean meal	6.5	6.5	6.5	
Vitamin/mineral premix[2]	2	2	2	
Control fat supplement	2.5			
CLA[3] supplement		2.5		
Calcium carbonate	0.5	0.5	0.5	
Analyzed chemical profile				
DM (g/kg)	873	871	870	445
Nutrients (g/kg of DM)				
Crude ash	65	69	64	62
Crude protein	182	180	182	124
Ether extract	50	44	20	28
ADF	134	133	134	208
NDF	259	260	265	405
Energy[4] (MJ/kg of DM)				
ME	13.9	13.7	13.5	11.9
NE_L	8.9	8.7	8.7	7.5
CLA (g/kg of DM)				
C18:2 *trans*-10, *cis*-12	0.0	1.7	0.0	0.0
C18:2 *cis*-9, *trans*-11	0.0	1.6	0.0	0.0

[1] Partial mixed ration (38% corn silage, 25% grass silage, 37% PMR-concentrate on DM basis).
[2] Per kg mineral feed: 140 g of Ca; 120 g of Na; 70 g of P; 40 g of Mg; 6 g of Zn; 5.4 g of Mn; 1 g of Cu; 100 mg of I; 40 mg of Se; 5 mg of Co; 1 000 000 IU of vitamin A; 100 000 IU of vitamin D_3; 1500 mg of vitamin E.
[3] Conjugated linoleic acid
[4] Calculation based on nutrient digestibilities measured with wethers (GfE, 1991).

Measurements, Slaughter and Sample Collection

The daily individual feed intake was recorded from calving until slaughter. Samples of the PMR were collected 4 times a wk directly after feeding and pooled over approximately 4 wk. Samples of the concentrates were taken once a wk and composited monthly. Milking took place 2 times daily at 0530 and 1530 h. Milk yield was recorded using automatic milk counters and BW was automatically recorded when leaving the milking parlor. Milk samples were taken twice a wk and stored at 4°C until analysis. At 1, 7, 14, 21, 28 DIM, and immediately before slaughter, blood samples were drawn from the vena jugularis. Furthermore BCS was recorded using a 5-point scale (Edmonson et al., 1989) and BFT was determined applying ultrasound (Staufenbiel, 1997) immediately before slaughter.

The slaughter took place in the slaughter facilities at the experimental station of the Institute of Animal Nutrition, FLI, next to the pens of the animals. After the morning milking the cow was transported to the slaughter facilities, weighed, stunned with a captive bolt gun and

exsanguinated. Blood was collected and weighed. Body parts were weighed as they were dissected from the animal and assigned to the nine different fractions named as meat, bones, hide, offal, mammary gland, retroperitoneal fat, mesenteric fat, omental fat and s.c. fat. The head, mammary gland, tail and feet (below carpal joint or hock) were detached first. The eyes and tongue were removed from the head, which was separated in 2 halves and the brain was removed. Claws were sawed off, weighed, and rejected. The full gastrointestinal tract, urinary and gall bladder were removed. The hide, heart, lung, liver, kidneys, uterus, spleen, thyroid gland, thymus and retroperitoneal fat were dissected and weighed during the slaughter process. After separation of the gastrointestinal tract in reticulo-rumen, omasum/ abomasum and intestine, these parts were weighed full before, and empty after, opening and cleaning of digests. Mesenteric fat adhering to the intestine and the omental fat, which included fat from the reticulo-rumen, omasum and abomasum, were cut off and weighed. The gastrointestinal tract fill was calculated as the difference in weights of the full and cleaned gastrointestinal tract. The carcass was divided longitudinally into 2 symmetric parts, weighed and stored at 4 °C. The offal fraction consisted of the organs, tongue, eyes, brain, empty gastrointestinal tract and blood. Immediately after slaughter the offal, mammary gland, retroperitoneal fat, mesenteric fat and omental fat fraction were homogenized by chopping 25 rounds per sample using a meat cutter. Two representative samples were taken and stored at -21°C until analysis. The hide was divided along the wither in two symmetric parts and the left part of the hide was first manually reduced to small pieces and then homogenized by the meat cutter (30 rounds) and sampled and stored at -21°C till analysis. The next day, carcass parts and the halves of the head were weighed once again for determination of water loss. Subcutaneous fat was dissected manually with a knife from the left carcass and weighed. The whole s.c. fat mass was calculated by multiplying the mass of the left part of the s.c. fat depot with the quotient resulted from weight of both carcass parts and weight of the left carcass. The meat from the left carcass, left side of the head, left feet (meat was assigned to the meat of the carcass) and whole tail was also separated from the bones manually with a knife and weighed. The s.c. fat and meat fraction were homogenized by the meat cutter (25 rounds) and duplicate samples were taken. The bones were weighed and stored at -21°C until sawed in small pieces and homogenized in a bone mill.

Chemical Analyses

The composition of the feedstuffs (DM, crude ash, CP, ether extract, NDF and ADF) was determined according to the methods of the Association of German Agricultural Analysis and Research Centres (VDLUFA, 1993). The analysis of fatty acid profile of fat supplements and feedstuffs is described elsewhere (von Soosten et al., 2011). Milk samples were analyzed for fat, protein and lactose using an infrared milk analyzer (Milkoscan FT 6000 combined with a Fossomatic 5000, Foss Electric, Hillerød, Denmark). Blood samples were analyzed for plasma concentrations of IGF-I in duplicates by a commercially available two-site-immunoradiometric assay (IRMA, DSL-5600 Active IGF-I-IRMA; Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Insulin-like-growth factor I was separated from its binding proteins by an acid-ethanol extraction procedure as described by the manufacturer and previously published (Lee et al., 1990; Taylor et al., 2004; Sander et al., 2011). The assay was tested for bovine plasma by determining the recovery and intra- and interassay coefficient of variation. The recovery was tested by adding 200 ng/ml IGF-I (obtained from the National Hormone & Peptide Program (NHPP), NIDDK, and Dr. Parlow) to five bovine plasma samples. Afterwards the acid-ethanol extraction and IRMA was performed according to instructions from the manufacturer. The recovery ranged between 96 and 112%. The intra-assay coefficient of variation was 1.5 - 3.5% and the inter-assay coefficient of variation was 1.5 - 8.5%.

Samples of the meat, bone, offal, mammary gland, retroperitoneal fat, mesenteric fat, omental fat and s.c. fat fraction were thawed and dried by lyophilisation for determination of dry matter content. Afterwards the samples were homogenized once again using a meat cutter. Exclusively the hide fraction was dried at 60°C in a forced-air oven and additionally homogenized with a mill (equipped with a 1 mm sieve). For exact DM determination of all fractions, the remaining moisture was determined in a drying cabinet at 103°C. Crude ash, CP and ether extract were analyzed according to the methods of the Association of German Agricultural Analysis and Research Centres (VDLUFA, 1993). Crude ash was determined by differential weighing before and after combustion in a muffle oven at 550°C for 5 hours. For determination of ether extract, samples were extracted with petrol ether and the Kjeldahl method was applied to determine CP. All fraction samples were analyzed for crude ash, CP and ether extract as triplicates and the average was taken for calculation of the fraction composition.

Calculations

The empty body mass (**EBM**) is defined as the whole body of the cow without claws off cut, digesta, and content of urinary and gall bladder, and was calculated as the sum of all body part weights, which were recorded during the slaughter process. The mass of the meat fraction of the whole animal was the sum of meat mass from head, carcass, and tail. The meat mass from the head was calculated by multiplying the meat mass of the left side of the head with the quotient resulting from the mass of both head parts and mass of the left part of the head. The meat mass from the carcass was calculated by multiplying the meat mass of the left carcass with the quotient resulting from mass of both carcass parts and left carcass. The bone mass from the carcass was calculated by multiplying the bone mass from the left carcass with the similar quotient used for calculation of the meat mass. The bone mass from the head was calculated by multiplying the bone mass of the left side of the head with the quotient resulted from the mass of both head parts and mass of the left part of the head. The mass of the bone fraction was calculated as the sum of the bone mass from the head, carcass and tail. The body composition, including water, ether extract, CP and crude ash was calculated by adding the contents of the fractions together. The fat free dry matter (**FFDM**) was calculated as the sum of CP and ash content in the EBM.

Applying the principles of the comparative slaughter technique (Bath et al., 1965) by assuming that the average EBM and average body composition of the initial slaughter group represented the EBM and body composition of the animals in the remaining 4 groups at 1 DIM, the average EBM and body composition of the IG served for calculation of the body composition at 1 DIM for the animals slaughtered after 42 or 105 DIM. The average body composition of the corresponding 42/CON and 42/CLA group served for the calculation of the body composition of the 105/CON and 105/CLA group at 42 DIM. The obtained body composition at 42 DIM for the animals actually slaughtered on 105 DIM was used to calculate the body fat and protein mobilization/accretion from 42 to 105 DIM. The body mass (fat or protein) mobilization/accretion was the difference of body mass at the end minus the calculated body mass at the start of the viewed periods from 1 to 42 DIM, from 1 to 105 DIM and from 42 to 105 DIM. The changes in BW were calculated by subtracting the BW of the animal at the start of a viewed period from the BW at the end of this period. Area under the curve (**AUC**) for weekly average BW data was calculated by using the trapezoidal rule (Jones, 1997) to obtain additional information about the change of BW from all BW data. Lower values for AUC indicated an intensified decrease of BW. The EBM change and the fat and

protein mobilization/accretion in the meat fraction were calculated with the same methods as for body mass mobilization/accretion.

The fat and protein mobilization/accretion from 1 DIM until slaughter were transformed to the corresponding energy equivalents by using the gross energy concentration of 39.8 kJ/g for fat and 23.8 kJ/g for protein, respectively (Brouwer, 1965). The daily mobilized/accreted energy in the form of body mass [E_G (MJ/d)] was calculated dividing the mobilized/accreted energy from 1 DIM until slaughter by the number of days from 1 DIM until slaughter.

Based on the equations published by the Gesellschaft für Ernährungsphysiologie (**GfE**) (GfE, 2001) the E_L was calculated with the following Equation 1.

$$E_L \text{ (MJ/kg)} = 0.38 \times \text{milk fat (\%)} + 0.21 \times \text{milk protein (\%)} + 0.95 \qquad (1)$$

The weekly mean of E_L/d (calculated with measurements on daily milk yield and milk fat and milk protein content twice per wk) was multiplied by seven to obtain the weekly E_L. Then the weekly E_L was cumulated over the period and divided by the number of days in this period to calculate an average value for daily E_L.

The daily ME intake (**MEI**) was cumulated over the period divided by the number of days in this period to obtain an average daily value for MEI.

The heat production (**HP)** was calculated using Equation 2.

$$\text{HP (MJ/d)} = \text{MEI (MJ/d)} - [E_L \text{ (MJ/d)} + E_G \text{ (MJ/d)}]. \qquad (2)$$

For calculation of the energetic efficiency (**EE**) according to (Weiss et al., 2008), during times of mobilization or accretion of body mass, the requirements for maintenance NE_M and lactation NE_L are needed and their estimation is described elsewhere (von Soosten et al., 2011). The daily requirements for NE_M and NE_L were cumulated from 1 DIM up to slaughter. In the case of body mass mobilization, E_G was multiplied by the factor 0.84 to consider that the mobilized energy from body mass is converted into E_L at a proportion of 84% (Moe et al., 1971). Afterwards this value was subtracted from NE_M and NE_L (Equation 3). In the case of body mass accretion, E_G was added to NE_M and NE_L (Equation 4). The result of adding/subtracting NE_M, NE_L and E_G was divided by DMI (Equation 3 and 4). The following equations were used:

During times of mobilization of body mass

$$EE \text{ (MJ } NE_L\text{/kg DMI)} = [NE_M \text{ (MJ } NE_L) + NE_L \text{ (MJ } NE_L) - [E_G \text{ (MJ) x 0.84]]/DMI (kg)} \qquad (3)$$

During times of accretion of body mass

$$EE\ (MJ\ NEL/kg\ DMI) = [NE_M\ (MJ\ NE_L) + NE_L\ (MJ\ NE_L) + E_G\ (MJ)]/DMI\ (kg) \quad (4)$$

The energy partitioning of the MEI into HP, E_L and E_G was calculated as an average of all animals, and separately for the CON and CLA group, during the different periods of the experiment.

Statistical Analyses

All statistical analyses were carried out using the software SAS version 9.1 (SAS Institute Inc., 2004). Data for all variables were tested for normal distribution with the Kolmogorov-Smirnov test and for homogeneity of variances with the Levene's test. Variables of body composition and the nine fractions were analyzed using the GLM procedure. The CON and CLA groups at the 42 and 105 DIM slaughter were compared with the IG by using the Dunnett test. For data of the CON and CLA groups at the 42 and 105 DIM slaughter without the IG, the GLM procedure was used with treatment (CON or CLA), DIM (42 or 105), and interactions of treatment x DIM as the fixed effects.

The IGF-I plasma concentrations were analyzed as repeated measures using the MIXED procedure. An autoregressive covariance structure was modelled using wk of lactation as the repeated effect (Littell et al., 1998). The model contained treatment and wk of lactation as the fixed effects. Cows were treated as random effect.

The mobilization/accretion of body mass and energy from 1 to 42 or 105 DIM and from 42 to 105 DIM for the CON and CLA group were analyzed by using the TTEST procedure. The variables HP, MEI, E_L, energetic efficiency and proportion of energetic variables on MEI, the changes of BW and EBM as well as the AUC of BW data were analyzed with the same procedure.

The BCS and BFT measured at the day of slaughter were correlated with total mass and fat mass of the EB and the nine fractions. The body fat mobilization in the different periods was correlated with the average value in the respective period of plasma NEFA or net energy balance data, which were obtained from our previous study (von Soosten et al., 2011). Pearson correlation coefficients were calculated by using the CORR procedure.

For all analyses, significance was declared when *P*-values were < 0.05 and a tendency was noted when $0.05 < P < 0.10$.

RESULTS

The BW recorded at the start of the experiment (1 DIM) showed that animals were representative in each group. The average BW at 1 DIM (means ± standard error (**SE**)) was 521 ± 15 kg for the IG, 515 ± 13 kg for the 42/CON group, 512 ± 24 kg for the 42/CLA group, 512 ± 16 kg for the 105/CON group and 496 ± 11 kg for the 105/CLA group.

Body Composition

The mean body composition of the 5 groups slaughtered at 3 different time points after calving and fed no fat supplement and the CLA supplement or the control fat supplement are presented in Table 2. Overall, CLA effects on body composition were not detectable. The EBM was not affected by CLA feeding or slaughter at different DIM. The water content of EBM presented as total mass of the animals slaughtered at 105 DIM was increased compared with the groups slaughtered at 42 DIM. However, expressed as proportion of EBM, this effect was not detectable and, consequently, the DM was not affected by CLA treatment or slaughter at different DIM. Ether extract, CP, ash and FFDM shown as percentage of DM were not influenced by CLA treatment or slaughter time. Additionally, ether extract, ash and FFDM shown as total mass or proportion of EBW remained unchanged. The CP content was not affected when expressed as total mass. Presented as proportion of EBM, CP showed a trend for an influence of CLA treatment ($P = 0.062$). The difference between DM mass and the sum of ether extract, CP and ash mass could be explained due to carbohydrates (approximately less than 0.5% according to Reid et al. (1955)) in the body of the animal which were not determined. The energy contents of the EBM were not different. The BCS and BFT were not affected by CLA treatment. However, compared with the IG, BCS tended to be deceased for the 105/CLA group. For BFT the 42/CON and 105/CLA group were decreased compared with the IG, whereas the 42/CLA group showed a trend for a decreased BFT.

Table 2. Empty body mass (EBM), chemical composition, energy content of EBM, BCS and back fat thickness (BFT; means) of cows slaughtered at different time points and fed no fat supplement (initial group, IG), the control (CON) diet, or conjugated linoleic acid (CLA)-supplemented diet

Variable	Initial group (IG)[1] (n = 5)	Treatment[2]				SEM[3]	*P*[4]		
		42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)		CLA	DIM	CLA*DIM
BW (kg)	521.0 ± 15.3	491.2	489.9	534.4	501.8	19.2	0.391	0.171	0.427
EBM (kg)	447.1 ± 11.9	397.5	402.3	434.1	412.7	15.6	0.600	0.152	0.411
Water									
Mass (kg)	266.5 ± 7.8	246.9	241.7	263.4	259.0	7.3	0.514	0.034	0.957
% of EBM	59.6 ± 1.1	62.1	60.2	60.8	62.9	1.2	0.943	0.548	0.109
DM									
Mass (kg)	180.6 ± 7.6	150.6	160.6	170.7	153.7	10.1	0.734	0.523	0.197
% of EBM	40.4 ± 1.1	37.9	39.8	39.2	37.1	1.2	0.943	0.547	0.109
Ether extract									
Mass (kg)	87.0 ± 7.1	61.1	70.5	75.7	60.8	8.5	0.746	0.775	0.173
% of EBM	19.4 ± 1.2	15.4	17.3	17.2	14.5	1.6	0.813	0.765	0.151
% of DM	47.9 ± 1.9	40.4	43.2	43.5	38.8	2.8	0.740	0.808	0.200
CP									
Mass (kg)	70.7 ± 1.4	65.4	66.8	67.7	68.7	1.9	0.534	0.300	0.927
% of EBM	15.8 ± 0.3	16.5	16.7	15.6	16.7	0.3	0.062	0.215	0.162
% of DM	39.4 ± 1.6	43.6	42.1	40.2	45.4	2.0	0.371	0.968	0.112
Ash									
Mass (kg)	20.7 ± 0.3	20.3	20.4	21.2	20.3	0.8	0.552	0.648	0.540
% of EBM	4.6 ± 0.1	5.1	5.1	4.9	4.9	0.2	0.909	0.257	0.775
% of DM	11.5 ± 0.4	13.6	12.8	12.6	13.4	0.7	0.975	0.764	0.272

(continued next page)

Table 2. (continued)

Variable	Initial group (IG)[1] (n = 5)	Treatment[2] 42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)	SEM[3]	*P*[4] CLA	DIM	CLA*DIM
FFDM[5]									
Mass (kg)	91.4 ± 1.3	85.8	87.2	88.9	89.0	2.4	0.762	0.342	0.793
% of EBM	20.5 ± 0.4	21.6	21.7	20.5	21.6	0.4	0.170	0.176	0.249
% of DM	50.9 ± 1.9	57.2	54.9	52.8	58.7	2.6	0.493	0.913	0.130
Energy									
EBM (MJ)	5142.9 ± 283.1	3991.0	4394.0	4622.8	4055.8	364.3	0.825	0.692	0.202
DM (MJ/kg)	28.4 ± 0.4	26.5	27.2	26.8	26.3	0.6	0.916	0.662	0.326
EBM (MJ/kg)	11.5 ± 0.4	10.0	10.9	10.6	9.7	0.6	0.994	0.616	0.162
BCS	3.05 ± 0.05	2.90	3.00	3.05	2.85†	0.08	0.515	1.000	0.063
BFT (cm)	2.84 ± 0.04	2.26**	2.45†	2.53	2.26**	0.13	0.778	0.778	0.105

† Indicates means are tended to be significantly different ($0.05 < P < 0.10$) from those of the IG (Dunnett test).

* and ** indicates means are significantly different from those of the IG (Dunnett test) ($P < 0.05$) and ($P < 0.01$), respectively.

[1] Mean ± SE of cows slaughtered at 1 DIM (served to calculate baseline values for calculation of body fat and protein mobilization/accretion by using the principle of the comparative slaughter technique). Cows of the IG received no fat supplement and no CLA.

[2] Treatment: Cows of the CLA groups consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON groups received a control fat supplement, in which the CLA's were substituted by stearic acid.

[3] Pooled SEM included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG.

[4] Statistical analyses with the GLM procedure included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG. *P*-values in the CLA and DIM column are related to the fixed effects of CLA supplementation and slaughter time at different DIM, respectively. *P*-values in the CLA x DIM column describe the interactions between the fixed effects.

[5] Fat free dry matter (sum of CP and ash)

Composition of the Tissue Fractions

The composition of the meat, bone, offal, hide and mammary gland fraction is shown in Table 3. Overall no CLA effects were found on the composition of these fractions. Total mass of the meat fraction in the groups slaughtered at 42 DIM tended to be decreased compared with the IG and were increased for the animals slaughtered at 105 DIM. The other variables for the meat fraction (ether extract, CP, ash, energy in MJ/kg and proportion of empty body energy) remained unchanged. The proportion of empty body energy of the meat fraction amounted to approximately half of the total empty body energy (average across all groups 45.3 %).

Apart from DM and CP content, the bone fraction was not influenced by the different treatments. The CP content was lower at 105 DIM in the CON and CLA group. The interaction between CLA and DIM for bone DM is due to a low value for the 42/CON group.

The ash contents of the offal fraction for the 42/CON and 105/CLA groups were higher than for the IG. Additionally, the energy value per kg was decreased in all groups compared with the IG. The proportion of empty body energy showed interactions between CLA treatment and DIM. The hide fraction did not differ in any of the presented variables. The total mass of the mammary gland fraction in the 42/CON group tended to be decreased and in the 42/CLA, 105/CON and 105/CLA group was decreased compared with the IG. Furthermore, the slaughter at different DIM affected the CP content and the proportion of mammary gland energy content of total empty body energy. The sum of the percentage of ether extract, CP, and ash is not 100%, because of the proportion of carbohydrates in the different fractions.

The composition of the retroperitoneal, mesenteric, omental and s.c. fat fractions are shown in Table 4. Overall no CLA and DIM effects, or interactions between both, were found on the composition of the adipose depot fractions. The most pronounced effects were observed for the retroperitoneal fat depot fraction. Mass was decreased for the 42/CON, 105/CON and, 105/CLA groups compared to the IG, whereby mass of the 42/CLA group tended to be decreased. This also resulted in a lower proportion of empty body energy in the same groups. The DM content was lower in the 105/CLA group and tended to be lower in the 42/CON group compared with the IG. While the omental fat fraction remained unchanged, the mesenteric fat fraction was affected for DM, CP and proportion of empty body energy (**PEBE**). The content of DM was lower in the 42/CON, 105/CON and 105/CLA group compared with the IG. For PEBE, the groups slaughtered at 105 DIM were lower or tended to be lower. The effect on CP was only visible by a trend for a higher CP content in the

105/CLA group compared with the IG. The s.c. fat fraction did not differ in any of the determined variables.

Table 3. Total mass, DM, chemical composition of DM and energy content (means) in the meat, bone, offal, hide and mammary gland fraction of cows slaughtered at different time points and fed no fat supplement (initial group, IG), the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet

Variable	Initial group (IG)[1] (n = 5)	Treatment[2]				SEM[3]	*P*[4]		
		42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)		CLA	DIM	CLA*DIM
Meat									
Total mass (kg)	208.3 ± 4.1	184.7†	187.0†	213.6	198.0	8.1	0.420	0.025	0.283
DM (%)	36.1 ± 1.5	32.4	34.1	33.9	31.6	1.3	0.828	0.717	0.157
Ether extract (%)	48.3 ± 3.1	41.8	44.1	44.6	39.4	3.2	0.654	0.756	0.261
CP (%)	48.3 ± 3.2	53.2	52.0	47.7	55.9	3.0	0.263	0.789	0.138
Ash (%)	2.5 ± 0.1	2.9	2.6	2.7	3.0	0.2	0.908	0.695	0.132
Energy (MJ/kg)	30.7 ± 0.5	29.3	29.9	29.1	29.0	0.6	0.700	0.360	0.572
PEBE[5] (%)	45.6 ± 1.2	44.4	44.3	46.5	45.6	1.0	0.587	0.098	0.683
Bone									
Total mass (kg)	49.7 ± 0.8	50.1	49.1	49.8	48.3	1.5	0.394	0.721	0.876
DM (%)	73.0 ± 0.5	73.0	75.9	76.9	75.5	0.9	0.402	0.060	0.030
Ether extract (%)	23.4 ± 1.0	24.4	24.0	27.1	25.3	1.1	0.332	0.085	0.535
CP (%)	25.5 ± 0.5	25.7	26.4	24.3	25.2	0.5	0.128	0.025	0.899
Ash (%)	48.3 ± 1.1	47.3	47.0	46.8	46.7	1.0	0.820	0.739	0.941
Energy (MJ/kg)	15.4 ± 0.3	15.8	15.8	16.6	16.1	0.4	0.523	0.194	0.487
PEBE[5] (%)	11.0 ± 0.8	14.6	13.8	14.2	15.0	1.2	0.995	0.780	0.516
Offal									
Total mass (kg)	90.5 ± 2.9	86.3	85.1	90.3	93.8	3.8	0.769	0.110	0.542
DM (%)	25.6 ± 0.6	24.7	25.2	26.0	25.0	0.8	0.776	0.483	0.350
Ether extract (%)	43.7 ± 0.8	36.2	37.8	39.1	36.7	2.3	0.852	0.714	0.409
CP (%)	49.4 ± 0.9	53.3	52.7	48.9	54.1	2.0	0.268	0.461	0.164
Ash (%)	3.3 ± 0.1	4.1*	3.8	3.7	4.1*	0.2	0.614	0.759	0.078
Energy (MJ/kg)	29.2 ± 0.2	27.1*	27.6*	27.2*	27.5*	0.5	0.503	0.984	0.859
PEBE[5] (%)	13.2 ± 0.4	14.5	13.6	14.1	16.2	0.7	0.427	0.135	0.047

(continued next page)

Table 3. (continued)

Variable	Initial group (IG)[1] (n = 5)	Treatment[2] 42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)	SEM[3]	*P*[4] CLA	DIM	CLA*DIM
Hide									
Total mass (kg)	40.8 ± 1.5	35.4	35.6	38.5	37.8	1.6	0.879	0.128	0.768
DM (%)	34.1 ± 0.7	35.2	35.5	35.6	33.9	0.9	0.451	0.511	0.305
Ether extract (%)	21.8 ± 1.7	14.8	16.6	22.5	15.4	3.3	0.429	0.341	0.196
CP (%)	78.4 ± 2.2	85.3	82.5	75.4	81.0	3.6	0.712	0.130	0.261
Ash (%)	2.0 ± 0.1	2.1	1.7	2.0	1.9	0.1	0.103	0.832	0.293
Energy (MJ/kg)	27.4 ± 0.2	26.3	26.3	27.0	25.4	0.6	0.262	0.901	0.243
PEBE[5] (%)	7.4 ± 0.2	8.2	7.6	8.1	8.2	0.3	0.388	0.447	0.327
Mammary Gland									
Total mass (kg)	27.8 ± 3.3	20.5†	19.9*	19.6*	15.6**	2.1	0.290	0.229	0.431
DM (%)	26.1 ± 1.7	25.6	26.1	26.2	23.4	1.8	0.554	0.570	0.382
Ether extract (%)	57.0 ± 2.8	53.4	54.4	50.7	46.5	3.2	0.625	0.110	0.416
CP (%)	33.9 ± 2.9	33.9	34.8	37.1	41.9	2.3	0.228	0.039	0.403
Ash (%)	3.6 ± 0.3	3.4	3.3	3.6	4.0	0.3	0.531	0.143	0.381
Energy (MJ/kg)	30.7 ± 0.4	29.3	29.9	29.0	28.5	0.8	0.935	0.287	0.484
PEBE[5] (%)	4.3 ± 0.6	3.9	3.6	3.2	2.6	0.4	0.312	0.037	0.681

† Indicates means are tended to be significantly different ($0.05 < P < 0.10$) from those of the IG (Dunnett test).

* and ** indicates means are significantly different from those of the IG (Dunnett test) ($P < 0.05$) and ($P < 0.01$), respectively.

[1] Mean ± SE of cows slaughtered at 1 DIM. Cows of the IG received no fat supplement and no CLA.

[2] Treatment: Cows of the CLA groups consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON groups received a control fat supplement, in which the CLA's were substituted by stearic acid.

[3] Pooled SEM included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG.

[4] Statistical analyses with the GLM procedure included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG. *P*-values in the CLA and DIM column are related to the fixed effects of CLA supplementation and slaughter time at different DIM, respectively. *P*-values in the CLA x DIM column describe the interactions between the fixed effects.

[5] PEBE (Proportion of empty body energy) meant the percentage of empty body energy which is contained in the fraction.

Table 4. Total mass, DM, chemical composition of DM and energy content in the four adipose depot fractions of cows slaughtered at different time points and fed no fat supplement (initial group, IG), the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet

Variable	Initial group (IG)[1] (n = 5)	Treatment[2]				SEM[3]	*P*[4]		
		42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)		CLA	DIM	CLA*DIM
Retroperitoneal fat									
Total mass (kg)	8.8 ± 1.0	4.6*	5.8†	5.6*	4.3**	1.0	0.961	0.789	0.218
DM (%)	89.6 ± 1.1	83.7†	86.3	84.5	79.0**	2.1	0.499	0.136	0.069
Ether extract (%)	97.1 ± 0.5	96.6	96.4	96.5	92.7	1.6	0.243	0.259	0.297
CP (%)	1.9 ± 0.2	2.5	2.8	2.5	3.4	0.5	0.296	0.608	0.586
Ash (%)	0.1 ± 0.0	0.2	0.2	0.1	0.2	0.0	0.435	0.698	0.591
Energy (MJ/kg)	39.0 ± 0.2	39.0	39.0	38.9	37.6	0.5	0.240	0.212	0.256
PEBE[5] (%)	5.9 ± 0.4	3.7**	4.3†	3.9*	3.1**	0.5	0.882	0.289	0.177
Omental fat									
Total mass (kg)	11.0 ± 1.2	7.6	9.1	8.5	7.4	1.1	0.860	0.743	0.258
DM (%)	81.4 ± 0.7	73.6	76.5	76.4	70.9	3.1	0.689	0.663	0.201
Ether extract (%)	95.8 ± 0.7	94.3	96.1	94.9	93.6	0.9	0.800	0.284	0.010
CP (%)	2.1 ± 0.1	4.0	3.0	3.3	4.0	0.6	0.734	0.812	0.177
Ash (%)	0.2 ± 0.1	0.3	0.2	0.3	0.4	0.1	0.762	0.404	0.083
Energy (MJ/kg)	38.6 ± 0.3	38.4	38.9	38.5	38.1	0.3	0.875	0.197	0.134
PEBE[5] (%)	6.7 ± 0.3	5.3	6.1	5.4	4.9	0.5	0.789	0.275	0.251
Mesenteric fat									
Total mass (kg)	5.9 ± 0.3	5.0	5.9	4.4	4.5	0.5	0.436	0.116	0.472
DM (%)	77.1 ± 1.6	67.4*	72.7	67.8*	64.8**	2.6	0.679	0.177	0.135
Ether extract (%)	95.6 ± 0.9	93.1	94.0	93.2	92.0	1.2	0.884	0.398	0.377
CP (%)	3.3 ± 0.5	4.9	3.9	5.0	5.6†	0.6	0.694	0.122	0.191
Ash (%)	0.3 ± 0.0	0.5	0.3	0.4	0.4	0.1	0.340	0.798	0.127
Energy (MJ/kg)	38.7 ± 0.3	38.1	38.3	38.2	37.8	0.4	0.750	0.641	0.544
PEBE[5] (%)	3.5 ± 0.2	3.2	3.7	2.5*	2.7†	0.3	0.206	0.009	0.649

(continued next page)

Table 4. (continued)

Variable	Initial group (IG)[1] (n = 5)	Treatment[2] 42/CON (n = 5)	42/CLA (n = 5)	105/CON (n = 5)	105/CLA (n = 5)	SEM[3]	*P*[4] CLA	DIM	CLA*DIM
s.c. fat									
Total mass (kg)	4.3 ± 0.4	3.3	4.9	3.8	3.0	0.7	0.580	0.334	0.117
DM (%)	75.7 ± 2.1	69.2	73.3	73.3	68.5	3.3	0.929	0.909	0.196
Ether extract (%)	90.6 ± 1.3	87.2	86.3	86.0	85.1	2.4	0.729	0.630	0.998
CP (%)	8.7 ± 1.3	9.2	9.5	9.3	9.4	1.7	0.927	0.990	0.957
Ash (%)	0.4 ± 0.0	0.5	0.4	0.2	0.5	0.1	0.424	0.298	0.090
Energy (MJ/kg)	38.0 ± 0.3	36.8	36.5	36.4	36.0	0.9	0.733	0.590	0.983
PEBE[5] (%)	2.4 ± 0.1	2.1	2.9	2.2	1.8	0.4	0.614	0.202	0.168

† Indicates means are tended to be significantly different ($0.05 < P < 0.10$) from those of the IG (Dunnett test).

* and ** indicates means are significantly different from those of the IG (Dunnett test) ($P < 0.05$) and ($P < 0.01$), respectively.

[1] Mean ± SE of cows slaughtered at 1 DIM. Cows of the IG received no fat supplement and no CLA.

[2] Treatment: Cows of the CLA groups consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON groups received a control fat supplement, in which the CLA's were substituted by stearic acid.

[3] Pooled SEM included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG.

[4] Statistical analyses with the GLM procedure included data from the CON and CLA groups slaughtered at 42 and 105 DIM without the IG. *P*-values in the CLA and DIM column are related to the fixed effects of CLA supplementation and slaughter time at different DIM, respectively. *P*-values in the CLA x DIM column describe the interactions between the fixed effects.

[5] PEBE (Proportion of empty body energy) meant the percentage of empty body energy which is contained in the fraction.

Correlation of BCS and BFT with Total Mass and Fat Mass of the Empty Body and the Fractions

Table 5 shows the correlations of BCS and BFT with total mass or fat mass of the empty body and the 9 fractions for all animals regardless of group affiliation and grouped by slaughter at DIM. Including all animals, BCS and BFT was significantly correlated with total mass and fat mass of the empty body, meat, mammary gland, retroperitoneal fat, omental fat, and s.c. fat. Across early lactation the correlations at 1, 42 and 105 DIM were not consistent. At 1 DIM only significant correlations for the retroperitoneal and omental total mass and fat mass with BCS were detectable. At 42 DIM, no significant correlations were found. However, at 105 DIM, correlations of BCS and BFT with total mass and fat mass of the empty body, meat, retroperitoneal fat, omental fat and s.c. fat were significant.

Table 5. Pearson correlation coefficients between BCS or back fat thickness (BFT) and total or fat mass for empty body composition or the different fractions (presented for all animals and for animals slaughtered at 1, 42 and 105 DIM; merged data of the control (CON) and conjugated linoleic acid (CLA) groups)

	All (n=25)		1 DIM (IG[1]; n=5)		42 DIM (n=10)		105 DIM (n=10)	
Item	BCS	BFT	BCS	BFT	BCS	BFT	BCS	BFT
EBM								
Total mass (kg)	0.427*	0.459*	0.873	-0.369	-0.198	-0.163	0.651*	0.720*
Ether extract (kg)	0.616**	0.582**	0.911	0.330	0.101	0.018	0.798**	0.814**
Meat								
Total mass (kg)	0.460*	0.500*	0.654	-0.622	-0.025	0.079	0.718*	0.796**
Ether extract (kg)	0.624**	0.599**	0.674	0.471	0.218	0.105	0.770**	0.794**
Bone								
Total mass (kg)	-0.067	0.103	-0.902	-0.433	-0.422	-0.173	0.408	0.509
Ether extract (kg)	-0.253	-0.347	-0.630	-0.602	-0.551†	-0.580†	0.197	0.124
Offal								
Total mass (kg)	-0.137	-0.127	0.691	-0.532	-0.571†	-0.475	0.041	0.058
Ether extract (kg)	0.335	0.338	0.930	-0.111	-0.379	-0.304	0.691*	0.699*
Hide								
Total mass (kg)	0.205	0.214	0.728	-0.611	-0.137	-0.310	0.220	0.292
Ether extract (kg)	0.462*	0.412*	0.850	0.062	-0.066	-0.053	0.742*	0.728*
Mammary Gland								
Total mass (kg)	0.565*	0.506*	0.847	0.106	0.145	-0.086	0.297	0.319
Ether extract (kg)	0.565**	0.506**	0.974	0.242	-0.053	-0.123	0.798**	0.771**

(continued next page)

Table 5. (continued)

	All (n=25)		1 DIM (IG; n=5)		42 DIM (n=10)		105 DIM (n=10)	
Item	BCS	BFT	BCS	BFT	Item	BCS	BFT	BCS
Retroperitoneal fat								
Total mass (kg)	0.640***	0.665***	0.902*	0.414	0.242	0.167	0.854**	0.841**
Ether extract (kg)	0.642***	0.660***	0.917*	0.387	0.236	0.142	0.866**	0.863**
Omental fat								
Total mass (kg)	0.641***	0.540**	0.973**	-0.053	0.232	-0.016	0.791**	0.840**
Ether extract (kg)	0.668***	0.605**	0.990**	0.012	0.311	0.157	0.810**	0.845**
Mesenteric fat								
Total mass (kg)	0.333	0.354	0.762	0.603	-0.047	-0.038	0.572†	0.621†
Ether extract (kg)	0.436*	0.490*	0.718	0.552	0.076	0.096	0.676*	0.742*
s.c. fat								
Total mass (kg)	0.546**	0.445*	0.664	0.029	0.464	0.335	0.614†	0.653*
Ether extract (kg)	0.600**	0.494*	0.837	0.169	0.478	0.350	0.666*	0.698*

†$P < 0.1$, * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$

[1] IG = initial group

Energy Metabolism, Mobilization and Accretion of Body Fat and Protein

In Table 6, the change of BW, change of EBM, AUC of BW data, the mobilized body fat, accreted body protein, and energy variables from 1 until 42 DIM are shown. No significant effect of CLA on body fat mobilization and protein accretion was observed. A trend for a decreased mobilization of body mass (fat and protein) was detected. The mobilized energy from body mass, HP, energetic efficiency, MEI and E_L remained unchanged in CLA fed cows.

Table 6. Change in BW and empty body mass (EBM), mobilization (negative values) and accretion (positive values) of body fat and protein with assessment for energy metabolism from 1 until 42 DIM of cows fed the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet (means ± SE)

	Treatment[1]					
	42/CON (n = 5)		42/CLA (n = 5)		Diff [2]	P-value
BW-change (kg)	-29.1	± 16.5	-22.9	± 9.4	6.2	0.754
BW-AUC (kg x wk)[3]	2957	± 93	2980	± 118	23	0.885
EBM-change (kg)	-44.5	± 13.2	-37.3	± 8.8	7.3	0.659
Fat (kg)	-24.1	± 4.0	-14.3	± 5.2	9.8	0.173
Protein (kg)	2.8	± 1.0	3.6	± 1.5	0.8	0.680
Fat and Protein (kg)	-21.3†	± 3.5	-10.7†	± 3.8	10.5	0.073
Energy[4]						
Fat (MJ/d)	-22.8	± 3.8	-13.5	± 4.9	9.3	0.173
Protein (MJ/d)	1.6	± 0.5	2.0	± 0.9	0.4	0.680
E_G[5] (MJ/d)	-21.2	± 3.5	-11.5	± 4.1	9.7	0.108
Energy metabolism						
ME intake (MJ/d)	180.9	± 11.2	175.0	± 7.2	-5.9	0.670
E_L (MJ/d)	79.1	± 6.3	69.6	± 5.5	-9.5	0.290
HP (MJ/d)	123.0	± 8.7	116.9	± 6.3	-6.1	0.585
Energetic efficiency[6]	6.4	± 0.2	6.7	± 0.5	0.4	0.520

† Indicates that the marked means are tended to be significantly ($P < 0.10$) different from each other.

[1] Cows of the CLA group consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group received a control fat supplement, in which the CLA's were substituted by stearic acid.

[2] Difference = CLA – CON

[3] Area under the curve: lower values represented an intensified decrease of BW.

[4] Represents the daily energy mobilized (negative) or retained (positive values) from the body components.

[5] E_G meant the energy mobilized from (negative) or retained in (positive) body mass (fat and protein).

[6] Describes the conversion of 1 kg DMI into NE_L.

From 1 to 105 DIM (Table 7) fat mobilization was not different between the CON and CLA groups, whereas for protein a higher accretion in the CLA group was observed. Transformed to energetic equivalent, the retained energy in protein was also higher for CLA cows.

The MEI tended to be lower in the CLA group and HP was also lower in the CLA group. The energetic efficiency did not differ in this period. However, a trend for a decreased MEI and lower HP was detectable in CLA-supplemented cows.

Table 7. Change in BW and empty body mass (EBM), mobilization (negative values) and accretion (positive values) of body fat and protein with assessment for energy metabolism from 1 until 105 DIM of cows fed the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet (means ± SE)

	Treatment[1]					
	105/CON (n = 5)		105/CLA (n = 5)		Diff [2]	P-value
BW-change (kg)	22.9	± 14.5	6.8	± 19.8	-16.1	0.531
BW-AUC[3] (kg x wk)	7762	± 232	7329	± 297	-433	0.283
EBM-change (kg)	-5.7	± 12.8	-13.6	± 13.3	-7.9	0.684
Fat (kg)	-9.3	± 9.3	-21.2	± 6.4	-11.9	0.321
Protein (kg)	-1.0[b]	± 1.0	3.3[a]	± 1.3	4.3	0.027
Fat and protein (kg)	-10.3	± 8.6	-17.8	± 5.3	-7.6	0.474
Energy[4]						
Fat (MJ/d)	-2.7	± 2.7	-6.2	± 1.9	-3.5	0.321
Protein (MJ/d)	-0.2[b]	± 0.4	0.8[a]	± 0.3	1.0	0.027
E_G[5] (MJ/d)	-2.9	± 2.6	-5.4	± 1.6	-2.5	0.433
Energy metabolism						
MEI (MJ/d)	202.2†	± 3.7	187.2†	± 5.6	-15.0	0.057
E_L (MJ/d)	79.2	± 2.7	77.1	± 2.5	- 2.1	0.589
HP (MJ/d)	125.9[a]	± 1.3	115.5[b]	± 3.7	-10.4	0.029
Energetic efficency[6]	6.6	± 0.1	6.8	± 0.1	0.2	0.244

[ab] different letters denotes significance ($P < 0.05$).

† Indicates that the marked means are tended to be significantly ($P < 0.10$) different from each other.

[1] Cows of the CLA group consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group received a control fat supplement, in which the CLA's were substituted by stearic acid.

[2] Difference = CLA - CON

[3] Area under the curve: lower values represented an intensified decrease of BW.

[4] Represents the daily energy mobilized (negative) or retained (positive values) from the body components.

[5] E_G meant the energy mobilized from (negative) or retained in (positive) body mass (fat and protein).

[6] Describes the conversion of 1 kg DMI into NE_L.

From 42 up to 105 DIM (Table 8) no differences in mobilization of body reserves or influences of CLA on variables of the energy metabolism were observed. For the changes in BW, AUC for BW data and changes of EBM, no differences between the CLA and CON group were observed in any of the periods. Furthermore the IGF-I blood plasma concentration increased with progress in lactation but was not influenced by CLA treatment during the first 105 DIM (Figure 1).

Table 8. Change in BW and empty body mass (EBM), mobilization (negative values) and accretion (positive values) of body fat and protein with assessment for energy metabolism from 42 DIM until 105 DIM of cows fed the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet (means ± SE)

	Treatment[1]					
	105/CON (n = 5)		105/CLA (n =5)		Diff [2]	P-value
BW-change (kg)	20.2	± 4.8	21.4	± 12.6	1.2	0.933
BW-AUC[3] (kg x wk)	4710	± 142	4410	± 198	-300	0.253
EBM-change (kg)	17.6	± 8.7	16.3	± 4.4	-1.3	0.897
Fat (kg)	11.5	± 9.1	-5.6	± 5.1	-17.2	0.138
Protein (kg)	-1.0	± 1.6	2.7	± 1.4	3.7	0.124
Fat and protein (kg)	10.6	± 8.5	-2.9	± 3.9	-13.5	0.189
Energy[4]						
Fat (MJ/d)	7.3	± 5.7	-3.6	± 3.2	-10.8	0.138
Protein (MJ/d)	-0.4	± 0.6	1.0	± 0.5	1.4	0.124
E_G[5] (MJ/d)	6.9	± 5.5	-2.5	± 2.8	-9.4	0.164
Energy metabolism						
MEI (MJ/d)	219.4	± 4.7	202.1	± 10.2	-17.3	0.161
E_L (MJ/d)	82.4	± 2.3	78.3	± 3.7	- 4.1	0.384
HP (MJ/d)	130.1	± 2.9	126.3	± 6.9	- 3.8	0.625
Energetic efficency[6]	6.8	± 0.2	6.6	± 0.2	- 0.2	0.521

[1] Cows of the CLA group consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group received a control fat supplement, in which the CLA's were substituted by stearic acid.
[2] Difference = CLA – CON
[3] Area under the curve: lower values represented an intensified decrease of BW.
[4] Represents the daily energy mobilized (negative) or retained (positive values) from the body components.
[5] E_G meant the energy mobilized from (negative) or retained in (positive) body mass (fat and protein).
[6] Describes the conversion of 1 kg DMI into $NE_{L.}$

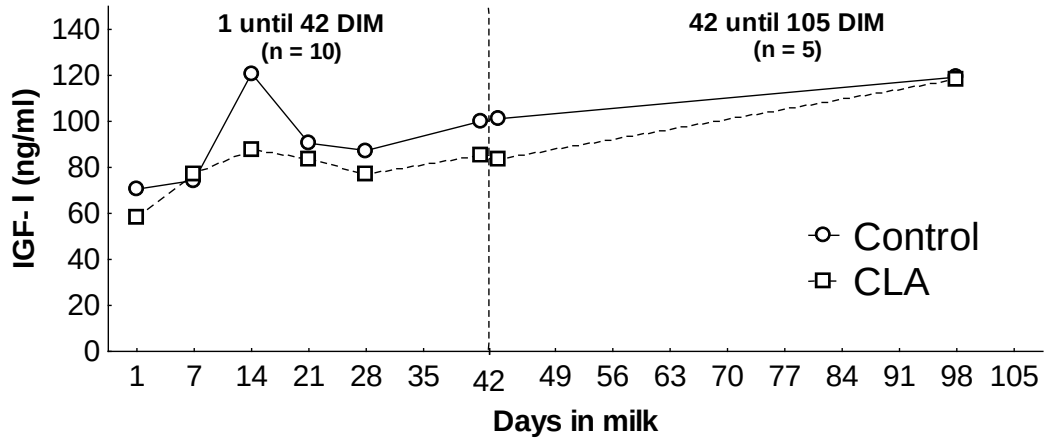

Figure 1. Development of IGF-I plasma concentrations (means) during the trial. From 1 until 42 DIM each group consists of 10 animals and from 42 until 105 DIM samples of 5 animals in each group are available, because of the slaughter after 42 DIM. Cows received from 1 DIM until slaughter a control fat supplement (CON) or a CLA supplement (CLA). Animals of the CLA group consumed 6 g/d each of *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA. No differences between the groups were observed from 1 until 42 DIM ($P = 0.219$) and the SEM averaged 6.9 and ranged from 5.4 to 10.0 ng/ml. From 42 until 105 DIM no differences between the groups were observed ($P = 0.566$) and the SEM averaged 9.5 and ranged from 9.6 to 9.4 ng/ml. With progress of DIM the IGF-I values were increased from 1 until 42 DIM ($P < 0.001$) and a trend for an increase was noted from 42 until 105 DIM ($P = 0.070$).

The mobilization of fat and energy from fat of the meat fraction was not influenced by CLA supplementation during the first 42 DIM. In the period from 42 until 105 DIM a trend to a higher fat, body mass and energy mobilization from fat and body mass was observed in the CLA group (Table 9).

Table 9. Mobilization (negative values) or accretion (positive values) of body fat and protein and its energy (means) in the meat fraction during three time periods in early lactation of cows fed the control (CON) diet or conjugated linoleic acid (CLA)-supplemented diet

	1 – 42 DIM				42 – 105 DIM			
	Treatment[1]				Treatment[1]			
	42/ CON	42/ CLA	SEM[2]	*P*	105/ CON	105/ CLA	SEM[2]	*P*
Fat (kg)	-11.4	-7.6	2.0	0.215	8.4†	-3.1†	4.2	0.088
Protein (kg)	-3.8	-2.6	1.8	0.648	2.3	1.7	1.0	0.633
Fat and protein (kg)	-15.2	-10.1	2.2	0.143	10.7†	-1.4†	4.1	0.070
Fat (MJ/d)	-10.8	-7.2	1.9	0.215	-3.8†	-1.9†	2.6	0.088
Protein (MJ/d)	-2.1	-1.5	1.0	0.648	0.9	0.6	0.4	0.633
E_G[3] (MJ/d)	-13.0	-8.6	1.8	0.133	6.2†	-1.3†	2.6	0.076

	1 – 105 DIM			
	Treatment[1]			
	105/CON	105/CLA	SEM[2]	*P*
Fat (kg)	-2.9	-9.5	4.4	0.313
Protein (kg)	-1.3	0.2	1.1	0.365
Fat and protein (kg)	-4.2	-9.4	4.7	0.459
Fat (MJ/d)	-1.1	-3.6	1.7	0.313
Protein (MJ/d)	-0.5	0.0	0.2	0.361
E_G[3] (MJ/d)	-1.4	-3.6	1.7	0.395

† Indicates that the marked means are tended to be significantly ($P < 0.10$) different from each other.

[1] Cows of the CLA group consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group received a control fat supplement, in which the CLA's were substituted by stearic acid.

[2] Pooled standard error of the mean.

[3] E_G meant the energy mobilized from (negative) or retained in (positive) body mass (fat and protein).

The body fat mobilization for 42/CON cows from 1 until 42 DIM was not correlated with plasma NEFA concentration (Figure 2A). Conversely, for the 42/CLA cows this correlation was negative (Figure 2B). In the same period body fat mobilization tended to be negatively correlated with net energy balance in the 42/CON group (Figure 2C) and tended to be positively correlated with net energy balance in the 42/CLA group (Figure 2D). No significant correlations were observed for the same variables from 1 until 105 DIM and from 42 until 105 DIM.

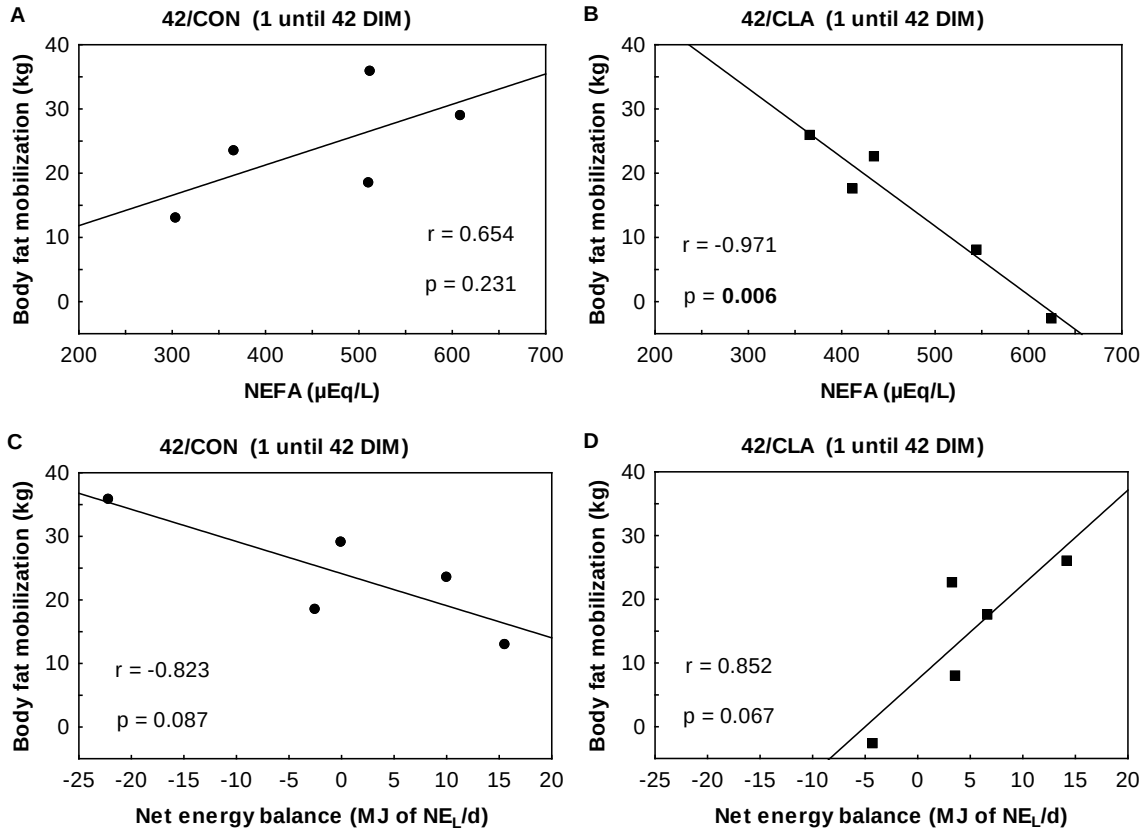

Figure 2. Relationship (Pearson correlation coefficient r) between body fat mobilization and indicators of body fat mobilization (blood plasma NEFA concentration and calculated net energy balance) from 1 until 42 DIM of cows fed the control fat preparation (42/CON, n = 5) or the conjugated linoleic acid (CLA) supplement (42/CLA, n = 5).

Energy Partitioning

Calculations for the proportions of HP, E_L and E_G of the MEI are presented in Table 10. Within the 3 periods, the CLA and CON groups did not differ in percentage of HP, E_L or E_G of MEI. The proportion of HP of MEI averaged 67.5% over the 42/CLA and 42/CON group from 1 until 42 DIM and was higher compared to the average HP proportion from 42 until 105 DIM, and from calving until 105 DIM, 60.9% (P = 0.017) and 62.0% (P = 0.027), respectively. The E_G proportion of MEI from calving until 42 DIM (-9.3%) is lower than in the period from 42 to 105 DIM and from calving until 105 DIM, 0.9% (P < 0.001) and -2.2% (P = 0.002). The proportion of E_L of MEI was 41.7% from calving to 42 DIM, 38.2% from 42 to 105 DIM and 40.2% from calving until 105 DIM, and showed no differences between the periods.

Table 10. Partitioning of ME intake (MEI) into heat production (HP), milk energy, and body mass during 3 time periods and with or without conjugated linoleic acid (CLA) supplementation in early lactation (percentage values of MEI)

	1 – 42 DIM				42 – 105 DIM			
	Treatment[1]				Treatment[1]			
	42/ CON	42/ CLA	SEM[2]	P	105/ CON	105/ CLA	SEM[2]	P
HP[3], (% of MEI)	67.9	67.1	3.3	0.866	59.4	62.5	1.6	0.217
E_L[4], (% of MEI)	44.0	39.6	2.8	0.308	37.5	38.8	0.9	0.338
E_G[5], (% of MEI)	-11.9	-6.7	2.3	0.155	3.0	-1.3	2.0	0.157

	1 – 105 DIM			
	Treatment[1]			
	105/CON	105/CLA	SEM[1]	P
HP[3], (% of MEI)	62.3	61.7	0.8	0.583
E_L[4], (% of MEI)	39.2	41.3	1.2	0.239
E_G[5], (% of MEI)	-1.5	-3.0	1.1	0.379

[1] Cows of the CLA group consumed 6 g/d *trans*-10, *cis*-12 CLA and 6 g/d *cis*-9, *trans*-11 CLA. Cows of the CON group received a control fat supplement, in which the CLA's were substituted by stearic acid.
[2] Pooled standard error of the mean.
[3] HP/MEI x 100.
[4] E_L/MEI x 100.
[5] E_G/MEI x 100 (negative value indicated that energy was mobilized from body mass).

DISCUSSION

CLA Effects on Fat Metabolism in Dairy Cows

In the present study the body fat content at 42 and 105 DIM in CLA-supplemented cows were not different compared to the respective CON group (Table 2). This suggested CLA supplementation during the first 42 or 105 DIM did not affect the mechanisms in fat metabolism active in regulation of body fat content in early-lactation dairy cows. The unchanged plasma NEFA concentration and energy balance of cows used in the present study (von Soosten et al., 2011) support this conclusion and it appears that body fat mobilization, viewed as a major cause for the body composition at 42 or 105 DIM, was not influenced by CLA supplementation in early lactation. In several studies plasma NEFA concentration and energy balance were not changed (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2007). The study of Sinclair et al. (2010) reported no changes in carcass composition in CLA-fed lactating ewes. None of these studies evaluated body fat mobilization. In growing mice, fed higher laboratory dosages of CLA, a marked reduction in body fat content was shown (Delany et al., 1999; Park et al., 1997; Wang and Jones, 2004). However, the effects of CLA on fat metabolism are not well investigated in ruminants and the transmissibility from mice to ruminants is not possible because of differences in fat metabolism between mice and ruminants described by Pearce (1983). Body condition score and BFT are commonly used as indicators for body composition to estimate the mobilization of body reserves in lactating dairy cows. No correlations of BCS and BFT with total mass and fat mass of the empty body and the different fractions were observed at 42 DIM in the present study (Table 5). This suggested that the assessment of development of body composition and the different fat depots in early lactation by BCS or BFT, which mainly reflect the changes in the s.c. fat depot, do not adequately illustrate the different changes in adipose depots and body fat mobilization during early lactation.

The present study was designed to calculate values for body fat mobilization. An initial body composition at 1 DIM could be calculated for each animal slaughtered after 42 or 105 DIM including body composition data of the IG and applying the principle of the comparative slaughter technique according to Bath et al. (1965). This initial body composition offers the opportunity to determine body fat mobilization. By using the comparative slaughter technique it is important to be aware that for calculation of fat or protein mobilization/accretion the animals were slaughtered at different stages. The suggestion of the average body composition of the IG for the animals slaughtered at 42 or 105 DIM to calculate a start body composition

at 1 DIM, of the animals actually slaughtered later, assumed that the animals slaughtered at 42 and 105 DIM underwent similar changes in body composition to the animals of the IG. This aspect of the method also has to be considered in calculation of the body composition at 42 DIM of the animals slaughtered after 105 DIM. Therefore we only used primiparous cows with a small variation in age and BW at 1 DIM to create optimal prerequisites for similar changes in body composition during the trial irrespective of treatment.

Compared to the 42/CON group the mobilized body fat mass was numerically decreased (40.7%) in the 42/CLA group from 1 to 42 DIM (Table 6) and accounted with 93.3% for the trend of reduced body mass mobilization in the CLA group. Conversely, there seems to be little evidence that plasma NEFA concentrations of cows used in the present study (von Soosten et al., 2011) corroborated the trend for lower body mass mobilization. The correlation of body fat mobilization with plasma NEFA concentration during the first 42 DIM for the 42/CON cows showed a relationship of higher body fat mobilization associated with higher plasma NEFA concentration (Figure 2A). Furthermore, this relationship and the relationship between body fat mobilization and negative energy balance are reported by Vernon (1980). In contrast the body fat mobilization of the 42/CLA cows from 1 until 42 DIM was negatively correlated with plasma NEFA concentration (Figure 2B) and a correlation between body fat mobilization and net energy balance was not apparent for the 42/CLA group (Figure 2D), whereas in the 42/CON group this relationship was evident (Figure 2C). Explanations for these observations are rather difficult; conceivable could be CLA effects on lipolysis and lipogenesis in the different adipose tissues of the dairy cow, which could have caused the observed effects on body mass mobilization. In dairy cows used in the present study the retroperitoneal adipose depot mass tended to be less reduced in CLA-fed animals (von Soosten et al., 2011). For the same animals used in the present study Akter et al. (2011) measured adipocyte area, and animals of the CLA group showed a decreased adipocyte area in s.c. and mesenteric adipose tissue at 42 and 105 DIM, as well as in omental and retroperitoneal fat at 105 DIM. However, the correlations between adipocyte area and plasma NEFA concentrations for the CON cows and the IG were strongly positive for the retroperitoneal fat depot ($r = 0.698$) and the visceral fat depots ($r = 0.602$). The adipocyte area of the CLA cows was not correlated with plasma NEFA concentration. This does not deliver an explanation for the different correlations of body fat mobilization and plasma NEFA concentration, but it supports and is in accordance to the calculated correlations in the present study.

In a short-term abomasal infusion study of trans-10, cis-12 CLA (7.5 g/d) with dairy cows Harvatine et al. (2009) observed increased expression of lipid synthesis-related genes in s.c. adipose tissue. Furthermore, the expression of peroxisome proliferator-acivated receptor gamma (PPARγ), which is involved in lipid deposition in adipose tissue, was increased. For cows used in the present study, Saremi et al. (2011) showed an increased mRNA abundance of PPARγ2 in visceral adipose tissue for the CLA treated animals.

Overall, no effects of CLA supplementation on body fat content and mobilization were observed, the trend for decreased body mass mobilization in CLA-supplemented cows is mainly explained through the numerically reduced body fat mobilization. This and the changed relationships of body fat mobilization with plasma NEFA concentrations and energy balance in CLA fed cows suggested a CLA influence on fat metabolism in the early lactation dairy cow.

CLA Effects on Protein Metabolism in Dairy Cows

In the present investigation CLA supplementation enhanced protein accretion from 1 to 105 DIM (Table 7). Protein turnover comprises protein synthesis and degradation. That means the observed protein accretion is the net balance between protein synthesis and protein degradation (Stangl, 2010). Feeding of CLA to double-muscled Piemontese young bulls improved feed efficiency. Despite feeding a low protein diet containing 108 g of CP/kg DM to the animals, the ADG was not decreased in the CLA group. This suggests that CLA affects protein turnover and compensated the lack of dietary CP (Schiavon et al., 2010). The possible CLA effects on protein anabolism might be mediated by anabolic hormones like IGF-I. This growth factor is part of the somatotrophic axis, which describes the interaction of GH, GH receptors in the liver and IGF-I synthesized and secreted by the liver (Lucy et al., 2001). The study by Castaneda-Gutierrez et al. (2007) showed increased plasma IGF-I concentrations during supplementation of 7.1 g/d each of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA from 20 until 56 DIM. For cows of the present trial IGF-I values were not influenced by CLA treatment (Figure 1). This might be because primiparous cows have not been completing growth and therefore the IGF-I in blood plasma concentration is higher (Weber et al., 2007). Nevertheless, IGF-I could stimulate the protein synthesis (Svanberg et al., 1996) and further research is needed to clarify an effect of CLA on protein metabolism in growing ruminants and in the periparturient period of dairy cows.

CLA Effects on Energy Metabolism and Energy Partitioning in Dairy Cows

Significant differences in energy partitioning between the CON and CLA group were not detectable (Table 10). The average daily energy mobilization during the first 42 DIM was numerically lower (9.3 MJ/d) in CLA fed cows. Additionally, considering the energy accreted in body protein, the energy mobilized from body mass was 9.7 MJ/d lower in this period compared to the CON group (Table 6). The PEBE showed that most of total body energy originated from the meat fraction (Table 3) and 4.4 MJ/d less energy was mobilized from the meat fraction in the CLA group in the first 42 DIM (Table 9). These effects are not associated with a decreased E_L or higher MEI in CLA feed cows. On the contrary, the E_L was equal and DMI tended to be reduced by CLA treatment (Table 6 and 7). This is in line with other studies observing no influence on E_L (Bernal-Santos et al., 2003, Sinclair et al., 2010) due to repartitioning of energy spared by reduction of milk fat content to a higher milk yield. Furthermore, a reduced DMI was shown by Moallem et al. (2010), Pappritz et al. (2011) and in a meta-analysis by Harvatine et al. (2009) for CLA-supplemented dairy cows.

The HP from 1 until 42 DIM was unchanged and from calving until 105 DIM was decreased in the CLA group of the present study (Table 6 and 7). In CLA-fed mice, a higher HP was observed and was explained by concurrent marked reductions of adipose depots or reduction in body fat content (West et al., 1998; West et al., 2000). The converse effects on HP in dairy cows of the present study could be related to higher CLA dosages fed to mice (Bauman et al., 2008) and differences between the fat metabolism in mice and ruminants (Vernon, 1980). However, the trend for a reduced HP in CLA fed cows (Table 7) could be due to lower DMI which led to lower production of metabolic heat. Higher DMI is associated with a higher heat increment of feeding (Wenk et al., 2001). Nevertheless values for the HP measured in this trial are in a range with HP measured by van Knegsel et al. (2007) in dairy cows.

The effects of CLA on energy partitioning in the first 105 DIM are of minor importance. The numerically lower amount of energy needed from body reserves (E_G) is a result of the trend for a lower body mass mobilization from 1 until 42 DIM and an improved conversion of the ME into the products could be suggested during the first 42 DIM.

CONCLUSION

The results of the present study indicated no effects of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA supplementation on body composition of dairy cows. The slightly decreased body mass mobilization from 1 until 42 DIM in the CLA group; the increased protein accretion, decreased HP, and a trend to lower DMI from 1 until 105 DIM for CLA-supplemented dairy cows in relation to the unchanged $E_{L,}$ indicate a lower energy expense by equal E_L and more energy retained in body protein. This suggested an improved utilization of the ME in CLA-fed early-lactation dairy cows.

ACKNOWLEDGEMENTS

The authors would like to thank the Deutsche Forschungsgemeinschaft (DFG, Bonn, Germany) for financial support (PAK 286/1, DA 558/5-1). Furthermore, the assistance of the co-workers of the Institute of Animal Nutrition and the Experimental Station of the Friedrich-Loeffler-Institute (FLI) in Brunswick Germany, in performing the experiment and analyses, is gratefully acknowledged.

REFERENCES

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

Bath, D. L., M. Ronning, J. H. Meyer, and G. P. Lofgreen. 1965. Caloric equivalent of live weight loss of dairy cattle. J. Dairy Sci. 48:374-380.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation - A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E., J. W. Perfield, K. J. Harvatine, and L. H. Baumgard. 2008. Regulation of fat synthesis by conjugated linoleic acid: Lactation and the ruminant model. J. Nutr. 138:403-409.

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

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

Brouwer, E. 1965. Report of sub-committe on constants and factors. In: K. L. Baxter (Ed.) Energy metabolism - Proceedings of the 3rd symposium. London - New York. Pages 441-443.

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

Delany, J. P., F. Blohm, A. A. Truett, J. A. Scimeca, and D. B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 276:R1172-R1179.

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

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.

GfE. 1991. Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern (Guidelines for determining the digestibility of crude nutrients in ruminants). J. Anim. Physiol. Anim. Nutr. 65:229–234.

GfE. 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder (Recommendations of energy and nutrient supply for dairy cows and breeding cattle). DLG-Verlag, Frankfurt am Main, Germany.

Harvatine, K. J., J. W. Perfield, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-854.

Jones, N.H. 1997. Finding the area under a curve using JMP and a trapezoidal rule. SAS Inst., Inc., Cary, NC.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using sas procedures. J. Anim. Sci. 76:1216-1231.

Lofgreen, G. P. and K. K. Otagaki. 1960. The net energy of blackstrap molasses for fattening steers as determined by a comparative slaughter technique. J. Anim. Sci. 19:392-403.

Lucy, M.C., H. Jiang, and Y. Kobayashi. 2001. Changes in the somatotrophic axis associated with the initiation of lactation. J. Dairy Sci. 84(E Suppl.):E113-E119.

Lee, C.Y. and D.M. Henricks. 1990. Comparisons of various acidic treatments of bovine serum on insulin-like growth factor-I immunoreactivity and binding activity. J. Endocrinol. 172: 139-148.

Medeiros, S. R., D. E. Oliveira, L. J. M. Aroeira, M. A. McGuire, D. E. Bauman, and D. P. D. Lanna. 2010. Effects of dietary supplementation of rumen-protected conjugated linoleic acid to grazing cows in early lactation. J. Dairy Sci. 93:1126-1137.

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

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1971. Energetics of body tissue mobilization. J. Dairy Sci. 54:548-553.

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

Odens, L. J., R. Burgos, B. C. Pollard, M. L. Innocenti, S. H. Baker, S. R. Sanders, J. K. Kay, M. L. Rhoads, C. E. Moore, M. J. VanBaale, and L. H. Baumgard. 2006. Effects of

varying CLA doses on production and bioenergetic variables during the transition period. J. Anim Sci. 84:112-112.

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

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

Pearce, J. 1983. Fatty acid synthesis in liver and aipose tissue. Proc. Nutr. Soc. 42:263-271.

Reid, J. T., G. H. Wellington, and H. O. Dunn. 1955. Some relationships among the major chemical components of the bovine body and their application to nutritional investigations. J. Dairy Sci. 38:1344-1359.

Sander A.K., M. Piechotta, G. Schlamberger, H. Bollwein, M. Kaske, A. Sipka, H.J. Schuberth. (2011). Ex vivo phagocytic overall performance of neutrophilic granulocytes and the relation to plasma insulin-like growth factor-I concentrations in dairy cows during the transition period. J. Dairy Sci. 94:1762-1771.

Saremi B., H. Sauerwein, D. von Soosten, S. Dänicke, M. Mielenz. 2011. Adiponectin system and peroxisome proliferator-activated receptor gamma2 (PPARγ2) mRNA abundance in different bovine fat depots considering conjugated linoleic acids (CLA) or lactation stage related changes. J. Dairy Sci. 94(E-Suppl. 1):E343. (Abstr.)

SAS Institute Inc. 2004. SAS/STAT 9.1 User's guide. SAS Inst., Inc., Cary, NC.

Schiavon, S., F. Tagliapietra, M. Dal Maso, L. Bailoni, and G. Bittante. 2010. Effects of low-protein diets and rumen-protected conjugated linoleic acid on production and carcass traits of growing double-muscled piemontese bulls. J. Anim. Sci. 88:3372-3383.

Sinclair, L. A., W. M. P. B. Weerasinghe, R. C. Wilkinson, M. J. de Veth, and D. E. Bauman. 2010. A supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

Stangl, G. 2010. Das Zusammenspiel zwischen Proteinauf- und -Abbau – Neue Erkenntnisse zum Proteinturnover (The balance between protein synthesis and protein degradation - novel insights into protein turnover). Übers. Tierernährg. 38:1-32.

Staufenbiel, R. 1997. Konditionsbeurteilung von Milchkühen mit Hilfe der sonographischen Rückenfettdickenmessung (Evaluation of body condition in dairy cows by ultrasonographic measurement of back fat thickness). Prakt. Tierarzt 78:87-92.

Svanberg, E., H. Zachrisson, C. Ohlsson, B. M. Iresjo, and K. G. Lundholm. 1996. Role of insulin and IGF-1 in activation of muscle protein synthesis after oral feeding. Am. J. Physiol. 270:E614-E620.

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

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of energy value of cows milk. J. Dairy Sci. 48:1215-1223.

van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. J. Dairy Sci. 90:1467-1476.

VDLUFA. 1993. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch). Vol. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt. Germany.

Vernon, R. G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19:23-106.

von Soosten, D., U. Meyer, E. M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of *trans*-10, *cis*-12 conjugated linoleic acid on performance, adipose depot weights and liver weight in early-lactation dairy cows. J. Dairy Sci. 94:2859-2870.

Wang, Y. W. and P. J. H. Jones. 2004. Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr. 79:1153S-1158S.

Weber, W. J., C. R. Wallace, L. B. Hansen, H. Chester-Jones, and B. A. Crooker. 2007. Effects of genetic selection for milk yield on somatotropin, insulin-like growth factor-1, and placental lactogen in holstein cows. J. Dairy Sci. 90:3314-3325.

Weiss, W. P., D. J. Wyatt, and T. R. McKelvey. 2008. Effect of feeding propionibacteria on milk production by early lactation dairy cows. J.Dairy Sci. 91:646-652.

Wenk, C., P. C. Colombani, J. van Milgen, and A. Lemme. 2001. Glossary: Terminology in animal and human energy metabolism. In: A. Chwalibog and K. Jakobsen (Ed.) 15th Symp. Energy Metab. Anim. Snekkersten, Denmark. Pages 409-421.

West, D. B., F. Y. Blohm, A. A. Truettt, and J. P. DeLany. 2000. Conjugated linoleic acid persistently increases total energy expenditure in akr/j mice without increasing uncoupling protein gene expression. J. Nutr. 130:2471-2477.

West, D. B., J. P. Delany, P. M. Camet, F. Blohm, A. A. Truett, and J. Scimeca. 1998. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 44:R667-R672.

6. Paper III

Effect of conjugated linoleic acid supplementation on the fatty acid composition of ileal chyme, faeces and different tissues of dairy cows.

D. von Soosten*, R. Kramer†, G. Jahreis†, U. Meyer*, G. Flachowsky* and S. Dänicke*

*Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Brunswick, Germany
†Institute of Nutrition, Friedrich Schiller University Jena, Dornburger Str. 24, Jena, Germany

Archives of Animal Nutrition
Submitted

ABSTRACT

The objective of the study was to investigate the effect of *trans*-10,*cis*-12 (*t*10,*c*12) and *cis*-9,*trans*-11 (*c*9,*t*11) conjugated linoleic acids (CLA) supplementation on the fatty acid profile of the ileal chyme, the faeces and the body of dairy cows, as well as the transfer of supplemented *t*10,*c*12 CLA into the body of the dairy cow during the first 105 DIM. Therefore, a slaughter experiment was conducted with 25 first lactation German Holstein heifers. The experiment started at 1 DIM with the slaughter of five animals of an initial group (IG) receiving no CLA supplementation. After 42 DIM on a CLA-supplemented (CLA) or control diet (CON) five more cows were slaughtered from each feeding treatment (42/CLA and 42/CON). The remaining five cows on each feeding treatment were slaughtered after 105 DIM (105/CLA and 105/CON). The animals of the CLA groups consumed 6.0 g/d of the *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA. In the CON diet a stearic acid-based control fat supplement was included. Immediately before slaughter a sample of faeces was taken. During the slaughter process ileal chyme was sampled. The empty body mass (EBM) was recorded and partitioned to nine fractions (retroperitoneal fat, omental fat, mesenteric fat, s.c. fat, meat, bone, offal, hide and mammary gland). The fatty acid distribution of the ileal chyme, faeces and the fractions were determined. CLA supplementation increased *t*10,*c*12 CLA and *c*9,*t*11 CLA in the ileal chyme and faeces. In the retroperitoneal, mesenteric, s.c., offal and mammary gland fraction the amount of *t*10,*c*12 CLA was increased after CLA supplementation. However, the transfer efficiency into these fractions was less than 0.1% of consumed *t*10,*c*12 CLA. The *c*9,*t*11 CLA isomer was detected in all fractions and across all groups, but the amount of *c*9,*t*11 CLA was not changed due to CLA supplementation. At 105 DIM the desaturase indices were affected in the retroperitoneal fat, mesenteric fat, bone, hide and mammary gland fraction. This suggested a higher activity of Δ^9-desaturase in these fractions with progressive DIM.

Key words: conjugated linoleic acid, fatty acid composition, fat depot, dairy cow

INTRODUCTION

In lactating cows conjugated linoleic acid (**CLA**), especially the *trans*-10,*cis*-12 (***t*10,*c*12**) isomer, reduces milk fat synthesis (Loor et al., 1998). Technically produced CLA mixes, containing equal amounts of *t*10,*c*12 and *cis*-9,*trans*-11 (***c*9,*t*11**) CLA, are supplemented to dairy cows with the intention of disburdening the energy metabolism in early-lactation. The tendency to an increased retroperitoneal fat depot weight after 42 DIM (von Soosten et al., 2011); decreased adipocyte sizes in retroperitoneal, omental and mesenteric fat depots after 105 DIM (Akter et al., 2011); and to decreased body mass mobilization in the first 42 DIM in CLA supplemented dairy cows (von Soosten et al., 2012), indicated that *t*10,*c*12 and *c*9,*t*11 CLA supplementation affected fat and energy metabolism. The *t*10,*c*12 and *c*9,*t*11 CLA isomers are intermediates of biohydrogenation of dietary unsaturated fatty acids by rumen bacteria. The fatty acid *c*9,*c*12 C18:2 (linoleic acid) is converted to *c*9,*t*11 CLA. The rumen microbial enzyme linolate isomerase is responsible for pasting a conjugated double bond onto linoleic acid. The synthesised *c*9,*t*11 CLA is the major CLA isomer in the fat of ruminants and labelled as rumenic acid (Kramer et al., 1998). In the rumen, *c*9,*t*11 CLA undergoes the next step of biohydrogenation, the hydrogenation to *t*11 C18:1 (vaccenic acid). Alternatively, this isomer could passes the rumen unmetabolized and is available for resorption in the duodenum and the direct transfer into the products of the cow (Bauman et al., 1999). Vaccenic acid is either converted to C18:0 (stearic acid) in the last step of biohydrogenation, or is available (after resorption in the duodenum) to different tissues of the dairy cow as a substrate for endogenous *c*9,*t*11 CLA synthesis by Δ^9-desaturase depending on tissue specific activities (Bauman et al., 1999). Feeding 70% concentrate and 30% meadow hay in combination with linseed oil supplementation to cows fitted with a cannula at the proximal duodenum, resulted in an increased flow of *c*9,*t*11 and *t*10,*c*12 CLA in the duodenal chyme compared to cows receiving no linseed oil supplementation and fed 30% concentrate and 70% meadow. The flow for *c*9,*t*11 and *t*10,*c*12 CLA was 1.7-fold and 14-fold increased, respectively (Flachowsky et al., 2006). Supplementing rumen-protected *c*9,*t*11 and *t*10,*c*12 CLA to dairy cows fitted with a cannula at the proximal duodenum increased the duodenal availability for both isomers. The duodenal availability of the *c*9,*t*11 isomer was 0.4 to 0.5 g/d and 4 to 5-fold increased compared to an unsupplemented control group. No *t*10,*c*12 CLA reached the duodenum without supplementation of this isomer, whereas 0.4 to 0.5 g/d of the *t*10,*c*12 isomer were available at the duodenum when supplementing this isomer (Pappritz et al., 2012).

Currently it is not clear to what extent the duodenal available *t*10,*c*12 CLA isomer is included into the fat depots and body mass of dairy cows. Furthermore, it needs to be clarified if the CLA effects on fat depots and body mass mobilization could be directly caused by the included isomers in the respective tissue or be caused indirectly by the CLA-induced MFD in the mammary gland. Therefore, in the present study we examined the impact of ruminal biohydrogenation on the supplemented *t*10,*c*12 and *c*9,*t*11 CLA isomers and the influence of *t*10,*c*12 and *c*9,*t*11 CLA supplementation on the fatty acid composition and the transfer of *t*10,*c*12 CLA into different fat depot and body mass fractions of the dairy cow.

MATERIALS AND METHODS

Animals, Experimental Design and Feeding

In compliance with the European Union Guidelines concerning the protection of experimental animals, the study was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (**FLI**), Brunswick, Germany, and was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany (File Number 33.11.42502-04-071/07). The whole experiment is described in full detail by von Soosten et al. (2011, 2012). In brief, primiparous lactating German Holstein cows were slaughtered after 1, 42 and 105 DIM. For this purpose twenty-five animals were assigned to five groups with five animals in each group. First an initial group (IG) receiving no CLA supplementation was slaughtered at 1 DIM. The remaining twenty animals were allocated to one of two feeding treatments (10 animals in each treatment) receiving a control (CON treatment) or CLA-supplemented (CLA treatment) diet beginning at calving and ceasing at experimental slaughter. Five animals of the control treatment (42/CON) and five animals of the CLA treatment (42/CLA) were slaughtered after 42 DIM. The remaining five animals of the control (105/CON) and CLA (105/CLA) treatment were slaughtered after 105 DIM. The control fat supplement (Silafat, BASF SE, Ludwigshafen, Germany) or the CLA supplement (Lutrell Pure, BASF SE, Ludwigshafen, Germany) were mixed into a concentrate feed which was provided by a computerized concentrate feeding station (3.5 kg/d on a DM basis). The animals of the CLA groups consumed (calculated based on the analyzed proportion in concentrates) 6.0 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA. In the control fat supplement these isomers were substituted by stearic acid. Together with feeding the different supplemented concentrates all animals received a partial mixed ration (**PMR**) comprising 38% corn silage (*Zea mays*), 25% grass silage and 37% concentrate (on a DM basis) for ad libitum intake from calving until slaughter (Table 1).

Table 1. Ingredients and chemical composition of the concentrates and partial mixed ration (PMR[1])

	Concentrate			PMR
Variable	CON	CLA	PMR	
Ingredient (%)				
Wheat grain	39.5	39.5	41	
Sugar beet pulp, dried	29	29	30	
Rapeseed meal	20	20	20	
Soybean meal	6.5	6.5	6.5	
Vitamin/mineral premix[2]	2	2	2	
Control fat supplement	2.5			
CLA[3] supplement		2.5		
Calcium carbonate	0.5	0.5	0.5	
Analyzed chemical profile				
DM (g/kg)	873	871	870	445
Nutrients (g/kg of DM)				
Crude ash	65	69	64	62
Crude protein	182	180	182	124
Ether extract	50	44	20	28
ADF	134	133	134	208
NDF	259	260	265	405
Energy[4] (MJ/kg of DM)				
ME	13.9	13.7	13.5	11.9
NE_L	8.9	8.7	8.7	7.5
CLA (g/kg of DM)				
C18:2 *t*10,*c*12	0.0	1.7	0.0	0.0
C18:2 *c*9,*t*11	0.0	1.6	0.0	0.0
Selected fatty acids (g/kg of DM)				
C18:2 *c*9,*c*12 [5]	6.7	7.8	8.2	9.9
C18:3 *c*9,*c*12,*c*15 [6]	0.7	0.7	0.8	4.4

[1] Partial mixed ration (38% corn silage, 25% grass silage, 37% PMR-concentrate on DM basis).
[2] 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.
[3] Conjugated linoleic acid
[4] Calculation based on nutrient digestibilities measured with wethers (GfE, 1991).
[5] Linoleic acid
[6] Linolenic acid

Measurements, Slaughter and Sample Collection

Samples of the PMR were collected four times a wk directly after feeding and pooled over approximately 4 wk. Samples of the concentrates were taken once a wk and composited monthly. Immediately before slaughter samples of the faeces were collected from the rectum and stored at -21°C. The slaughter took place in the slaughter facilities at the experimental station of the Institute of Animal Nutrition next to the pens of the animals. After the morning milking the cows were transported to the slaughter facilities, stunned with a captive bolt gun and exsanguinated. The blood was collected and weighed. Ileum (from the end of the plica ileocaecalis to the ileocaecal junction) was quickly dissected and chyme was collected and stored at -21°C for later analysis. Body parts were weighed as they were dissected from the animal and assigned to nine different fractions named as meat, bones, hide, offal, mammary

gland, retroperitoneal fat, mesenteric fat, omental fat and subcutaneous fat. The head, mammary gland, tail and feet (below carpal joint or hock) were detached first. The eyes and tongue were removed from the head, which was separated into two halves and the brain was removed. Claws were sawed off, weighed and removed. The full gastrointestinal tract, urinary and gall bladder were removed. The hide, heart, lung, liver, kidneys, uterus, spleen, thyroid gland, thymus and retroperitoneal adipose depot were dissected and weighed during the slaughter process. After separation of the gastrointestinal tract in reticulo-rumen, omasum/ abomasum and intestine, these parts were weighed full and after opening and cleaning of digests. Adherent mesenteric fat at the intestine and the omental adipose depot, which included fat from the reticulo-rumen, omasum and abomasum, were cut off and weighed. The carcass was divided into two parts, weighed and stored at 4°C. The offal fraction consists of the organs, tongue, eyes, brain, empty gastrointestinal tract and blood. Immediately after the slaughter the offal, mammary gland, retroperitoneal fat, mesenteric fat and omental fat fraction were homogenized in a meat cutter by 25 rounds per sample. Two representative samples were taken and stored at -21°C till analysis. The hide was first manually reduced to small pieces and then homogenized with the meat cutter by 30 rounds and then sampled. Carcass parts and the halves of the head were weighed once again on the next day for determination of water loss. Subcutaneous fat was dissected manually with a knife from the left carcass and weighted. The meat from the left carcass, left side of the head, left feet (meat was assigned to the meat of the carcass) and whole tail was also separated from the bones manually using a knife and weighed. The s.c. and meat fraction were homogenized in a meat cutter by 25 rounds and a double sample was taken. The bones were weighed and stored at -21°C until sawed in small pieces and homogenized in a bone mill.

Chemical Analyses

The composition of the feedstuffs (DM, ash, CP, ether extract, NDF and ADF) was determined according to the suggestions of the Association of German Agricultural Analysis and Research Centres (VDLUFA, 1993).

The feedstuffs, ileal chyme and faeces were analyzed for acid insoluble ash (**AIA**) content according to the methods described by Remling et al. (Remling et al., 2011). Samples were ashed for 5 h at 550°C in a muffle oven and ashes were boiled for 15 minutes with 4 N HCl. Afterwards the samples were filtrated by using an ash-free filter and washed neutrally with boiled water. The dried filters were ashed in the muffle oven for 5 h at 550°C to obtain the AIA.

Samples of the ileal chyme, faeces, meat, bone, offal, mammary gland, retroperitoneal fat, mesenteric fat, omental fat and s.c. fat fraction were thawed and dried by lyophilisation for determination of dry matter content. Afterwards the ileal chyme and the faeces were ground and the remainding samples were homogenized once again with a meat cutter. Exclusively the hide fraction was dried at 60°C in a forced-air oven and additionally homogenized with a mill (equipped with a 1 mm sieve). For exact dry matter determination for ileal chyme, faeces and all fractions remainder moisture was ascertained by 103°C in a drying oven. Ether extract was analyzed according to the methods of the Association of German Agricultural Analysis and Research Centres (VDLUFA, 1993) by extraction with petroleum ether. The analysis of the fractions and their composition (DM, ether extract, CP and ash) is described in detail elsewhere (von Soosten et al., 2012).

The fatty acid distribution of fat supplements, feedstuffs, ileal chyme, faeces, the meat, bone, offal, hide and mammary gland fraction was analyzed by extracting the lipid content according to Folch et al. (1957). For the omental, mesenteric, retroperitoneal and s.c. fat depot ,fraction lipid content was extracted with petrol ether. The lipid extract was incubated with Boron trifluoride (BF_3) to produce fatty acid methyl esters (FAME), which were purified by thin-layer chromatography (SIL G-25 UV_{254}, MACHERY-NAGEL, Germany) and quantified by gas chromatography (**GC**) (GC-17A Version 3, Schimadzu Corp., Kyoto, Japan) equipped with an auto sampler and flame ionisation detector. The applied GC procedures were as previously described by Degen et al. (2011a, 2011b).

Calculations

The apparent ileal digestibility for *t*10,*c*12 CLA was calculated using Equation 1.

Apparent digestibility (%) =
(((*t*10,*c*12 feed [%] / AIA feed [%])-(*t*10,*c*12 ileal chyme [%] / AIA ileal chyme [%]))
/ (*t*10,*c*12 feed [%] / AIA feed [%])) x 100 [1]

In Equation 1 the *t*10,*c*12 CLA and AIA data of the ileal chyme were substituted by data of the faeces to calculate the total *t*10,*c*12 CLA apparent digestibility in the whole digestive tract.

The calculation for the indices of Δ^9-desaturase (indicator for activity of stearoyl CoA desaturase) from the fatty acid composition was adapted from Jaudszus et al. (2010). The

desaturase index 1 using C16:0 as a substrate was calculated according to Equation 2 and the desaturase index 2 using C18:0 as a substrate was calculated using Equation 3.

$$\text{Desaturase index 1} = \text{C16:0} / (\text{C16:0} + c9\ \text{C16:1}) \quad [2]$$

$$\text{Desaturase index 2} = \text{C18:0} / (\text{C18:0} + c9\ \text{C18:1}) \quad [3]$$

Assuming that 90% of the fat mass were fatty acids, the total *t*10,*c*12 and *c*9,*t*11 CLA amount in the fat mass of the fractions was calculated by multiplying the fat mass of the fraction with the factor 0.9. The fat masses of the fractions are presented in our previous paper (von Soosten et al., 2012).

The total amount (mg) of *t*10,*c*12 CLA in the fractions at 42 and 105 DIM in the CLA groups is assumed as the deposited amount of *t*10,*c*12 CLA in the fractions during the supplementation time, because for the IG no *t*10,*c*12 CLA was contained in any of the fractions.

The *t*10,*c*12 CLA transfer efficiency is defined as the quotient of total *t*10,*c*12 CLA (mg) in the fraction and consumed amount of this isomer (252,000 mg in the 42 CLA group or 630,000 mg in the 105 CLA group) during the supplementation time multiplied by 100 to obtain percentage values.

Statistical Analyses

Data of the fatty acid pattern of the ileal chyme and faeces were analyzed with the GLM procedure as a 3-factorial design with supplementation (CON or CLA), DIM (42 or 105), measurement site (**MS**, ileal chyme or faeces), interactions of supplementation x DIM, interactions of supplementation x MS, interactions of DIM x MS and interactions of supplementation x DIM x MS as the fixed effects. The fatty acid composition, the desaturase indices and the total/deposited CLA amount of the nine fractions of the CON and CLA groups at the 42 and 105 DIM slaughter without the IG were analyzed by using the GLM procedure with treatment (CON or CLA), DIM (42 or 105), and interactions of treatment x DIM as the fixed effects. For all analyses, significance was declared when *P*-values were < 0.05 and a tendency was noted when $0.05 < P < 0.10$. All statistical analyses were carried out using the software SAS version 9.1 (SAS Institute Inc, 2004).

RESULTS AND DISCUSSION

Fatty acid composition of the ileal chyme and faeces

Supplementation of CLA increased the proportion of *c*9,*t*11 and *t*10,*c*12 CLA of total fatty acids in ileal chyme and faeces. Data for fatty acid composition of the CLA groups indicated a 2–fold increase of *c*9,*t*11 CLA. In CLA-supplemented cows values for *t*10,*c*12 CLA were remarkably higher compared to the CON groups (Table 2). This suggested that supplemented rumen-protected CLA bypassed the rumen and is available for absorption in the small intestine.

However, the selected intermediates of biohydrogenation and the sum of *trans* fatty acids were increased in CLA-supplemented cows (Table 2). The *t*11 C18:1 fatty acid (vaccenic acid) is a major monoenoic rumen fatty acid (Katz et al., 1966) originating from the incomplete ruminal biohydrogneation of linoleic acid to stearic acid. First the *c*9,*t*11 CLA isomer is produced by the microbial enzyme linolate isomerase from linoleic acid. The next step of biohydrogenation is the hydrogenation of *c*9,*t*11 CLA to *t*11 C18:1 before this isomer is saturated to stearic acid (Bauman et al., 1999). The control fat and CLA supplement contained no linoleic acid and the contents in the CON and CLA concentrate as well as the PMR were similar (Table 1). Therefore, higher contents of vaccenic acid in the ileal chyme and faeces of CLA-supplemented cows suggested that the supplemented *c*9,*t*11 CLA was partially hydrogenated to vaccenic acid in the rumen.

The *t*10 C18:1 isomer is a metabolic product of the rumen bacteria R7/5, gram negative rod or 2/9/1 gram negative vibrio by using linoleic or linolenic acid as a substrate (Hazlewood et al., 1976) and the conversion of *c*9 C18:1 (oleic acid) to *t*10 C18:1 was shown (Mortimer and Niehaus, 1972). Furthermore, Mortimer and Niehaus (1974) proposed pathways of the ruminal biohydrogenation of linoleic acid to stearic acid, in which the *t*10,*c*12 CLA biohydrogenation intermediate is hydrogenated to *t*10 C18:1 octadecenoic acid. The *t*10,*c*12 CLA is also produced from linolenic acid (Lee et al., 2011). The absence of *t*10,*c*12 CLA in the digests of the CON groups suggested that the higher amounts of *t*10 C18:1 in the ileal chyme and faeces for CLA cows was the result of conversion of *t*10,*c*12 CLA and/or oleic acid originating from the CLA supplement into *t*10 C18:1.

Table. 2 Fatty acid pattern of the ileal chyme and feaces in dependence of CLA supplementation, slaughter at different DIM and measurement site (MS; ileal chyme and faeces). Values (LS means) represent % of total fatty acid methyl esters

Variable	SUP[1]		DIM		MS		SEM[2]	*P*						
	CON	CLA	42	105	ileal chyme	faeces		SUP	DIM	MS	SUP* DIM	SUP* MS	DIM* MS	SUP* DIM*MS
Selected CLA isomers														
C18:2 *c*9,*t*11	0.11	0.22	0.18	0.15	0.14	0.20	0.02	<0.001	0.254	0.009	0.017	0.396	0.118	0.055
C18:2 *t*10,*c*12	<0.00	0.07	0.04	0.03	0.03	0.04	<0.01	<0.001	0.543	0.571	0.404	0.735	0.334	0.234
Selected intermediates of biohydrogenation														
C18:1 *t*10	0.21	0.46	0.28	0.39	0.28	0.39	0.05	0.002	0.146	0.133	0.365	0.614	0.996	0.911
C18:1 *t*11	0.81	1.29	0.99	1.11	0.68	1.42	0.08	<0.001	0.280	<0.001	0.562	0.056	0.969	0.762
∑C18:1 *t*FA	2.08	3.41	2.48	3.01	2.14	3.34	0.16	<0.001	0.025	<0.001	0.777	0.153	0.839	0.596
Fatty acids														
C16:0	13.88	14.74	15.13	13.50	13.50	15.12	0.29	0.043	<0.001	<0.001	0.100	0.033	0.254	0.735
C18:0	63.15	49.35	54.61	57.90	55.28	57.22	1.59	<0.001	0.148	0.388	0.395	0.910	0.497	0.515
SFA	87.53	78.05	82.50	83.08	78.17	87.40	1.47	<0.001	0.778	<0.001	0.592	0.136	0.492	0.694
MUFA	8.81	13.56	11.67	10.70	13.26	9.10	0.70	<0.001	0.326	<0.001	0.323	0.827	0.243	0.811
PUFA	3.67	8.40	5.84	6.23	8.57	3.50	0.97	0.001	0.775	0.001	0.924	0.038	0.845	0.671

[1] Supplementation: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the LS mean

The apparent ileal digestibility, determined using the AIA method for the *t*10,*c*12 CLA isomer averaged in the CLA groups 99.0% (99.2 ± 0.4% for the 42/CLA and 98.7 ± 0.3% for the 105/CLA group). The total apparent digestibility in the whole digestive tract averaged 99.3% in the CLA groups (99.3 ± 0.1% for the 42/CLA and 99.3 ± 0.2% for the 105/CLA group). Pappritz et al. (2012) determined that 0.1 g/d was excreted with faeces when cows consumed 8.0 g/d of this isomer. This corresponds to an apparent digestibility in the whole digestive tract of 98.8% and confirms the values obtained in the present study. Furthermore, in the same study by Pappritz et al. (2012) with duodenal fistulated cows, of the consumed 8 g/d *t*10,*c*12 CLA, 0.4 g/d reached the duodenum and suggested that 95% of supplemented CLA were biohydrogenated in the rumen. Assuming the same conditions in the present trial (cows consumed 6 g/d *t*10,*c*12 CLA), the amount of *t*10,*c*12 CLA available for absorption in the duodenum could be approximately 0.3 g/d. Calculation of duodenally available *t*10,*c*12 CLA according to equations published by de Veth et al. (de Veth et al., 2004) as previously described by von Soosten et al (von Soosten et al., 2011) revealed 1.1 g/d *t*10,*c*12 CLA available at the duodenum. In combination with the ileal digestibility of 99.0% for this isomer, 0.06 g/d *t*10,*c*12 CLA reached the ileum. The difference between *t*10,*c*12 CLA reaching the duodenum and ileum represented the amount of absorbed *t*10,*c*12 CLA in the small intestine. Therefore, these calculations suggested that the absorbed *t*10,*c*12 CLA amounts in the small intestine ranged from 0.24 until 1.04 g/d in the present study.

Further investigations concerning with the influence of ruminal biohydrogenation on rumen-protected CLA are rare. Wynn et al. (2006) demonstrated, using wethers fitted with ruminal and duodenal cannulas, how stable a rumen-protected CLA supplement, containing the *t*10,*c*12 and *c*9,*t*11 CLA isomer, was against ruminal degradation. The ruminal hydrogenation of the *t*10,*c*12 and *c*9,*t*11 CLA isomers was 34%.

In accordance with the higher content of stearic acid in the control fat supplement and the oleic acid, *t*10,*c*12 and *c*9,*t*11 CLA amount in the CLA supplement (von Soosten et al., 2011), the saturated fatty acids (**SFA**) of CLA supplemented cows were decreased in the ileal chyme and faeces. Concomitantly the monounsaturated fatty acids (**MUFA**) and polyunsaturated fatty acids (**PUFA**) in the CLA groups were increased compared to the CON group (Table 2).

An effect of MS for fatty acid composition of ileal chyme and faeces was observed for *c*9,*t*11 CLA, vaccenic acid, SFA, MUFA and PUFA (Table 2). The increased amounts of SFA in association with decreased MUFA and PUFA in the faeces suggested further alterations of fatty acids by microbial activity in the large intestine (Doreau et al., 1994).

Fatty acid composition of the fat depot fractions

The fatty acid compositions of the retroperitoneal, omental, mesenteric and s.c. fat depot fractions studied are presented in Tables 3 to 6. The *c*9,*t*11 CLA isomer was found in all fat depot fractions for each group and the amount of this isomer markedly exceeded the amount of *t*10,*c*12 CLA in all fat depot fractions. No effect of CLA supplementation was observed on the amount of *c*9,*t*11 CLA in any of the four fat depot fractions. The *t*10,*c*12 CLA isomer was not detectable in the fat depot fractions of the IG and CON groups. Except for the 105/CON group, marginal amounts of this isomer were detectable in the retroperitoneal fat depot fraction. For the 42/CLA and 105/CLA groups, *t*10,*c*12 CLA was detected in this fat depot (Table 3). In the omental, mesenteric and s.c. fat depot, *t*10,*c*12 CLA was found only in the 105/CLA group. This indicated that supplemented *t*10,*c*12 CLA was transferred into all fat depots, but amounts are only marginal. For example the total amount deposited into the retroperitoneal fat depot during the trial was 53 mg and 318 mg in the 42/CLA and 105/CLA group, respectively. In relation to the consumed amount of *t*10,*c*12 CLA the transfer efficiency into all fat depots was negligible and ranged from 0% in the omental, mesenteric and s.c. fat depot after 42 d of CLA supplementation to 0.06% in the omental fat depot after 105 d of CLA supplementation. To our knowledge, studies concerning the impact of CLA-supplementation on the fatty acid composition of fat depot fractions as in the present study are not available for lactating dairy cows. In lactating ewes (Sinclair et al., 2010) observed increasing amounts of *c*9,*t*11 CLA and *t*10,*c*12 CLA in the perirenal fat after CLA supplementation. Ewe lambs also showed an increase of *c*9,*t*11 and *t*10,*c*12 CLA in the perirenal, omental and mesenteric fat depot for CLA supplemented groups (Wynn et al., 2006). A reason for the lack of effect of CLA supplementation in the present study on the *c*9,*t*11 CLA amount in the fat depots could be the endogenous synthesis of this isomer via Δ^9-desaturase, which exceeded the deposition of supplemented c9,*t*11 CLA considerably. This synthesis seems to be the major source of *c*9,*t*11 CLA in fat originating from ruminants (Griinari et al., 1999, Griinari et al., 2000).

The calculated desaturase indices 1 and 2 (1 substrate C16:0; 2 substrate C18:0 for Δ^9-desaturase) could give insight into the activity of Δ^9-desaturase in the 4 fat depot fractions. No effects of CLA were observed for the two desaturase indices in the 4 fat depot fractions (Table 3 to 6) and suggested no CLA effects on the Δ^9-desaturase activity. This is consistent with the unchanged Δ^9-desaturase mRNA levels in perirenal, omental and s.c. fat depots of ewe lambs fed *c*9,*t*11 and *t*10,*c*12 CLA (Wynn et al., 2006).

Table 3. Fatty acid composition (selected fatty acids) of the **retroperitoneal** fat depot fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n =5)	42/CLA (n =5)	105/CON (n =5)	105/CLA (n =5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.15	0.18	0.19	0.19	0.20	0.02	0.414	0.569	0.921
C18:2 *t*10,*c*12	0.00	0.00	<0.01	<0.01	0.01	0.00	0.005	0.013	0.038
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.22	0.22	0.25	0.24	0.35	0.03	0.058	0.089	0.258
C18:1 *t*11	1.07	1.00	1.08	0.91	0.92	0.07	0.554	0.117	0.687
∑C18:1 *t*FA	2.63	2.50	2.73	2.32	2.55	0.13	0.110	0.215	0.993
Selected fatty acids									
C16:0	26.48	23.80	24.07	24.84	25.10	0.62	0.651	0.089	0.997
C16:1 *c*9	1.36	1.01	1.08	1.49	1.46	0.10	0.876	0.001	0.662
C18:0	29.86	32.99	32.48	29.35	29.50	0.96	0.868	0.006	0.755
C18:1 *c*9	29.58	30.02	29.78	32.41	31.06	1.03	0.483	0.114	0.621
SFA	63.70	63.59	63.46	60.82	61.80	1.05	0.704	0.061	0.620
MUFA	35.14	35.00	35.08	37.81	36.66	1.05	0.637	0.066	0.591
PUFA	1.16	1.41	1.47	1.37	1.54	0.10	0.266	0.875	0.568
Desaturase index 1[4]	0.95	0.96	0.96	0.94	0.94	0.00	0.897	0.001	0.637
Desaturase index 2[5]	0.50	0.52	0.52	0.47	0.49	0.01	0.752	0.019	0.659
Total CLA amount/included [6] into the fat depot fraction									
*c*9,*t*11 (mg)	9,673	6,006	8,062	7,654	6,141	1,461	0.863	0.931	0.267
*t*10,*c*12 (mg)	0	0	52	33	318	40	0.002	0.004	0.020
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fat depot fraction									
%			0.02		0.05				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Table 4. Fatty acid composition (selected fatty acids) of the **omental** fat depot fraction. As well as the total amount and transfer efficiency of supplemented *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.13	0.12	0.15	0.15	0.12	0.02	0.988	0.805	0.219
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	<0.01	0.00	0.332	0.332	0.332
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.28	0.24	0.26	0.28	0.33	0.02	0.109	0.026	0.639
C18:1 *t*11	1.06	0.93	1.07	0.88	0.90	0.06	0.257	0.106	0.373
∑C18:1 *t*FA	3.00	2.78	2.98	2.58	2.80	0.13	0.121	0.158	0.903
Selected fatty acids									
C16:0	27.59	26.56	25.71	26.45	27.45	0.61	0.901	0.223	0.169
C16:1 *c*9	1.19	1.09	1.11	1.33	1.26	0.08	0.729	0.043	0.622
C18:0	30.66	31.53	32.15	28.42	30.23	0.93	0.240	0.022	0.559
C18:1 *c*9	27.66	28.60	28.17	31.61	28.32	1.07	0.118	0.180	0.221
SFA	65.58	65.03	65.05	61.68	64.96	1.13	0.187	0.170	0.190
MUFA	33.27	33.86	33.66	37.08	33.86	1.12	0.170	0.168	0.222
PUFA	1.15	1.11	1.30	1.23	1.17	0.14	0.682	0.994	0.433
Desaturase index 1[4]	0.96	0.96	0.96	0.95	0.96	0.00	0.729	0.080	0.288
Desaturase index 2[5]	0.53	0.52	0.53	0.47	0.52	0.02	0.131	0.051	0.323
Total CLA amount/deposited [6] into the fat depot fraction									
*c*9,*t*11 (mg)	9,895	5,999	9,247	8,570	5,839	1,795	0.898	0.836	0.151
*t*10,*c*12 (mg)	0	0	0	0	173	77	0.332	0.332	0.332
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fat depot fraction									
%			0.00		0.03				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Table 5. Fatty acid composition (selected fatty acids) of the **mesenteric** fat depot fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.10	0.14	0.16	0.19	0.21	0.02	0.397	0.017	0.954
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	0.02	<0.01	<0.001	<0.001	<0.001
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.30	0.26	0.29	0.32	0.38	0.03	0.155	0.015	0.567
C18:1 *t*11	1.12	0.99	1.11	0.95	0.96	0.07	0.375	0.217	0.485
∑C18:1 *t*FA	3.03	2.80	3.00	2.73	2.81	0.12	0.231	0.274	0.644
Selected fatty acids									
C16:0	26.99	25.69	25.23	25.21	25.86	0.66	0.879	0.913	0.396
C16:1 *c*9	1.15	1.02	1.05	1.27	1.25	0.09	0.979	0.030	0.816
C18:0	32.18	33.14	33.47	30.11	31.20	1.05	0.538	0.032	0.741
C18:1 *c*9	26.49	27.57	26.96	30.62	28.29	1.21	0.248	0.093	0.494
SFA	66.59	65.68	65.90	61.98	64.16	1.32	0.382	0.059	0.474
MUFA	32.33	33.05	32.69	36.31	33.95	1.28	0.315	0.104	0.458
PUFA	1.08	1.28	1.42	1.72	1.89	0.11	0.197	0.001	0.902
Desaturase index 1[4]	0.96	0.96	0.96	0.95	0.95	<0.01	0.982	0.025	0.612
Desaturase index 2[5]	0.55	0.55	0.55	0.50	0.52	0.02	0.331	0.045	0.588
Total CLA amount/deposited [6] into the fat depot fraction									
*c*9,*t*11 (mg)	4,099	3,910	6,265	5,025	5,406	1,132	0.285	0.919	0.437
*t*10,*c*12 (mg)	0	0	0	0	383	26	<0.001	<0.001	<0.001
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fat depot fraction									
%			0.00		0.06				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Table 6. Fatty acid composition (selected fatty acids) of the **s.c.** fat depot fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.56	0.49	0.53	0.55	0.50	0.05	0.853	0.773	0.331
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	<0.01	<0.01	<0.001	<0.001	<0.001
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.20	0.20	0.21	0.23	0.24	0.02	0.447	0.098	0.907
C18:1 *t*11	0.70	0.65	0.70	0.62	0.58	0.06	0.875	0.238	0.466
∑C18:1 *t*FA	1.95	1.88	1.93	1.83	1.80	0.10	0.923	0.419	0.726
Selected fatty acids									
C16:0	25.47	24.46	24.76	23.97	24.41	0.78	0.631	0.583	0.932
C16:1 *c*9	6.65	5.15	5.79	6.57	5.74	0.67	0.893	0.345	0.307
C18:0	10.65	12.62	12.14	11.06	13.42	1.09	0.431	0.903	0.237
C18:1 *c*9	42.95	44.55	43.62	44.49	43.29	1.22	0.431	0.886	0.921
SFA	42.18	42.82	42.90	40.53	43.90	1.70	0.342	0.717	0.365
MUFA	55.78	55.34	55.11	57.39	54.21	1.69	0.347	0.749	0.413
PUFA	2.04	1.85	1.99	2.09	1.90	0.10	0.814	0.472	0.134
Desaturase index 1[4]	0.79	0.83	0.81	0.79	0.81	0.02	0.852	0.330	0.287
Desaturase index 2[5]	0.20	0.22	0.22	0.20	0.24	0.02	0.380	0.877	0.309
Total CLA amount/deposited [6] into the fat depot fraction									
*c*9,*t*11 (mg)	14,453	9,156	15,228	13,357	8,380	2,743	0.860	0.669	0.088
*t*10,*c*12 (mg)	0	0	0	0	73	11	0.011	0.011	0.011
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fat depot fraction									
%			0.00		0.01				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Interestingly, an effect of DIM for both desaturase indices was shown. In the retroperitoneal and mesenteric fat fraction at 105 DIM, the amount of *c*9 C16:1 (possible product of Δ^9-desaturase) was increased and the desaturase index 1 was decreased (here lower values indicated a higher activity of Δ^9-desaturase). Additionally, the decreased amount of C18:0 in combination with the numerically increased *c*9 C18:1 fatty acid (possible product of Δ^9-desaturase) in the fatty acid profile of the retroperitoneal fat resulted in a lower desaturase index 2 for the 105 DIM groups as compared to the 42 DIM groups. These time effects indicated an increased activity of Δ^9-desaturase with progressive DIM in the retroperitoneal and mesenteric fat depot fraction. These desaturase indices in the omental fat fraction tended to be decreased and were not changed for the s.c. fat depot (Table 4 and 6). Although no time dependent changes were found in the s.c. fat fraction on the desaturase index 2, its value was one half of the destaturase index 2 of the retroperitoneal, omental, and mesenteric fat fraction. Concomitantly, the amount of *c*9,*t*11 CLA was 2-fold higher in the s.c. fat fraction as compared to the omental, mesenteric and retroperitoneal fat fraction. This suggests differences in activity of Δ^9-desaturase between the fat depots. The Δ^9-desaturase activity seems to be constant during early lactation in the s.c. fat fraction, whereby its activity increased during early lactation in the retroperitoneal, mesenteric and slightly in the omental fat fraction. For Angus-Wagyu heifers, the study by Beaulieu et al. (2002) showed higher contents of *c*9,*t*11 CLA in the s.c fat than in the mesenteric and perirenal fat. Furthermore the calculated desaturase index of the s.c. fat suggested an increased Δ^9-desaturase activity compared to the mesenteric and perirenal fat depot. This confirms the assumption of different Δ^9-desaturase activity between s.c. and visceral fat (retroperitoneal, omental and mesenteric fat) observed in the present study.

Fatty acid composition of the offal, bone, hide, meat and mammary gland faction

In the offal fraction higher proportions of *c*9,*t*11 CLA were observed after 105 DIM. The *t*10,*c*12 CLA isomer was not detectable or only marginally evident in the 105/CLA group. For the selected intermediates of biohydrogenation, the *t*10 C18:1 fatty acid was increased after 105 DIM and the sum of C18:1 *trans* fatty acids was increased in the 42 and 105 CLA group. The total amount of *c*9,*t*11 CLA was numerically higher after 105 DIM (Table 7).

Table 7. Fatty acid composition (selected fatty acids) of the **offal** fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.14	0.10	0.12	0.14	0.15	0.01	0.179	0.002	0.737
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	<0.01	<0.01	0.332	0.332	0.332
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.26	0.25	0.29	0.30	0.37	0.03	0.092	0.046	0.575
C18:1 *t*11	1.02	0.97	1.08	0.91	0.96	0.06	0.212	0.156	0.635
∑C18:1 *t*FA	2.80	2.75	3.06	2.68	2.91	0.12	0.038	0.381	0.753
Selected fatty acids									
C16:0	27.45	26.73	25.99	26.55	26.74	0.61	0.658	0.641	0.449
C16:1 *c*9	2.13	1.74	1.94	2.22	2.19	0.18	0.661	0.080	0.554
C18:0	24.73	26.78	26.00	23.72	24.33	1.13	0.943	0.068	0.576
C18:1 *c*9	31.70	31.68	32.09	34.25	32.70	1.19	0.663	0.235	0.459
SFA	59.54	60.53	59.28	57.02	58.30	1.39	0.991	0.155	0.413
MUFA	38.70	38.14	39.24	41.44	39.97	1.41	0.903	0.202	0.410
PUFA	1.76	1.33	1.48	1.54	1.73	0.13	0.204	0.094	0.908
Desaturase index 1[4]	0.93	0.94	0.93	0.92	0.92	0.01	0.554	0.101	0.452
Desaturase index 2[5]	0.44	0.46	0.45	0.41	0.43	0.02	0.857	0.106	0.517
Total CLA amount/deposited [6] into the fraction									
*c*9,*t*11 (mg)	12,932	6,753	8,724	11,600	12,425	1,319	0.342	0.009	0.694
*t*10,*c*12 (mg)	0	0	0	0	118	53	0.332	0.332	0.332
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fraction									
%			0.00		0.02				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Comparable studies providing data of the fatty acid composition of a fraction similar to the offal fraction of the present study are not available for ruminants. Nevertheless, the study by Sinclair et al. (2010) showed an uptake of *c*9,*t*11 CLA and *t*10,*c*12 CLA in the liver and heart, which are part of the offal fraction in the present study.

The CLA supplementation did not affect *c*9,*t*11 CLA in the bone fraction and no *t*10,*c*12 CLA was contained in the bone fraction (Table 8). For the *c*9 C18:1 (oleic acid) intermediate of biohydrogenation, an interaction of CLA supplementation and DIM was observed. The *t*10 C18:1, *t*11 C18:1 and the sum of *trans* fatty acids in the bone fraction were increased due to CLA supplementation. The SFA and the MUFA were not affected by CLA supplementation and DIM, but an interaction between these factors was observed. In the CLA group the SFA were increased with DIM and for the MUFA the content decreased with DIM, whilst in the CON groups the opposite was found. The desaturase index in the bone fraction was not affected by CLA supplementation. After 105 DIM the desaturase index 2 was decreased and an interaction of DIM with CLA supplementation was observed. With CLA supplementation the desaturase index increased with DIM and for the CON group this was opposite. The total CLA amount and inclusion of the supplemented CLA was not affected by any of the factors. Effects of CLA supplementation on fatty acid composition of dairy cows bones have not been well investigated. In mice CLA supplementation improved bone mass, but fatty acid composition was not investigated (Park et al., 2011).

The fatty acid pattern of the hide fraction was not altered by CLA supplementation (Table 9). Differences were shown between the fatty acid composition of 105 DIM slaughtered animals and 42 DIM slaughtered animals. The desaturase indices indicated an increased activity of the Δ^9-desaturase after 105 DIM. The fatty acids related to these indices were changed accordingly. The higher Δ^9-desaturase activity could be the reason for higher *c*9,*t*11 CLA amounts in the hide faction after 105 DIM.

Table 8. Fatty acid composition (selected fatty acids) of the **bone** fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.33	0.32	0.36	0.36	0.37	0.02	0.112	0.220	0.493
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	0.00	0.00	n.c.	n.c.	n.c.
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.25	0.24	0.29	0.27	0.36	0.03	0.024	0.099	0.502
C18:1 *t*11	0.99	0.98	1.09	0.91	1.06	0.05	0.033	0.359	0.762
∑C18:1 *t*FA	2.55	2.48	2.84	2.45	2.91	0.13	0.004	0.905	0.677
Selected fatty acids									
C16:0	20.28	19.67	19.48	20.36	20.80	0.40	0.734	0.017	0.421
C16:1 *c*9	2.14	2.27	2.37	2.44	2.43	0.10	0.701	0.322	0.608
C18:0	21.35	20.37	18.59	16.82	18.91	0.59	0.780	0.009	0.003
C18:1 *c*9	44.12	45.63	46.54	47.95	44.59	0.88	0.111	0.804	0.010
SFA	46.96	45.20	43.41	42.18	45.15	0.89	0.457	0.417	0.007
MUFA	51.16	52.92	54.40	55.50	52.49	0.90	0.346	0.672	0.011
PUFA	1.88	1.88	2.20	2.32	2.36	0.09	0.078	0.005	0.154
Desaturase index 1[4]	0.90	0.90	0.89	0.89	0.90	0.00	0.733	0.879	0.401
Desaturase index 2[5]	0.33	0.31	0.29	0.26	0.30	0.01	0.378	0.044	0.002
Total CLA amount/deposited [6] into the fraction									
*c*9,*t*11 (mg)	25,002	26,012	29,740	33,438	30,859	2,343	0.822	0.108	0.227
*t*10,*c*12 (mg)	0	0	0	0	0	0	n.c.	n.c.	n.c.
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fraction									
%			0		0				

n.c. = not calculated (*t*10,*c*12 CLA was not detectable); [1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.; [2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Table 9. Fatty acid composition (selected fatty acids) of the **hide** fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.19	0.14	0.08	0.23	0.21	0.03	0.212	0.002	0.440
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	0.00	0.00	n.c.	n.c.	n.c.
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.18	0.14	0.15	0.20	0.22	0.02	0.396	0.004	0.921
C18:1 *t*11	0.50	0.41	0.43	0.46	0.47	0.04	0.686	0.388	0.905
∑C18:1 *t*FA	1.54	1.36	1.38	1.49	1.59	0.08	0.534	0.081	0.627
Selected fatty acids									
C16:0	27.83	31.60	32.05	26.53	26.33	1.14	0.919	<0.001	0.793
C16:1 *c*9	6.60	4.51	5.30	6.96	5.73	0.58	0.701	0.021	0.093
C18:0	10.22	13.14	12.28	9.81	11.65	0.72	0.487	0.011	0.069
C18:1 *c*9	41.49[a]	36.14	35.97	42.41	42.34	1.30	0.933	<0.001	0.970
SFA	44.65	53.03	52.70	42.72	44.82	1.89	0.655	<0.001	0.541
MUFA	54.20	45.70	46.44	56.03	53.73	1.84	0.687	<0.001	0.438
PUFA	1.15	1.27	0.87	1.25	1.46	0.14	0.470	0.053	0.037
Desaturase index 1[4]	0.81	0.88	0.86	0.79	0.82	0.02	0.745	0.002	0.158
Desaturase index 2[5]	0.20	0.27	0.26	0.19	0.22	0.01	0.573	0.001	0.216
Total CLA amount/deposited [6] into the fraction									
*c*9,*t*11 (mg)	5,004	2,335	1,862	7,050	4,054	1,480	0.297	0.048	0.444
*t*10,*c*12 (mg)	0	0	0	0	0	0	n.c.	n.c.	n.c.
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fraction									
%			0		0				

n.c. = not calculated (*t*10,*c*12 CLA was not detectable); [1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.; [2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

No effect of CLA supplementation was observed on the fatty acid composition of the meat fraction (Table 10). The supplemented CLA was not deposited in this fraction. The *c*9,*t*11 CLA was found in all groups and amounts were equal across the groups. The *t*10,*c*12 CLA isomer was not detectable in the meat fraction and the transfer efficiency into this fraction was 0%. However, in studies with lactating ewes (Sinclair et al., 2010) and ewe lambs (Wynn et al., 2006), CLA supplementation increased the *c*9,*t*11 and *t*10,*c*12 CLA amount in the *longissimus* muscle.

Table 10. Fatty acid composition (selected fatty acids) of the **meat** fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.32	0.29	0.32	0.34	0.34	0.02	0.592	0.183	0.463
C18:2 *t*10,*c*12	0.00	0.00	0.00	0.00	0.00	0.00	n.c.	n.c.	n.c.
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.95	0.89	0.98	0.86	0.88	0.03	0.141	0.086	0.446
C18:1 *t*11	0.95	0.89	0.98	0.86	0.88	0.06	0.364	0.278	0.565
∑C18:1 *t*FA	2.56	2.47	2.62	2.41	2.56	0.11	0.171	0.594	0.989
Selected fatty acids									
C16:0	25.28	24.62	24.46	24.93	24.91	0.65	0.890	0.554	0.917
C16:1 *c*9	3.41	2.90	3.12	3.64	3.26	0.22	0.744	0.086	0.227
C18:0	18.01	18.99	18.75	16.47	17.81	0.77	0.501	0.047	0.338
C18:1 *c*9	39.16	40.24	39.65	41.16	40.12	0.94	0.430	0.501	0.825
SFA	49.76	49.62	49.64	47.45	48.90	1.07	0.527	0.220	0.535
MUFA	47.91	48.23	48.07	50.21	48.52	1.08	0.430	0.303	0.514
PUFA	2.33	2.15	2.30	2.34	2.57	0.12	0.113	0.053	0.718
Desaturase index 1[4]	0.88	0.89	0.89	0.87	0.88	0.01	0.802	0.106	0.172
Desaturase index 2[5]	0.31	0.32	0.32	0.29	0.31	0.01	0.426	0.085	0.442
Total CLA amount/deposited [6] into the fraction									
*c*9,*t*11 (mg)	106,543	67,053	85,315	104,092	80,505	13,600	0.860	0.294	0.178
*t*10,*c*12 (mg)	0	0	0	0	0	0	n.c.	n.c.	n.c.
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fraction									
%			0		0				

n.c. = not calculated (*t*10,*c*12 CLA was not detectable); [1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.; [2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Table 11. Fatty acid composition (selected fatty acids) of the **mammary gland** fraction. As well as the total amount of supplemented CLA and transfer efficiency of *t*10,*c*12 CLA into the fraction of cows slaughtered at different time points and fed no fat supplement (IG), the CON diet or CLA supplemented diet. Values (means) represent % of total fatty acid methyl esters

Variable	Initial group (IG) (n=5)	Treatment[1]				SEM[2]	*P*[3]		
		42/CON (n=5)	42/CLA (n=5)	105/CON (n=5)	105/CLA (n=5)		CLA	DIM	CLA*DIM
Selected CLA isomers									
C18:2 *c*9,*t*11	0.24	0.26	0.32	0.34	0.40	0.03	0.039	0.007	0.980
C18:2 *t*10,*c*12	0.00	0.00	0.01	0.00	0.01	<0.01	0.002	0.603	0.603
Selected intermediates of biohydrogenation									
C18:1 *t*10	0.22	0.26	0.34	0.31	0.51	0.09	0.188	0.283	0.585
C18:1 *t*11	0.77	0.82	0.91	0.74	0.90	0.05	0.030	0.439	0.571
∑C18:1 *t*FA	2.29	2.43	2.81	2.39	2.91	0.20	0.055	0.885	0.744
Selected fatty acids									
C16:0	31.38	29.08	27.52	28.66	27.10	0.55	0.021	0.494	1.000
C16:1 *c*9	2.37	2.12	2.33	2.77	2.70	0.17	0.716	0.011	0.434
C18:0	17.43	17.50	18.35	15.48	16.59	0.78	0.095	0.003	0.810
C18:1 *c*9	32.18	33.54	33.54	35.37	35.99	1.24	0.796	0.089	0.796
SFA	59.00	57.59	56.48	54.27	53.03	1.27	0.320	0.009	0.957
MUFA	39.05	40.39	41.10	43.28	44.10	1.35	0.540	0.029	0.963
PUFA	1.96	2.02	2.42	2.45	2.86	0.21	0.072	0.056	0.983
Desaturase index 1[4]	0.93	0.93	0.92	0.91	0.91	0.01	0.269	0.007	0.439
Desaturase index 2[5]	0.35	0.34	0.35	0.30	0.32	0.02	0.338	0.004	0.979
Total CLA amount/deposited [6] into the fraction									
*c*9,*t*11 (mg)	8,603	6,397	8,288	7,820	6,344	1,194	0.865	0.832	0.181
*t*10,*c*12 (mg)	0	0	270	0	88	36	<0.001	0.039	0.039
Transfer efficiency of supplemented *t*10, *c*12 CLA into the fraction									
%			0.08		0.01				

[1] Treatment: CLA = cows consumed 6 g/d *t*10,*c*12 CLA and 5.7 g/d *c*9,*t*11 CLA; CON = cows received a control fat supplement.
[2] Pooled standard error of the mean; [3] Statistical analyses without data of the IG.
[4] Desaturase index 1: C16:0/(C16:0 + C16:1 *c*9); [5] Desaturase index 2: C18:0/(C18:0 + C18:1 *c*9)
[6] The amount of the *t*10,*c*12 CLA isomer in the fraction for the 42 and 105 DIM groups represents the deposited amount of this isomer into the fraction, because the amount of this isomer in the IG was zero.

Data for the mammary gland fraction could be influenced by residual milk which remained when preparing the mammary gland for analysis. The CLA supplementation increased the content of *c*9,*t*11 and *t*10,*c*12 CLA in the mammary gland fraction (Table 11). The transfer efficiency of *t*10,*c*12 CLA was low, with 0.08% and 0.01% for the 42/CLA and 105/CLA group, respectively. The desaturase indices were not altered by CLA supplementation. Therefore, the increased amounts of *c*9,*t*11 CLA in the CLA groups indicated that the supplemented *c*9,*t*11 CLA isomer was transferred into the mammary gland fraction. After 105 DIM the time effect on the desaturase indices indicated a higher activity of Δ^9-desaturase, which is confirmed by increased amounts of *c*9,*t*11 CLA in the 105/CON and 105/CLA groups.

CONCLUSION

Supplementation of *t*10,*c*12 and *c*9,*t*11 CLA increased the amount of these isomers in the ileal chyme and faeces, despite ruminal biohydrogenation of the majority of consumed *t*10,*c*12 CLA. The supplemented *t*10,*c*12 CLA isomer was deposited in the retroperitoneal, mesenteric, s.c. and mammary gland fraction, but the transfer efficiencies of less than 0.1% of consumed *t*10,*c*12 CLA into these fractions were very low. A transfer of the *t*10,*c*12 CLA isomer into the meat fraction was not observed. Overall, the accumulated *t*10,*c*12 CLA transfer efficiency across all fractions revealed 0.10% of consumed *t*10,*c*12 CLA from 1 until 42 DIM and 0.18% from 1 until 105 DIM. The Δ^9-desaturase indices were not affected by CLA supplementation. However, the desaturase indices suggested an increased activity of Δ^9-desaturase in the retroperitoneal, mesenteric and mammary gland fraction with progressive DIM.

ACKNOWLEDGEMENTS

The authors would like to thank the Deutsche Forschungsgemeinschaft (DFG) for financial support (PAK 286/1, DA 558/5-1). Furthermore, the assistance of the co-workers of the Institute of Animal Nutrition and the experimental station of the Friedrich-Loeffler-Institute (FLI) in Brunswick Germany in performing the experiment and analyses is gratefully acknowledged.

REFERENCES

Akter, S., S. Dänicke, U. Mueller, D. von Soosten, J. Rehage, and H. Sauerwein. 2011. Physiological and conjugated linoleic acids-induced changes of adipocyte size in different fat depots of dairy cows during early lactation. J. Dairy. Sci. 94:2859-2870.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 1999. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci. Available: http://www.asas.org/symposia/9899proc/0937.pdf.

Beaulieu, A. D., J. K. Drackley, and N. R. Merchen. 2002. Concentrations of conjugated linoleic acid (cis-9, trans-11-octadecadienoic acid) are not increased in tissue lipids of cattle fed a hiph-concentrate diet supplemented with soybean oil. J. Anim. Sci. 80:847-861.

de Veth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of cla on milk fat synthesis in dairy cows: Comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-372.

Degen, C., J. Ecker, S. Piegholdt, G. Liebisch, G. Schmitz, and G. Jahreis. 2011a. Metabolic and growth inhibitory effects of conjugated fatty acids in the cell line ht-29 with special regard to the conversion of *t*11,*t*13-cla. Biochim. Biophys. Acta 1811:1070-1080.

Degen, C., A. Lochner, S. Keller, K. Kuhnt, S. Daenicke, and G. Jahreis. 2011b. Influence of in vitro supplementation with lipids from conventional and alpine milk on fatty acid distribution and cell growth of ht-29 cells. Lipids Health Dis. 10:131.

Doreau, M. and A. Ferlay. 1994. Digestion and utilization of fatty-acids by ruminants. Anim. Feed Sci. Tech. 45:379-396.

Flachowsky, G., K. Erdmann, L. Hüther, G. Jahreis, P. Moeckel, and P. Lebzien. 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. Arch. Anim. Nutr. 60:501-511.

GfE. 1991. Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. (Guidelines for determining the digestibility of crude nutrients in ruminants). J. Anim. Physiol. Anim. Nutr. 65:229-234.

Griinari, J. M. and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: M. P. Yurawecz, M. M. Mossoba,

J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (Ed.) Advances in conjugated linoleic acid research, Vol. 1. pp 180-200. AOCS Press, Champaign (IL).

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta(9)-desaturase. J. Nutr. 130:2285-2291.

Hazlewood, G. P., P. Kemp, D. Lander, and R. M. C. Dawson. 1976. C-18 unsaturated fatty-acid hydrogenation patterns of some rumen bacteria and their ability hydrolyze exogenous phospholipid. Br. J. Nutr. 35:293-297.

Jaudszus, A., P. Moeckel, E. Hamelmann, and G. Jahreis. 2010. Trans-10,cis-12-cla-caused lipodystrophy is associated with profound changes of fatty acid profiles of liver, white adipose tissue and erythrocytes in mice: Possible link to tissue-specific alterations of fatty acid desaturation. Ann. Nutr. Metab. 57:103-111.

Katz, I. and M. Keeney. 1966. Characterization of the octadecenoic acids in rumen digesta and rumen bacteria. J. Dairy. Sci. 49:962-966.

Kramer, J. K. G., P. W. Parodi, R. G. Jensen, M. M. Mossoba, M. P. Yurawecz, and R. O. Adlof. 1998. Rumenic acid: A proposed common name for the major conjugated linoleic acid isomer found in natural products. Lipids 33:835-835.

Lee, Y. and T. C. Jenkins. 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. J. Nutr. 141:1445-1450.

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

Mortimer, C. E. and W. G. Niehaus. 1974. Enzymatic interconversion of oleic acid, 10-hydroxyoctadecanoic acid, and trans-Δ^{10}-octadecenoic acid. J. Biol. Chem. 249:2833-2842.

Mortimer, C. E. and W. G. Niehaus. 1972. Enzymatic isomerization of oleic acid to trans-Δ^{10}-octadecenoic acid. Biochem. Biophys. Res. Commun. 49:1650-1656.

Pappritz, J., P. Lebzien, U. Meyer, G. Jahreis, R. Kramer, G. Flachowsky, and S. Dänicke. 2011. Duodenal availability of conjugated linoleic acids after supplementation to dairy cow diets. Eur. J. Lipid Sci. Technol. 113:1443-1455.

Park, Y., M. Terk, and Y. Park. 2011. Interaction between dietary conjugated linoleic acid and calcium supplementation affecting bone and fat mass. J. Bone Miner. Metab. 29:268-278.

Remling, N., S. Hachenberg, U. Meyer, M. Höltershinken, G. Flachowsky, and S. Dänicke. 2011. Influence of various amounts of fumaric acid on performance and parameters of the acid–base balance of growing bulls fed with grass or maize silage. Arch. Anim. Nutr. 65:386-401.

SAS Institute Inc. 2004. SAS/STAT 9.1 user's guide. SAS Inst., Inc., Cary, NC.

Sinclair, L. A., M. P. B. Weerasinghe, R. C. Wilkinson, M. J. de Veth, and D. E. Bauman. 2010. A supplement containing trans-10, cis-12 conjugated linoleic acid reduces milk fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

VDLUFA. 1993. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch). Vol. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt. Germany.

von Soosten, D., U. Meyer, M. Piechotta, G. Flachowsky, and S. Dänicke. 2012. Effect of conjugated linoleic acid supplementation on body composition, body fat mobilization, protein accretion and energy utilization in early-lactation dairy cows. J. Dairy Sci. 95:1222-1239.

von Soosten, D., U. Meyer, E. M. Weber, J. Rehage, G. Flachowsky, and S. Dänicke. 2011. Effect of trans-10, cis-12 conjugated linoleic acid on performance, adipose depot weights, and liver weight in early-lactation dairy cows. J. Dairy. Sci. 94:2859-2870.

Wynn, R. J., Z. C. T. R. Daniel, C. L. Flux, J. Craigon, A. M. Salter, and P. J. Buttery. 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.

7. General discussion

The aim of the present study was to investigate the effects of rumen protected *t*10,*c*12 and *c*9,*t*11 CLA supplementation on milk fat content, milk fat synthesis and body fat mobilization during early lactation, as well as the CLA effects on body fat content and especially on body fat depots in dairy cows. The core of this study was the determination of the body composition in early lactation dairy cows and the calculation of body fat and protein mobilization/accretion by applying the principle of the comparative slaughter technique (Paper II).

Taking into account the different metabolic status of tissues in the early lactation dairy cow, CLA effects are discussed according to the most important tissues: mammary gland, liver and fat depots. The relationships of fat and energy metabolism between these tissues and possible CLA effects on these relationships are considered in more detail here. Finally, CLA effects on the whole energy metabolism in early lactation dairy cows are discussed.

7.1 CLA effects on fat metabolism in the mammary gland

The milk fat reducing effect of the *t*10,*c*12 CLA isomer is well investigated in lactating dairy cows. Studies dealing with the effect of CLA supplementation on the synthesis of milk components (fat, protein and lactose) in early lactation are available. In these studies the daily milk fat synthesis was decreased and showed a repartitioning of the nutrients and energy from milk fat to milk lactose resulting in higher milk yield (Bernal-Santos et al., 2003, Odens et al., 2007). This is in line with findings in the current trial. From 1 until 42 DIM the CLA fed cows synthesised 100 g/d less milk fat than cows of the CON group. Milk protein synthesis was nearly comparable but 60 g/d more lactose was synthesized. From 42 until 105 DIM CLA supplemented cows synthesized 180 g/d less milk fat. The daily protein yield remained constant and 120 g/d more lactose was synthesized in the CLA group. The higher amounts of synthesized lactose in CLA supplemented cows indicated that nutrients and energy spared by milk fat synthesis was partitioned to higher milk yield which was slightly increased during the trial (Paper I). However, the markedly higher reduction in milk fat synthesis against the slightly higher milk lactose synthesis suggested that there is additional energy available from energy spared by reduced milk fat synthesis. This topic is discussed later in the energy metabolism section.

For synthesis of milk fat the mammary gland uses different energy sources and nutrients. Fatty acids for milk fat synthesis are formed by *de novo* synthesis in mammary epithelial cells or originate from preformed long chain fatty acids, after uptake by the mammary gland from circulation. The de novo synthesis of milk fatty acids from acetate and β-hydroxybutyrate (**BHBA**) amounts to 50% (Blum, 2004). Preformed fatty acids (e.g., VLDL secreted by the liver or NEFA) presented the other 50% as substrate for synthesized milk fatty acids. In established lactation, circulating NEFA accounted for 10% of milk fatty acids. The uptake of NEFA into the mammary gland is dependent on the plasma NEFA concentration. Therefore, in the very early lactation the increasing blood flow to the mammary gland and higher plasma NEFA concentration leads to an increased uptake of NEFA into the mammary gland. The milk fatty acids derived from plasma NEFA accounted for as much as 40% of milk fatty acids in very early lactation (Bell, 1995) and resulted in an attenuated contribution of de novo milk fat synthesis. These processes have to be considered when interpreting the milk fat reduction effects in the present study. At the beginning of the trial (from 1 until 21 DIM) the slow initial reduction of milk fat content could have been caused by a higher contribution of plasma NEFA for milk fat synthesis and masked the CLA effect on de novo fatty acid synthesis. During this period the blood plasma NEFA concentrations were particularly high (Paper I). Data for milk fatty acid composition from the present experiment are not available at this time, but data from the study of Pappritz et al. (2011b) supported this conclusion. A noticeable decrease of the stearic acid (C18:0) proportion in milk fat of the unsupplemented control group from 7 through to 28 and then to 84 DIM was shown with values of 12.8, 11.4 and 8.5%, respectively. Palmitic acid (C16:0) was increased over the same time points and values were 26.2 at 7 DIM, 28.7 at 28 DIM and 32.3 at 84 DIM. Stearic acid does not originate from de novo synthesis, while palmitic acid is partially synthesised de novo. For the CLA supplemented (8g/d *t*10,*c*12 CLA) group the increase of partially *de novo* synthesised palmitic acid proportion in milk fat was decelerated and values were 26.1 at 7 DIM, 27.5 at 28 DIM and 29.4 at 84 DIM. These results confirmed that CLA reduces milk fat synthesis by a reduction of de novo fatty acid synthesis, but in the very early lactation this was masked by increased uptake of NEFA from circulation. It was shown in a study by Harvatine and Bauman (2011) that CLA influenced both pathways of milk fat synthesis, but the reduction of milk fat synthesis was more pronounced for *de novo* synthesised fatty acids.

7.2 CLA effects on fat metabolism in the liver

With the onset of lactation, fatty liver (hepatic lipidosis) is a typical metabolic disorder in dairy cows. The NEB after parturition causes the mobilization of body fat and leads to elevated plasma NEFA concentrations. The NEFA are taken up by the liver, but uptake of NEFA exceeds the oxidation and release of lipids from liver through VLDL. The NEFA are esterified as TG and are accumulated in the liver. The development of hepatic lipidosis preceded ketosis and is related to decreased health status and reproductive performance (Grummer, 1993). In general, TG accumulation in the liver is an unavoidable process in the early lactation of the high yielding dairy cow. Accumulated TG are not being used efficiently for energy needs for maintenance or production (Wensing et al., 1997) and an important metabolic function of the liver, the hepatic gluconeogenesis, could be reduced (Rukkwamsuk et al., 1999). Therefore, the extent of TG accumulation in the liver determines if a clinical ketosis develops and the extent to which the general health status and the reproduction are concerned (Wensing et al., 1997).

The approach to avoid liver TG accumulation via reduction of body fat mobilization from adipose tissues and a concomitant decrease in plasma NEFA concentration and uptake of NEFA by the liver is described by Bobe et al. (2004). In the present trial, plasma NFEA concentrations were not reduced and the increase of liver weight until 42 and 105 DIM did not differ between the CLA and CON group (Paper I). Liver samples from the present experiment (Paper I) were taken at slaughter and analyzed - among other things - for dry matter and lipid content by our project partners at the Institute of Nutrition, Friedrich Schiller University Jena. A calculation of total liver lipid mass (Figure 3B) is possible based on the fresh liver weights (Paper I), the DM and total lipid contents (Figure 3A) (Kramer et al., 2011).

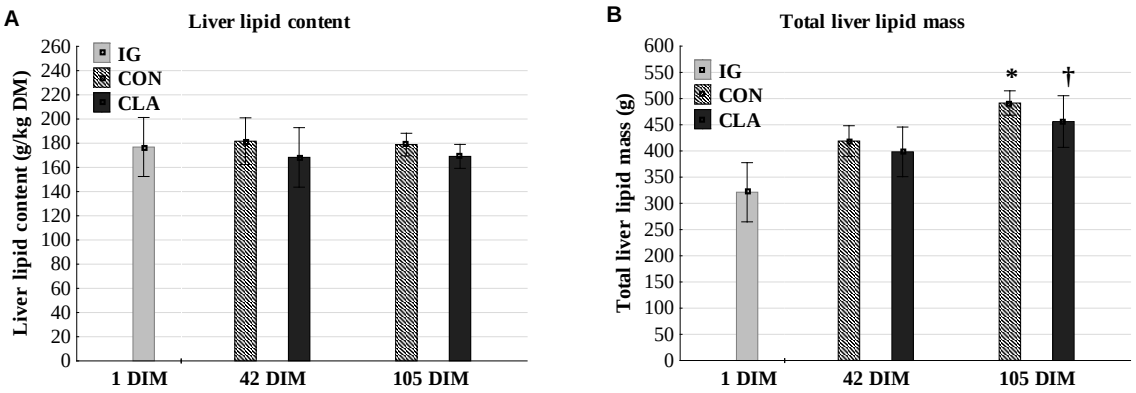

Figure 3: Liver lipid content (A) and total liver lipid mass (B) of dairy cows slaughtered at different days in milk (DIM) and with or without CLA feeding. Values represent means ± SEM. † indicates a tendency difference ($P < 0.1$) to the initial group (IG) (Dunnett test). * indicates a significant difference ($P < 0.05$) to the IG (Dunnett test).

Despite the fact that no significant differences for liver lipid content between the groups were found, values for the 42 and 105 CLA group were numerically lower compared with the IG, and the CON groups at 42 and 105 DIM (Figure 3A). The total liver lipid mass was increased at 42 and 105 DIM due to increased liver wet weights with continuous DIM. The increase in liver lipid mass (Figure 3B) in the period from calving (IG) to 42 DIM was numerically lower in CLA-fed cows (42/CLA) compared to the control cows (42/CON) and amounted to 6.4 %. The increase in liver lipid mass in the period from calving (IG) to 105 DIM tended to be lower in CLA-fed cows (105/CLA) compared to the control cows (105/CON) and amounted 11.0%. This suggested that TG accumulation in the liver is decreased during the first 105 DIM for CLA fed animals. This aspect, together with the trend for reduced body mass mobilization (mainly consisting of body fat mobilization) in CLA supplemented cows from 1 until 42 DIM (Paper II), and a tendency for a decelerated decrease of the retroperitoneal fat depot weight in the 42/CLA group, could be the cause for the lower TG accumulation in the livers of CLA fed animals. As noted above, the liver TG accumulation plays an important role in the development of ketosis during early lactation and the results of the present study suggested that CLA supplementation could reduce the risk of ketosis. The reason for unchanged plasma NEFA and BHBA concentrations in the present study (Paper I), despite apparent effects on body mass mobilization and TG accumulation, is not completely clear and was already discussed in Paper II for plasma NEFA concentrations. The plasma BHBA concentrations might be unchanged, because values for heifers in the present study were in the same range before and after parturition and the animals not susceptible for ketosis. The absence of an elevation of BHBA in blood plasma during early lactation is in contrast to pluriparous cows, which are more severe and over a longer time period in a NEB. However,

in the study by Odens et al. (2007), the plasma NEFA concentrations were reduced in CLA fed dairy cows and the study by Csillik et al. (2011) showed decreased plasma NEFA concentrations and decreased liver lipid accumulation from calving until the 5th week of lactation in dairy cows fed comparable CLA amounts to the present study.

Furthermore, the liver TG content is related to gluconeogenesis in the liver. Cows with high liver fat content (174 mg/g) showed increased fat mobilization in contrast to cows with low liver fat content (77 mg/g). The liver glycogen content was increased in cows with low fat liver content and the glycogen content correlated negatively with total fat concentration in the liver. However, the milk yield was not different between the two groups. This suggested effects on glucose metabolism in the liver (Hammon et al., 2009). In the present study the plasma glucose concentration was not influenced by CLA supplementation and suggested no influence on gluconeogenesis in the liver. However, the daily lactose synthesis was numerically increased by 60 g/d (1 until 42 DIM) and 120 g/d (42 until 105 DIM) in CLA fed cows (Paper I) and suggested that more glucose for lactose synthesis was available in CLA fed cows. Studies investigating the CLA impact on liver fat metabolism related to glucose metabolism are rare. The study of Hammon et al. (2011) deals with glucose metabolism of CLA fed dairy cows and lower glycogenolysis in combination with a higher lactose output and suggests a glucose-sparing effect in CLA fed cows.

Nevertheless, further research of possible CLA effects on liver lipid and glucose metabolism is necessary. Associations between a CLA influenced TG accumulation in the liver and effects on the gluconeogenic capacity of the liver as well as glycogen needed from the liver are conceivable. Interestingly, the fatty acid composition of the liver from animals in the present trial showed that no *t*10,*c*12 CLA was incorporated into liver tissue (Kramer et al., 2011). Conversely, marginal amounts of the *t*10,*c*12 CLA isomer were deposited in the different fat depots (Paper III).

7.3 CLA effects on fat metabolism in the fat depots

The NEB in early lactation dairy cows is balanced by mobilization of energy from fat tissue (Drackley, 1999). This period of NEB for primiparous cows in the present study lasted from calving until 14 DIM. Therefore, the decrease of fat depot weights was most pronounced from 1 until 42 DIM (Paper I). Although the meat fraction represents the highest proportion of total empty body energy, mobilization of energy from fat depots in relation to the total energy in the fat depots was more pronounced as the mobilization from the meat fraction. This is particularly obvious for the retroperitoneal fat depot and was indicated by the decreased proportions of empty body energy in the 42/CON, 105/CON and 105/CLA group compared to the IG (Paper II).

Studies for CLA effects on the dairy cows fat depot weights are not available and it is known from growing mice that the fat depots are markedly reduced by the *t*10,*c*12 CLA isomer. Concomitantly with the reduction of fat depots, in mice massive TG accumulation resulting in liver steatosis was observed (Tsuboyama-Kasaoka et al., 2000, Jaudszus et al., 2010). Those CLA effects on the fat depots were not confirmed in the present experiment for dairy cows from data of the fat depot weights (Paper I). Additionally, the liver fat mass (Figure 3B) showed that accumulation of TG was not increased in CLA fed animals. The reasons for detrimental effects on the adipose tissues for mice and cows could be higher experimental doses administrated in experiments with mice (Bauman et al., 2011) and the differences between the fat metabolism in mice and cows (Vernon, 1980). Additionally, the physiological stage of the animals could play a role for the species-specific CLA effects. In the mice, studies on the animals were in a physiological stage of growing with sufficient energy intake and the fat metabolism in the fat depots was programmed on lipogenesis. This implicated uptake of fatty acids from the circulation and storage as TG in the fat depots. This is different from the physiological stage of the early lactation dairy cow with a NEB. During this period lipolysis is the dominating metabolic process in the adipose depots of dairy cows (Bell, 1980). After the NEB changed into a positive range the lipogenesis plays the pivotal role in fat metabolism (McNamara, 1994). This could explain why the effects on the retroperitoneal fat depot were more pronounced in the first 42 DIM.

The different lipid metabolism in times of NEB and positive energy balance was also reflected by the transfer of the *t*10,*c*12 CLA isomer into the fat depots (Paper III). Cumulated for the retroperitoneal, omental, mesenteric and s.c. fat depot from 1 until 42 DIM, only 52 mg and from 1 until 105 DIM 947 mg *t*10,*c*12 CLA were transferred into the fat depots. The

transfer into the fat depots of the 105/CLA group was higher than into the depots of 42/CLA cows. The 105/CLA group was in a positive energy balance longer and therefore lipogenesis was the dominating process and with higher fatty acid uptake more *t*10,*c*12 CLA was transferred into the fat depots. However, in relation to the consumed *t*10,*c*12 CLA amounts the transfer of this isomer into the fat depots (0.15%) was very low. This is due to biohydrogenation of supplemented CLA in the rumen, which was shown in Paper III and in a study by Pappritz et al. (2011a). Therefore, post-treatment effects of incorporated CLA by release of these CLA into circulation after CLA depletion are not to be expected and this is in line with a further study by Pappritz et al. (2011b) where those aspects were investigated and after finishing CLA feeding the milk fat content was not reduced in a depletion period of 12 weeks.

7.4 CLA effects on energy metabolism

One of the main focuses of the present study was the investigation of the energy metabolism of dairy cows experiencing a CLA-induced MFD during early lactation. The energy spared by the reduced milk fat synthesis and excretion was mainly repartitioned to higher milk yield (Paper I). However, the retention of spared energy by reduced milk fat synthesis cannot be completely explained through higher milk lactose synthesis and milk yield. The lower energy concentration of milk lactose (16.6 kJ/g) compared to the energy value for milk fat (39.8 kJ/g), in combination with moderate higher synthesis of milk lactose and the reduction of milk fat synthesis, shows that more energy spared from milk fat synthesis is available as spent for higher milk lactose synthesis and milk yield. The trend for a lower body mass mobilization in the 42/CLA and an increased protein accretion in the 105/CLA group suggested that the spared energy by reduced milk fat synthesis, which is not used for higher milk yield, is used to counteract the energy mobilization from body stores of the dairy cow.

The CLA effects seem to be more pronounced in the very early lactation. This indicated the decelerated reduction of the retroperitoneal fat depot until 42 DIM (Paper I) and the tendency for reduced body mass mobilization, which was mainly caused by a 40% reduction of body fat mobilization in the 42/CLA group compared to the 42/CON group. Nevertheless, particularly in the first 42 DIM the energy retained in body mass exceeded the spared energy available from milk fat synthesis. Therefore, a lower ME expense in CLA fed animals up until 42 DIM in association with unchanged E_L and MEI, suggests an improved utilization of the ME into the products (Paper II).

A lower body mass mobilization was not observed for the 105/CLA group. Two reasons could be responsible for this. The animals of the 105 DIM groups were in a positive energy balance for a longer time than the animals of the 42 DIM groups. It was shown in Paper I that the energy balance of the animals already turned into a positive range after 14 DIM. Therefore, it seems that CLA effects on energy metabolism are more pronounced in times of NEB, when the mobilization of body reserves occurred. Secondly, the SEM for body fat mobilization in the 105/CON group indicated a high variation in this group, and a higher body fat mobilization in the 105/CLA group is elusive. However, higher protein accretion was observed in the 105/CLA group. The E_L of the 105/CLA group was equal to the 105/CON group and the DMI was slightly lower in the 105/CLA group. Therefore, more energy was retained in body protein despite less available energy from DMI, and it seems that the utilization of the ME was improved in the 105/CLA group (Paper II). In the study by

Shingfield et al. (2004) CLA supplementation during early lactation improved the N retention in pluriparous cows. In the first 15 weeks of lactation, N retention was 19 g/d for control cows and 42 g/d for the CLA supplemented group. Calculation of the daily N retention from 1 up to 105 DIM for the 105/CON and 105/CLA group by using data for protein accretion (Paper II) resulted in -1.5 g/d N retention in the 105/CON group and 5.0 g/d N retention for the 105/CLA group. Explanations for CLA effects on protein metabolism were previously mentioned in Paper II and might be related to CLA influences on the somatotrophic axis or the protein turnover of the dairy cow. Moreover, amino acids in early lactation are used intensively for gluconeogenesis (Drackley et al., 2001) and amino acids mobilized from muscle tissue or provided from the diet could be less used for gluconeogenesis, due to an increased gluconeogenic capacity of the liver, as mentioned above in the section on CLA effects on liver fat metabolism.

In the following the energy metabolism with the energy utilization of the ME for CON cows and CLA supplemented cows is discussed in more detail. The areas which will be addressed include the energy requirement for maintenance (E_M) and the efficiency of ME utilization for milk production (k_l). The HP is an important variable for these two areas of energy metabolism. The E_M and ME utilization for net energy (lactation (E_L) and body energy retention (E_G) as protein and fat) are closely associated with HP (Wenk et al., 2001). The values for numerically lower and decreased HP in the 42/CLA and 105/CLA group (Paper II), respectively, suggested that there could be CLA effects on E_M and k_l. Generally, the requirement for E_M is stated on net energy basis and is 0.29 MJ for 1 kg of metabolic body mass ($kg^{0.75}$) per day (GfE, 2001). Back calculated on the basis of ME, the daily ME energy requirement for maintenance (ME_M) is 0.49 $MJ/kg^{0.75}$. By using data on energy metabolism presented in Paper II the calculation of ME_M is possible with the linear regression method. Thereby, the ME_M was estimated by linear regression of ME intake on energy retention (**RE**). The RE was calculated by adding E_L and E_G. Both, the MEI and the RE are expressed on the basis of metabolic body mass. The ME_M estimated by using data of all animals, irrespective of CLA treatment (Figure 4), was 0.61 $MJ/kg^{0.75}$.

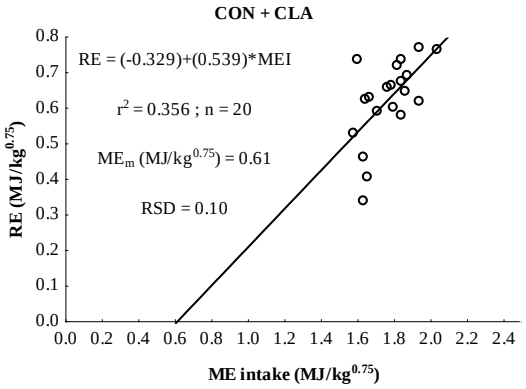

Figure 4: The metabolizable energy requirement for maintenance (ME_M) calculated by using the linear regression technique of metabolizable energy intake (MEI) on retained energy (RE). Data were pooled over experimental groups and periods (42/CLA, 42/CON, 105/CLA, 105/CON). RSD = residual standard deviation.

Interestingly, this ME_M exceeded the generally used value of 0.49 MJ/kg$^{0.75}$ in current energy feeding systems by 20%. Astonishingly, this is in line with the ME_M of 0.62 and 0.67 MJ/kg$^{0.75}$presented in recent studies by Agnew and Yan (2000) and Yan et al. (1997), respectively. An older study by Van Es (1975) showed for ME_M 0.49 MJ/kg$^{0.75}$. Reasons for these differences could be the fact that the basic studies for the currently used ME_M values in energy feeding systems were developed from data published 40 year ago. The increased milk yield, intensified fat mobilization, higher body protein content and a higher proportion of organs for digestion and metabolism could be the reason for the increased ME_M of today's dairy cows (Gruber et al., 2007).

Considering data of the CON or CLA animals separately, the estimation of ME_M resulted in numerically different values for ME_M of 0.68 and 0.64 MJ/kg$^{0.75}$ for CON and CLA animals, respectively (Figure 5).

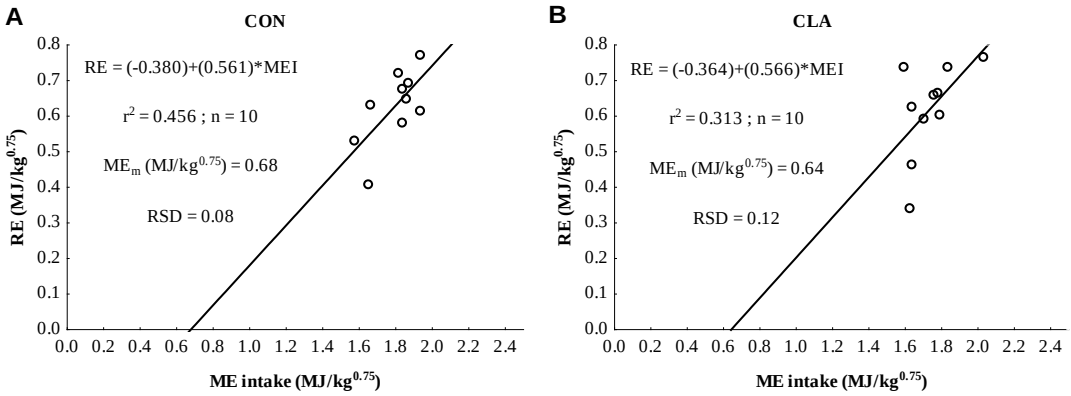

Figure 5: The metabolizable energy requirement for maintenance (ME_M) calculated by using the linear regression technique of metabolizable energy intake (MEI) on retained energy (RE) separated for the CON and CLA groups. A: includes pooled data of the 42/CON and 105/CON group. B: includes data of the 42/CLA and 105/CLA group. RSD = residual standard deviation.

It is notable that the ME_M for the CLA animals seems to be decreased by 5.9% compared to the CON group. This suggested that the ME_M could be reduced by CLA treatment. However, by interpreting these data, the low animal number and the low r^2 has to be mentioned and conclusions from these regressions should be made with caution.

Therefore, CLA effects on the ME_M are not unequivocally from the present study. Nevertheless, physiological reasons for CLA effects on the ME_M are also possible. For instance the protein turnover requires a considerable proportion of the ME_M and CLA effects on protein turnover could lead to a decreased ME_M. Furthermore, an association between the ME_M and the immune system could exist. Inflammatory conditions, which occur in early lactation, seem to increase the ME_M (Trevisi et al., 2007, Bertoni et al., 2008). In a study by Perdomo et al. (2011) it was shown that *t*10,*c*12 CLA attenuate experimentally induced inflammatory processes in growing dairy heifers. Therefore, the reduction of inflammatory processes in CLA supplemented dairy cows might be a further reason for a lower ME_M and could be a possible explanation for the slightly decreased body mass mobilization.

Besides the regressive ME_M estimation, an evaluation of the efficiency of utilization of ME for lactation (k_l) is also possible by the regressive partitioning method. A multiple regression model with E_L, E_G (Paper II) and the ME_M was used for calculation of k_l. A mean k_l value of 0.62 was calculated, pooled over all groups, as presented in Table 3, (Table 3; No. 1). A separate evaluation of the CON and CLA groups resulted in k_l estimates of 0.60 (Table 3; No. 2) and 0.64 (Table 3; No. 3), respectively. Although, the higher k_l in the CLA groups suggested an improvement of 6.7% for utilization of ME for milk production, the low proportions of variances accounted for the regression models of $r^2 = 0.52$ (CON + CLA groups), $r^2 = 0.63$ (CON groups) and $r^2 = 0.60$ (CLA groups) only allow a cautious interpretation.

Table 3. Summary of the efficiency of ME utilization for lactation (kl) obtained from data of the present study by multiple regression equations.

No.	Pooled groups	n	Model	k_l	r^2
1	42/CON 42/CLA 105/CON 105/CLA	20	MEI =((1/0.62)*E_L) + ((1/1.52)*E_G) + ME_M[a]	0.62	0.52
2	42/CON 105/CON	10	MEI = ((1/0.60)*E_L) + ((1/0.85)*E_G) + ME_M[b]	0.60	0.63
3	42/CLA 105/CLA	10	MEI = ((1/0.64)*E_L) + ((1/-)*E_G) + ME_M[c]	0.64	0.60

MEI = metabolizable energy intake
EL = milk energy
EG = energy in body mass
ME_M =metabolizable energy requirement for maintenance
[a] ME_M = 0.61 MJ/kg0.75 (obtained from linear regression; Figure 4)
[b] ME_M = 0.68 MJ/kg0.75 (obtained from linear regression; Figure 5A)
[c] ME_M = 0.64 MJ/kg0.75 (obtained from linear regression; Figure 5B)

Bennighoff et al. (2010) concluded that a more efficient utilization could be caused by a lower energy expense for synthesis of milk fat. Not the reduction of milk fat synthesis is responsible in this case, but rather the shift of the fatty acid pattern to more long chain fatty acids, also observed by Pappritz et al. (2011a), seems to be the reason. Long chain fatty acids are taken up to the mammary gland directly form the blood. This requires less energy compared to the *de novo* synthesis of short chain fatty acids. Nevertheless, if CLA has the ability to improve the utilization of the ME for E_L, the spared energy - due to this improvement - is not needed to be mobilized from the body reserves and fat depots in periods of NEB. This could be a further reason for the tendency for reduced body mass mobilization and the higher weight of the retroperitoneal fat depot observed in the first 42 DIM for CLA fed dairy cows.

8. Conclusion

CLA supplementation reduced milk fat content, milk energy concentration and milk fat yield. Energy was spared from milk fat synthesis and excretion. Nonetheless, the spared energy from milk fat synthesis was partially repartitioned to increased lactose synthesis and milk yield. Therefore the milk energy output and the calculated energy balance were not changed.

The typical increase of liver weight and the decrease of fat depot weights during early lactation were more pronounced in the first 42 DIM. CLA supplementation dose not reinforce these processes. On the contrary, this study suggested that the mobilization of the retroperitoneal fat depot is decelerated and the liver TG accumulation was attenuated. This suggested that CLA could have a preventative effect against massive TG accumulation in the liver.

No distinct CLA effect on the body composition of dairy cows was found. Only for the body protein content a slight increase was observed. The calculation of body fat mobilization and body protein accretion, by using the principle of the comparative slaughter technique, resulted in higher protein accretion for animals of the CLA group slaughtered at 105 DIM and a trend for reduced body mass mobilization (mainly in form of body fat) in the first 42 DIM.

The CLA effects on body mass mobilization and protein accretion are directly associated to the energy metabolism and suggested an improved utilization of the ME in early lactation. The energy expenses from body mass and slightly decreased DMI were lower, whereas the E_L remained unchanged and energy accreted in body protein was increased for CLA supplemented animals. The causes for this improvement of the utilization could be related with a lower ME_M and improved k_l in CLA supplemented dairy cows.

The transfer of the *t*10,*c*12 CLA isomer into the different fractions of the animals was marginal, probably due to ruminal biohydrogenation of supplemented CLA. Only 0.02% after 42 DIM and 0.15% after 105 DIM of consumed *t*10,*c*12 CLA were transferred into the fat depots. No transfer into the meat, bone and hide fraction was detectable. The transfer was more pronounced in periods of positive energy balance for CLA animals slaughtered at 105 DIM.

Overall, from the present work can be concluded, that CLA effects on fat and energy metabolism are not only caused by the energy spared for milk fat synthesis due to the *t*10,*c*12 CLA induced MFD. This spared energy is mainly repartitioned to higher milk yield and is not completely available for reduction of body mass mobilization in early lactation. Obviously, additional CLA effects on fat and energy metabolism in the mammary gland, liver and adipose depots seems to be responsible for an improvement for ME utilization for milk production and a lower ME_M. The mechanisms for those effects are not distinctly clarified and further research is needed on these topics.

9. Summary

Early lactation dairy cows are naturally in a period of a negative energy balance. Non available energy from feed intake is mobilized from body reserves. Massive mobilization of body mass in early lactation, in form of body fat, increases the risk of metabolic diseases like ketosis. Lost of milk production, lower reproductiveness and earlier exit of the dairy cow from production with high economical losses are the consequence.

Since identification of the *t*10,*c*12 conjugated linoleic acid (CLA) isomer and its capability to reduce milk fat content, the possibility for a targeted reduction of milk fat content for dairy cows existed. Conjugated linoleic acid supplementation is used with the intention to reduce the energy requirement for milk production via a reduced milk fat synthesis to disburden the energy metabolism and to improve the calculated energy balance of the early lactation dairy cow. This should be reflected in the body composition and the body fat mobilization during early lactation, but these aspects have not yet been investigated. Therefore, the aim of the present study was to investigate changes in body composition, body mass mobilization and especially the body fat mobilization, as well as the energy metabolism under conditions of a CLA induced reduction of milk fat synthesis during early lactation.

For this purpose a slaughter experiment with 25 primiparous German Holstein cows was conducted. The animals were divided into 5 groups and each group contained 5 animals. An initial group (IG) was slaughtered at 1 day in milk (DIM) and this group received no CLA supplementation. Further 10 animals were slaughtered after 42 DIM, whereby 5 of these 10 animals received from 1 until 42 DIM a CLA supplement (42/CLA group) and the remainder 5 animals received a control fat supplement (42/CON group). The remaining 10 animals were slaughtered at 105 DIM and five animals received from 1 until 105 DIM the CLA supplement (105/CLA group) and 5 animals received the control fat supplement (105/CON group). The animals of the CLA and control group received 3.5 kg pelleted concentrate per day (on dry matter (DM) basis). Either the CLA supplement (contained *t*10,*c*12 CLA and *c*9,*t*11 CLA in equal parts) or the control fat supplement (CLA's were substituted by stearic acid) was contained in this concentrate. On the basis of analyzed CLA contents in the concentrate the animals of the CLA group consumed 6 g/d *t*10,*c*12 CLA and 6 g/d *c*9,*t*11 CLA. Additionally, the animals of the CLA and control groups were fed a partial mixed ration comprising of 38% maize silage, 25% grass silage and 38% concentrate (on DM basis) for *ad libitum* consumption.

The overall performance level of the animals during the present experiment was characterized by an average daily milk yield of 25.7 kg and an average milk fat content of 3.9%. In the period from 1 until 42 DIM the average milk fat content of the animals in the CLA groups (42/CLA and 105/CLA group) was reduced by 14.1% and milk fat synthesis was numerical decreased by 9.8%. The remaining animals of the 105/CLA group showed from 42 until 105 DIM a reduction of milk fat content by 25.4% and a reduced milk fat synthesis by 17.1%. Despite of partial ruminal biohydrogenation of the rumen protected CLA, which was indicated by the fatty acid pattern of the ileal chyme, the reduction of milk fat synthesis was obvious in CLA supplemented dairy cows.

However, the CLA induced reduction of milk fat synthesis resulted not in an improvement for the calculated energy balance in CLA fed animals and it seems, that the energy metabolism is not influenced by CLA supplementation. This could be partially explained by a numerical increase of milk yield. From 1 until 42 DIM the average milk yield for the animals in the CLA groups (42/CLA and 105/CLA group) showed a numerical increase for milk yield by 1.1 kg or 4.7%. From 42 until 105 DIM a numerical increase of milk yield by 2.7 kg or 10.4% for CLA fed animals was obvious. It could be concluded that the saved energy from milk fat synthesis is available for higher milk yield and therefore for lactose synthesis. However, the energy content of 1 g milk fat is more than twice that of 1 g lactose and it is questionable if the higher milk synthesis could totally explain the retention of the energy saved from reduced milk fat synthesis. Moreover, dry matter intake (DMI) was slightly decreased in the CLA groups compared to the control groups with approximately equal milk energy output. The consideration of the body composition and the body mass mobilization enabled deeper insights into the energy metabolism and fat metabolism of the dairy cows.

No CLA effect on the body fat content was shown. In addition to that less than 0.2% of the supplemented *t*10,*c*12 CLA-Isomer was included into the body fat of the dairy cow and in the first 42 DIM marginal amounts of the *t*10,*c*12 CLA isomer were incorporated into the retroperitoneal fat depot. In the same period the weight reduction of this fat depot was slightly reduced. Furthermore, in the CLA groups a trend for a higher body protein content was observed.

Applying the principle of the comparative slaughter technique on the body composition data for calculation of body mass mobilization, a trend for reduced body mass mobilization was shown form 1 until 42 DIM in CLA supplemented cows. This trend for lower body mass mobilization could be mainly explained by a numerical reduction of body fat mobilization of 9.8 kg during this period. The trend for a lower fat accumulation in the liver for CLA

supplemented animals could also be associated with the trend for lower body mass mobilization. Additionally, a higher protein accretion was observed for CLA fed animals from 1 until 105 DIM. This is in accordance with the tendency for higher protein content in the CLA groups. The observation of several trends in this trial could be connected to the low animal number and the general variation for data of body composition and body mass mobilization in dairy cows.

Overall, the present experiment showed for CLA supplemented dairy cows in early lactation a lower energy requirement from body mass mobilization, which was more pronounced from 1 until 42 DIM. Additionally, more energy was accreted in body protein form 1 until 105 DIM. Moreover, slightly less energy was consumed with DMI. In association with repartitioning of the spared milk fat energy to higher milk yield and approximately equal milk energy output, with less energy mobilized from body fat and more energy retained in body protein, it can be suggested that CLA supplementation improved the utilization of the metabolizable energy (ME) in early lactation dairy cows.

Carrying on the energetically considerations with regression analysis, the results for the animals of the present trial suggested that the proportion of the ME expended for maintenance was 20% higher as in currently used energy evaluation systems. For CLA supplemented animals the ME needed for maintenance seems to be lower by 5.9%. Besides, it appears that the utilization of the ME for milk production was more efficient in CLA supplemented dairy cows. Further research on these aspects is needed in the future.

In conclusion the present experiment showed that CLA could partially relief the energy metabolism by reduction in milk fat synthesis and additionally by optimization of the energy metabolism in form of a more efficient utilization of the ME. This could reduce the risk of metabolic diseases in early lactation and makes the cow more able to tough the critical period of early lactation.

10. Zusammenfassung

In der Frühlaktation befinden sich Milchkühe natürlicherweise in einer Phase der negativen Energiebilanz. Energie, die nicht aus dem Futter zur Verfügung gestellt werden kann, wird aus den Körperreserven mobilisiert. Massive Mobilisierung von Körpermasse in der Frühlaktation, hauptsächlich in Form von Körperfett, erhöht das Risiko für die Milchkuh an Stoffwechselerkrankungen wie Ketose zu erkranken. Geringere Milchleistungen, verschlechterte Fruchtbarkeit und sogar frühzeitiges Ausscheiden der Milchkuh aus der Produktion, verbunden mit hohen ökonomischen Verlusten, können Folgen sein.

Seit Entdeckung der milchfettreduzierenden Wirkung des *t*10,*c*12 CLA Isomers besteht die Möglichkeit, den Milchfettgehalt gezielt zu beeinflussen. Durch Supplementierung dieser konjugierten Linolsäure soll über die geringere Milchfettsynthese ein geringerer Energiebedarf für die Milch und damit eine Entlastung des Energiehaushalts und somit eine verbesserte Energiebilanz erzielt werden. Dieses sollte sich in der Körperzusammensetzung und der Körpermassemobilisierung während der Frühlaktation wiederspiegeln, ist aber bisher bei Milchkühen nicht untersucht worden. Deshalb war es das Ziel dieser Studie, die Veränderungen der Körperzusammensetzung, die Körpermassemobilisierung und damit insbesondere die Körperfettmobilisierung, sowie den Energiehaushalt unter den Bedingungen einer CLA induzierten Reduzierung der Milchfettsynthese in der Frühlaktation zu untersuchen.

Hierzu wurde ein Schlachtversuch mit 25 erstlaktierenden Milchkühen der Rasse Deutsche Holstein durchgeführt. Die Tiere wurden in 5 Gruppen zu je 5 Tieren eingeteilt. Eine Startgruppe (Initial group, IG) wurde am 1. Tag nach der Abkalbung geschlachtet und erhielt keine CLA-Supplementation. Weitere 10 Tiere wurden am 42. Laktationstag geschlachtet, wobei jeweils 5 Tiere vom 1. bis zum 42. Laktationstag mit CLA gefüttert wurden (42/CLA Gruppe) und 5 Tiere in gleicher Menge im gleichen Zeitraum ein Kontrollfettsupplement erhielten (42/CON Gruppe). Die verbleibenden 10 Tiere wurden am 105. Laktationstag geschlachtet und ebenfalls erhielten 5 Tiere vom 1. bis zum 105. Laktationstag das CLA-Supplement (105/CLA Gruppe) und 5 Tiere das Kontrollfettsupplement (105/CON Gruppe). Über eine computergesteuerte Abruffütterungsstation erhielten die Tiere der CLA- und Kontroll-Gruppen 3,5 kg pelletiertes Kraftfutter pro Tag (auf Trockenmasse-Basis). Entweder das CLA-Supplement (enthielt *t*10,*c*12 CLA und *c*9,*t*11 CLA zu gleichen Teilen) oder das Kontrollfettsupplement (CLA's wurden durch Stearinsäure ersetzt) wurden diesem Kraftfutter zugesetzt. Auf Grundlage der analysierten CLA-Gehalte im Kraftfutter konsumierten die Tiere der CLA-Gruppen 6 g *t*10,*c*12 CLA und 6 g *c*9,*t*11 CLA pro Tag. Darüber hinaus wurde

den Tieren der CLA- und Kontroll-Gruppen eine Teil TMR bestehend aus 38% Maissilage, 25% Grassilage und 37% Kraftfutter (auf Trockenmasse-Basis) *ad libitum* gefüttert.

Das Leistungsniveau der Tiere über den gesamten Versuchszeitraum lag bei einer durchschnittlichen täglichen Milchleistung von 25,7 kg mit einem Milchfettgehalt von 3.9%.

Im Zeitraum vom 1. bis zum 42. Laktationstag zeigte sich bei den CLA-supplementierten Tieren (42/CLA- und 105/CLA-Gruppe) eine signifikante Reduzierung des Milchfettgehalts von 14,1% und eine um 9.8% numerisch geringere tägliche Milchfettsynthese. Die verbliebenen Tiere der 105/CLA Gruppe zeigten im Zeitraum von 42. bis zum 105. Laktationstag eine Verringerung des Milchfettgehaltes um 25,4% und eine Reduzierung der Milchfettsynthese von 17.1%. Diese deutlichen CLA-Effekte zeigten sich trotz teilweiser ruminaler Biohydrogierung der pansengeschützten CLA, welche aus dem analysierten Fettsäuremuster des Ileum Chymus abgeleitet werden konnte.

Die CLA bedingte reduzierte Milchfettsynthese war jedoch nicht in einer Verbesserung der kalkulierten Energiebilanz bei den CLA-supplementierten Tieren erkennbar. Obwohl es bei einem ersten Blick auf die kalkulierte Energiebilanz scheint, dass der Energiehaushalt der Milchkuh nicht beeinflusst ist, kann der nicht vorhandene Unterschied in der kalkulierten Energiebilanz zum Teil durch eine numerisch höhere Milchleistung erklärt werden. In dem Zeitraum vom 1. bis zum 42. Laktationstag war die tägliche Milchleistung, für die Tiere der 42/CLA- und der 105/CLA-Gruppe zusammen betrachtet, um 1.1 kg oder 4.7% numerisch erhöht und vom 42. bis zum 105. Laktationstag in der 105/CLA Gruppe um 2,7 kg oder 10.4% numerisch erhöht. Die eingesparte Energie für die Milchfettsynthese wird demnach für eine höhere Milchleistung, sprich Laktosesynthese verfügbar. Jedoch ist der Energiegehalt von 1 g Milchfett mehr als doppelt so hoch wie der von 1 g Laktose und deshalb ist es fraglich, ob der Verbleib der eingesparten Energie vollständig durch eine höhere Milchleistung erklärt werden kann. Zudem war die Trockenmasse-Aufnahme in den CLA-Gruppen gegenüber den Kontrollgruppen bei annährend gleichem Energieoutput über die Milch leicht verringert. Die Betrachtung der Körperzusammensetzung und des Körpermasseabbaus ergab hier weitere Einblicke in den Energiehaushalt und Fettstoffwechsel der Tiere.

Bei Betrachtung der Körperzusammensetzung zeigte sich zunächst kein CLA-Einfluss auf den Körperfettgehalt. Zudem wurden weniger als 0.2% des supplementierten *t*10,*c*12 CLA-Isomers in das Körperfett der Milchkuh eingelagert. Für das retroperitoneale Fettdepot, in das bereits nach 42 Laktationstagen geringe Mengen des supplementierten *t*10,*c*12 CLA-Isomers eingelagert wurden, scheint die Gewichtsabnahme vom 1. bis zum 42. Laktationstag in der

42/CLA Gruppe tendenziell verringert zu sein. Für den Körperproteingehalt deutete sich ein tendenziell höherer Gehalt in den CLA-Gruppen an.

Wird aus den Körperzusammensetzungen zu den verschiedenen Schlachtzeitpunkten nach dem Prinzip der vergleichenden Schlachttechnik die Mobilisierung der Körpermasse berechnet, zeigt sich bei den Tieren der 42/CLA Gruppe ebenfalls ein Trend zu einer geringeren Mobilisierung der Körpermasse für den Zeitraum vom 1. bis zum 42. Laktationstag. Dieser Trend zur geringeren Mobilisierung von Körpermasse in dem genannten Zeitraum ergibt sich aus einer 9.8 kg numerisch geringeren Mobilisierung des Körperfettes. Damit könnte auch der tendenziell etwas geringere Anstieg der Leberfettmasse in den CLA-Gruppen erklärt werden. Zudem war ein erhöhter Proteinansatz bei den CLA-Tieren vom 1. bis zum 105. Laktationstag feststellbar, was mit dem tendenziell erhöhten Körperproteingehalt der CLA-Tiere übereinstimmt. Die vermehrte Beobachtung von lediglich tendenziellen CLA-Effekten könnte durch die begrenzten Tierzahlen und der üblicherweise hohen Streuungen bei Daten zur Körperzusammensetzung und des Körpermasseabbaus bei Milchkühen begründet sein.

Letztlich scheint es, dass in diesem Versuch bei CLA-Supplementierung in der Frühlaktation weniger Energie aus den Körperfettreserven, besonders in den ersten 42 Laktationstagen, zur Verfügung gestellt werden musste und mehr Energie in Form von Protein in den ersten 105. Laktationstagen reteniert wurde. Zudem wurde auch tendenziell weniger Energie über die Trockenmasse aufgenommen. In Verbindung mit der Umverteilung der gesparten Energie zu leicht erhöhter Milchleistung und dem dadurch annähernd gleichem Milchenergie-Output, bei weniger Energie mobilisiert aus dem Körperfett und mehr Energie angesetzt in Form von Körperprotein, scheint CLA die Verwertung der metabolisierbaren Energie zu verbessern.

Eine weiterführende energetische Betrachtung mittels Regressionsanalysen lässt darauf schließen, dass der Anteil der metabolisierbaren Energie, welcher für Erhaltung aufgewendet wird, 20% höher ist, als in den derzeitigen Energiebewertungssystemen empfohlen und zudem bei den CLA-supplementierten Tieren um 5.9 % geringer zu sein scheint. Zudem deutet sich eine effizientere Verwertung der metabolisierbaren Energie für die Milchproduktion bei CLA-supplementierten Tieren an. Zukünftig sollten diese Aspekte weiter erforscht werden.

Abschließend kann aus dieser Studie geschlussfolgert werden, dass CLA-Supplementierung den Energiehaushalt zwar durch eine Reduzierung der Milchfettsynthese teilweise entlastet, aber ebenfalls durch eine effizientere Verwertung der metabolisierbaren Energie den Energiehaushalt der Milchkuh in der Frühlaktation optimiert. Dies kann dazu beitragen, das Risiko von Stoffwechselerkrankungen in der Frühlaktation zu verringern und der Milchkuh helfen diese kritische Phase besser zu durchstehen.

11. References

(Cited in Introduction, Background and General discussion)

Adewuyi, A. A., E. Gruys, and F. J. C. M. van Eerdenburg. 2005. Non esterified fatty acids (NEFA) in dairy cattle. A review. Veterinary Quarterly 27:117-126.

ADR. 2011. Jahresbericht. Available: Http://www.ADR-web.de/list_statistik.html.

Agnew, R. E. and T. Yan. 2000. Impact of recent research on energy feeding systems for dairy cattle. Livest. Prod. Sci. 66:197-215.

Andrew, S. M., D. R. Waldo, and R. A. Erdman. 1994. Direct analysis of body-composition of dairy-cows at 3 physiological stages. J. Dairy. Sci. 77:3022-3033.

Bath, D. L., M. Ronning, J. H. Meyer, and G. P. Lofgreen. 1965. Caloric equivalent of live weight loss of dairy cattle. 48:374-380.

Bauman, D. E. 2000. Regualtion of nutrient partitioning during lactation: Homeostasis and homeorhesis revisited. Pages 311-327. P. B. Cronje, ed. CABI Publishing, New York, NY., in Ruminant Physiology: Digestion, metabolism, Growth and Reproduction.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 1999. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci. Available: http://www.asas.org/symposia/9899proc/0937.pdf.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation - a review of mechanisms involving homeostasis and homeorhesis. J. Dairy. Sci. 63:1514-1529.

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

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

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

Bell, A. W. 1980. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. Prog. Lipid Res. 18:117-164.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition form late pregnancy to early lactation J. Anim. Sci. 73:2804-2819.

Belury, M. A. 2002. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. Annu. Rev. Nutr. 22:505-531.

Bennighoff, J., K. Metzger-Petersen, K.-H. Südekum, and A. Tröscher. 2010. Einfluss der konjugierten Linolsäure *trans*-10, *cis*-12 auf den Energieaufwand für die Synthese von Milchinhaltsstoffen bei Kühen. VDLUFA-Kongressband 122:142.

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

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy. Sci. 91:3300-3310.

Blum, J. W. 2004. Metabolic performances of the mammary gland. Übersichten zur Tierernährung 32:183-201.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy. Sci. 87:3105-3124.

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

Chilliard, Y., M. Cisse, R. Lefaivre, and B. Remond. 1991. Body-composition of dairy-cows according to lactation stage, somatotropin treatment, and concentrate supplementation. J. Dairy. Sci. 74:3103-3116.

Chin, S. F., W. Liu, J. M. Storkson, Y. L. Ha, and M. W. Pariza. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Compos. Anal. 5:185-197.

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

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

Corl, B. A., L. H. Baumgard, D. A. Dwyer, J. M. Griinari, B. S. Phillips, and D. E. Bauman. 2001. The role of δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA. The Journal of Nutritional Biochemistry 12:622-630.

Csillik, Z., V. Faigl, M. Keresztes, E. Galamb, A. Tröscher, H. Fébel, M. Kulcsár, F. Husvéth, and G. Huszenicza. 2011. Lipid-encapsulated conjugated linoleic acid (CLA) supplementation: Effects on changes in energy metabolism of the transition cow. 19. Schriftenreihe des Leibniz-Instituts für Nutztierbiologie: Oskar Kellner Symposium - Metabolic Flexibility in Animal and Human Nutrition. Page 130. Warnemünde. (Abstr.)

de Veth, M. J., J. M. Griinari, A. M. Pfeiffer, and D. E. Bauman. 2004. Effect of cla on milk fat synthesis in dairy cows: Comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-372.

Delany, J. P., F. Blohm, A. A. Truett, J. A. Scimeca, and D. B. West. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 276:R1172-R1179.

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

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84:E100-E112 (E. Suppl.)

Flachowsky, G., K. Erdmann, L. Hüther, G. Jahreis, P. Moeckel, and P. Lebzien. 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. Arch. Anim. Nutr. 60:501-511.

Flachowsky, G., P. Lebzien, and U. Meyer. 2009. Deduction of energy and nutrient requirements for high yielding cows. Züchtungskunde 81:429-441.

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

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

Goff, J. P. and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy. Sci. 80:1260-1268.

Griinari, J. M. and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson (Ed.) Advances in conjugated linoleic acid research, Vol. 1. pp 180-200. AOCS Press, Champaign (IL).

Gruber, L., A. Susenbeth, F.J. Schwarz, B. Fischer, H. Spiekers, U. Meyer, A. Chassot, T. Jilg, and Obermaier A. 2007. Bewertung des Nel-Systems und Schätzung des Energiebedarfs von Milchkühen auf der Basis von umfangreichen Fütterungsversuchen in Deutschland, Österreich und der Schweiz. 119. VDLUFA-Schriftenreihe 63, Kongressband 2007, 479-502.

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

Hammon, H. M., K. Hötger, S. Görs, M. Becker, C. Weber, A. Tröscher, and C. C. Metges. 2011. Glucose metabolism in dairy cows supplemented with rumen-protected conjugated linoleic acid (CLA) - first results. Proc. Soc. Nutr. Physiol. 20:70. (Abstr.)

Hammon, H. M., G. Stürmer, F. Schneider, A. Tuchscherer, H. Blum, T. Engelhard, A. Genzel, R. Staufenbiel, and W. Kanitz. 2009. Performance and metabolic and endocrine changes with emphasis on glucose metabolism in high-yielding dairy cows with high and low fat content in liver after calving. J. Dairy. Sci. 92:1554-1566.

Hartfoot, C. G. and G. P. Hazlewood. 1988. Lipid metabolism in the rumen. In: P. N. Hobson (Ed.) The rumen microbial ecosystem. Pages:285-322. Elsevier Applied Science Publishers, London.

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

Hattan, A. J., D. E. Beever, S. B. Cammell, and J. D. Sutton. 2001. Energy metabolism in high yielding dairy cows during early lactation. Energy metabolism in animals. Proceedings of the 15th symposium on energy metabolism in animals, Snekkersten, Denmark, 11-16 September 2000:325-328.

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

Jaudszus, A., P. Moeckel, E. Hamelmann, and G. Jahreis. 2010. *Trans*-10,*cis*-12-cla-caused lipodystrophy is associated with profound changes of fatty acid profiles of liver, white adipose tissue and erythrocytes in mice: Possible link to tissue-specific alterations of fatty acid desaturation. Ann. Nutr. Metab. 57:103-111.

Kepler, C. R. and S. B. Tove. 1967. Biohydrogenation of unsaturated fatty acids. J. Biol. Chem. 242:5686-5692.

Komaragiri, M. V. S., D. P. Casper, and R. A. Erdman. 1998. Factors affecting body tissue mobilization in early lactation dairy cows. 2. Effect of dietary fat on mobilization of body fat and protein J. Dairy. Sci. 81:169-175.

Komaragiri, M. V. S. and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein J. Dairy. Sci. 80:929-937.

Kramer, R., S. Dänicke, D. von Soosten, and G. Jahreis. 2011. Effects of rumen protected conjugated linoleic acids (cla) on fatty acid distribution of selected tissues of dairy cows. Proc. Soc. Nutr. Physiol. 20:95.

Lee, Y. and T. C. Jenkins. 2011. Biohydrogenation of linolenic acid to stearic acid by the rumen microbial population yields multiple intermediate conjugated diene isomers. J. Nutr. 141:1445-1450.

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

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

Loor, J. J., X. B. Lin, and J. H. Herbein. 2003. Effects of dietary *cis* 9, *trans* 11-18:2, *trans* 10, *cis* 12-18:2 or vaccenic acid (*trans* 11-18:1) during lactation on body composition, tissue fatty acid profiles, and litter growth in mice. Br. J. Nutr. 90:1039-1048.

McNamara, J. P. 1994. Lipid-metabolism in adipose-tissue during lactation - a model of a metabolic control-system. J. Nutr. 124:S1383-S1391.

Moe, P. W., H. F. Tyrrell, and W. P. Flatt. 1971. Energetics of body tissue mobilization. J. Dairy. Sci. 54:548-553.

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

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

Overton, T. R. and M. R. Waldron. 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. J. Dairy. Sci. 87:E105-E119.

Pappritz, J., P. Lebzien, U. Meyer, G. Jahreis, R. Kramer, G. Flachowsky, and S. Dänicke. 2011a. Duodenal availability of conjugated linoleic acids after supplementation to dairy cow diets. Eur. J. Lipid Sci. Technol. 113:1443-1455.

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

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

Park, Y., J. M. Storkson, K. J. Albright, W. Liu, and M. W. Pariza. 1999. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice Lipids 34:235-241.

Perdomo, M. C., J. E. Santos, and L. Badinga. 2011. *Trans*-10, *cis*-12 conjugated linoleic acid and the PPAR-γ agonist rosiglitazone attenuate lipopolysaccharide-induced TNF-α production by bovine immune cells. Domest. Anim. Endocrinol. 41:118-125.

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

Rukkwamsuk, T., T. Wensing, and M. J. H. Geelen. 1999. Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows. J. Dairy. Sci. 82:500-505.

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

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

Shingfield, K. J., D. E. Beever, C. K. Reynolds, S. K. Gulati, D. J. Humphries, B. Lupoli, G. Hervas, and M. J. Griinari. 2004. Effect of rumen protected conjugated linoleic acid on energy metabolism of dairy cows during early to mid-lactation. J. Dairy. Sci. 87(Suppl. 1):307. (Abstr.).

Smedman, A. and B. Vessby. 2001. Conjugated linoleic acid supplementation in humans - metabolic effects. Lipids 36:773-781.

Trevisi, E., A. Gubbiotti, and G. Bertoni. 2007. Effects of inflammation in peripartum dairy cows on milk yield, energy balance and efficiency. In: Energy and Protein Metabolism and Nutrition. EAAP publication No. 124. Wageningen Academic Publishers. Pages 395-396.

Tsuboyama-Kasaoka, N., M. Takahashi, K. Tanemura, H. J. Kim, T. Tange, H. Okuyama, M. Kasai, S. Ikemoto, and O. Ezaki. 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 49:1534-1542.

Tyrrell, H. F. and J. T. Reid. 1965. Prediction of energy value of cows milk. J. Dairy. Sci. 48:1215-1223.

Van Es, A.J.H. 1975. Feed evaluation for dairy cows. Livest. Prod. Sci. 2:95-107.

Vernon, R. G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19:23-106.

Wang, Y. W. and P. J. H. Jones. 2004. Dietary conjugated linoleic acid and body composition. Am. J. Clin. Nutr. 79:1153S-1158S.

Wenk, C., P. C. Colombani, J. van Milgen, and A. Lemme. 2001. Glossary: Terminology in animal and human energy metabolism. In: A. Chwalibog and K. Jakobsen (Ed.) 15th Symp. Energy Metab. Anim. Snekkersten, Denmark. Pages 409-421.

Wensing, T., T. Kruip, M. J. H. Geelen, G. H. Wentink, and A. M. van den Top. 1997. Postpartum fatty liver in high-producing dairy cows in practice and in animal studies. The connection with health, production and reproduction problems. Comp. Haematol. Int. 7:167-171.

West, D. B., F. Y. Blohm, A. A. Truettt, and J. P. DeLany. 2000. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. J. Nutr. 130:2471-2477.

West, D. B., J. P. Delany, P. M. Camet, F. Blohm, A. A. Truett, and J. Scimeca. 1998. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 44:R667-R672.

Yan, T., F. J. Gordon, R. E. Agnew, M. G. Porter, and D. C. Patterson. 1997. The metabolisable energy requirement for maintenance and the efficiency of utilisation of metabolisable energy for lactation by dairy cows offered grass silage-based diets. Livest. Prod. Sci. 51:141-150.

CURRICULUM VITAE

Dirk von Soosten
Born on 13 April 1983
In Bremerhaven, Germany

1995 - 2002	Niedersächsisches Internatsgymnasium Bad Bederkesa Qualification: Abitur
2002 - 2003	Alternative Service at Hospital Seepark in Debstedt
2003 - 2006	Bachelor studies of agricultural science at the Georg - August - University of Göttingen
2006 - 2008	Master studies of agricultural science at the Georg - August - University of Göttingen
Since Oktober 2008	Guest scientist at the Institute of Animal Nutrition, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute, Braunschweig, Germany

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation: „Investigations on the effects of conjugated linoleic acids on performance, body composition, body mass mobilization, energy utilization and fatty acid composition of different tissues in early lactation dairy cows" selbständig und nur unter der Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe. Die Arbeit lag bisher in gleicher oder ähnlicher Form keiner Prüfungsbehörde vor.

Halle/Saale, den 08.04.2012 _____

Dirk von Soosten

DANKSAGUNG

Hiermit bedanke ich mich herzlich bei all jenen, die mir bei der Vorbereitung und der Durchführung der Versuche, als auch bei der Erarbeitung meiner Dissertation geholfen und mich unterstützt haben.

Ein besonderer Dank gilt Herrn Prof. Dr. Dr. S. Dänicke für die Unterstützung und insbesondere für die intensiven und spannenden Diskussionen über die Zusammenhänge zwischen Körperzusammensetzung und Energiehaushalt der Milchkuh.

Für die wissenschaftliche und organisatorische Betreuung meiner Arbeit danke ich Herrn Dr. U. Meyer, der sich immer Zeit für mich genommen hat.

Bei Herrn Prof. Dr. G. Flachowsky bedanke ich mich für die Möglichkeit meine Arbeit am Institut für Tierernährung anfertigen zu können, für die kritische Durchsicht meiner Manuskripte und für die motivierenden Worte.

Gedankt sei außerdem der Deutschen Forschungsgemeinschaft (DFG) für die finanzielle Unterstützung dieser Arbeit. Zudem möchte ich mich bei allen weiteren Beteiligten dieses Forschungsprojektes für die gute Zusammenarbeit bedanken.

Ein großes Dankeschön gilt weiterhin allen Mitarbeitern des Instituts für Tierernährung, vom Stall über den Melkstand, ins Schlachthaus bis ins Labor, denn nur durch ihre Mithilfe war es möglich den aufwendigen Versuch durchzuführen und somit diese Arbeit zu verwirklichen. Dem CLA-Team des Instituts für Tierernährung, Julia und Lydia, danke ich für die großartige Zusammenarbeit und Lydia danke ich herzlich für die schöne Zeit im Büro.

Allen Gastwissenschaftlern der Tierernährung danke ich für die Hilfsbereitschaft während des Versuches, die schöne Zeit beim „Knochenpulen“ und die intensiven Feiern.

Darüber hinaus einen lieben Dank an meine Eltern, die mir quasi die Milchkuh in die Wiege legten und immer an mich geglaubt haben. Mein abschließendes größtes Dankeschön geht an meine liebe Frau, die in jeder Phase dieser Arbeit an meiner Seite war.