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Investigations on the effects of conjugated linoleic acids and dietary concentrate proportion on performance and various physiological parameters of periparturient dairy cows and their calves

Dissertation

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ABBREVIATIONS

a.p.	A*ntepartum*
ACC	Acetyl CoA carboxylase
ADF	Acid detergent fibre
AGPAT	Acylglycerol phosphate acyltransferase
Alb	Albumin
ANOVA	Analysis of variance
ASAT	Aspartate amino-transferase
ATP	Adenosine triphosphate
BCS	Body condition score
BF_3	Boron trifluoride
BFT	Body fat thickness
BHB	Beta-hydroxybutyrate
Bili	Total bilirubin
BW	Body weight
*c*9,*t*11	C*is*-9,*trans*-11
CA	Crude ash
CF	Crude fibre
Chol	Total cholesterol
CLA	Conjugated linoleic acids
CON	Control fat
ConA	ConcanavalinA
Conc	Concentrate
CP	Crude protein
CPT	Carnitine palmitoyltransferase
CS	Compound symmetry
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intakes
DMSO	Dimethyl sulfoxide
EE	Ether extract
FABP	Fatty acid binding protein
FAME	Fatty acid methyl esters

FAS	Fatty acid synthase
FBS	Foetal bovine serum
FCM	Fat-corrected milk yield
FLI	Friedrich-Loeffler-Institute
GGT	γ-glutamyl-transferase
GLDH	Glutamate dehydrogenase
Gluc	Glucose
GPAT	Glycerol phosphate acyltransferase
IGF-1	Insulin-like growth factor 1
LAVES	Lower Saxony State Office for Consumer Protection and Food Safety
LPL	Lipoprotein lipase
LSMeans	Least square means
LW	Live weight
ME	Metabolisable energy
mRNA	Messenger ribonucleic acid
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
NE_L	Net energy of lactation
NFC	Non-fibre carbohydrate
OD	Optical density
Oxa	Oxaloacetate
p.p.	*Postpartum*
PBMC	Peripheral blood mononuclear cells
PMR	Partial mixed ration
PPAR	Peroxisome proliferator-activated receptors
Prot	Total protein
PSEM	Pooled standard error of the mean
RMSE	Root mean square error
RpH	Rumen pH
RT	Rumen temperature
SARA	Sub-acute rumen acidosis
SCC	Somatic cell count
SE	Standard errors
SI	Simulations index

SREBP	Sterol regulatory element binding proteins
*t*10,*c*12	*Trans*-10,*cis*-12
TG	Triglyceride
Time-RpH<5.6	Time below rumen pH 5.6
Time-RpH<5.8	Time below rumen pH 5.8
Time-RpH<6.0	Time below rumen pH 6.0
TNF-α	Tumor necrosis factor-α
UCP2	Uncoupling protein 2
VDLUFA	Deutsche Landwirtschaftliche Untersuchungs- und Forschungsanstalten
VFA	Volatile fatty acids
VLDL	Very low density lipoprotein

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GENERAL DISCUSSION

INTRODUCTION

> *"Efficient milk production continues to require the dairy cow to experience gestation and parturition each year. The transition from the pregnant, nonlactating state to the nonpregnant, lactating state is too often a disastrous experience for the cow."* Goff & Horst (1997)

Dairy cows are physiologically subjected to enormous metabolic stress around parturition. Due to a limited feed intake potential around calving and a considerable increase of nutrient demands for milk production *postpartum* (p.p.), dairy cows are often unable to meet their energy requirements for both maintenance and milk production during early lactation (Bell 1995; Grummer 1995; Goff & Horst 1997). This causes a high energy deficit, which has been intensified by a steadily increasing milk production during the last decades. Germany itself registered an increase in annual herd performance of German Holstein cows from year 2002 (8092 kg) to 2012 (9097 kg) of 11 % (ADR 2013). Hence cows are dependent on the use of their body fat reserves to provide additional energy during early lactation (Drackley 1999), which in turn may lead to health and fertility problems (Butler et al. 1981; Grummer 1995; Goff & Horst 1997; Beam & Butler 1999; Drackley 1999). Moreover, an increased hepatic uptake of non-esterified fatty acids (NEFA) may cause an enhanced susceptibility to ketosis and a fatty liver may occur if the lipid infiltration becomes more severe (Drackley 1999).

An approach to reduce the energy deficit at the onset of lactation is to decrease the milk energy content and therefore the milk energy output. Milk fat presents with 50 % the largest proportion of total milk energy and is, as a consequence, the energetically most expensive milk component to synthesize (Tyrrell & Reid 1965). Special diets, such as low-fibre diets or dietary supplements containing polyunsaturated oils (for example plant or marine oils) or conjugated linoleic acids (CLA), are known to inhibit the milk fat production in dairy cows and thus induce a controlled milk fat reduction (Bauman & Griinari 2003). Especially, the *trans*-10, *cis*-12 CLA isomer is frequently added to dairy cow diets because of its milk fat reducing properties (Baumgard et al. 2000). It is supposed that CLA supplementation could have the potential to counteract a negative energy balance and thus reduce metabolic disturbances of early lactating cows due to a lesser extent of body fat mobilization. However, previous CLA studies during transition period or early lactation indicated that CLA supplements are unable to decrease the milk fat synthesis during the first weeks of lactation, whereas supplemented CLA during established lactation led to an immediate reduction in

milk fat synthesis. The reasons are widely unknown. However, the impact of a CLA addition before calving has been insufficiently investigated. Moreover, CLA effects on energy balance and lipid metabolism of dairy cows were contradictory during transition period and less information is available whether CLA affects these parameters after terminated supplementation, especially after a longer period of supplementation. Understanding better the lipid metabolism modifying properties of added CLA by influencing the metabolic situation of cows immediately before calving could be helpful to get more information about how CLA influences an energy deficit and thus metabolism of dairy cows immediately after calving and during early lactation. It is hypothesized that CLA effects are more pronounced in energetically overfed cows during late pregnancy. Hence, the relationship between dietary concentrate proportion and supplemented CLA in diets immediately before calving should be examined in more detail. Furthermore, dairy cows are immune-compromised around calving and it is known that their immune function is sensitive to fatty acids. However, there is also a lack of information about the impact of CLA on the bovine immune system during transition period, which is influenced by strong fluctuations of NEFA. Moreover, the mode of action of CLA on rumen metabolism has been rarely investigated and less is known about physiological effects of maternal supplemented CLA during late pregnancy on the offspring.

Therefore, it is an ongoing need for investigations that examine the effects of supplemented CLA on energy and lipid metabolism, on the bovine immune function, on rumen metabolism of periparturient dairy cows fed various concentrate feed proportions *antepartum* (a.p.) and on calf metabolism and development.

BACKGROUND

1 Conjugated linoleic acids

1.1 Chemical structure and biosynthesis

CLA are a group of positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12 $C_{18:2}$), characterized by having two conjugated unsaturated double bonds at various carbon positions in the fatty acid chain, for example at carbon atoms 7 and 9, 8 and 10, 9 and 11 or 10 and 12. Each double bond can be *cis* or *trans* configured so that *cis*-*trans*, *trans*-*cis*, *cis*-*cis* or *trans*-*trans* configurations are possible (Bauman et al. 1999; Khanal & Dhiman 2004). Within a number of potential CLA isomers, the *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA isomers belong to the most important ones for ruminants and are presented in Figure 1.

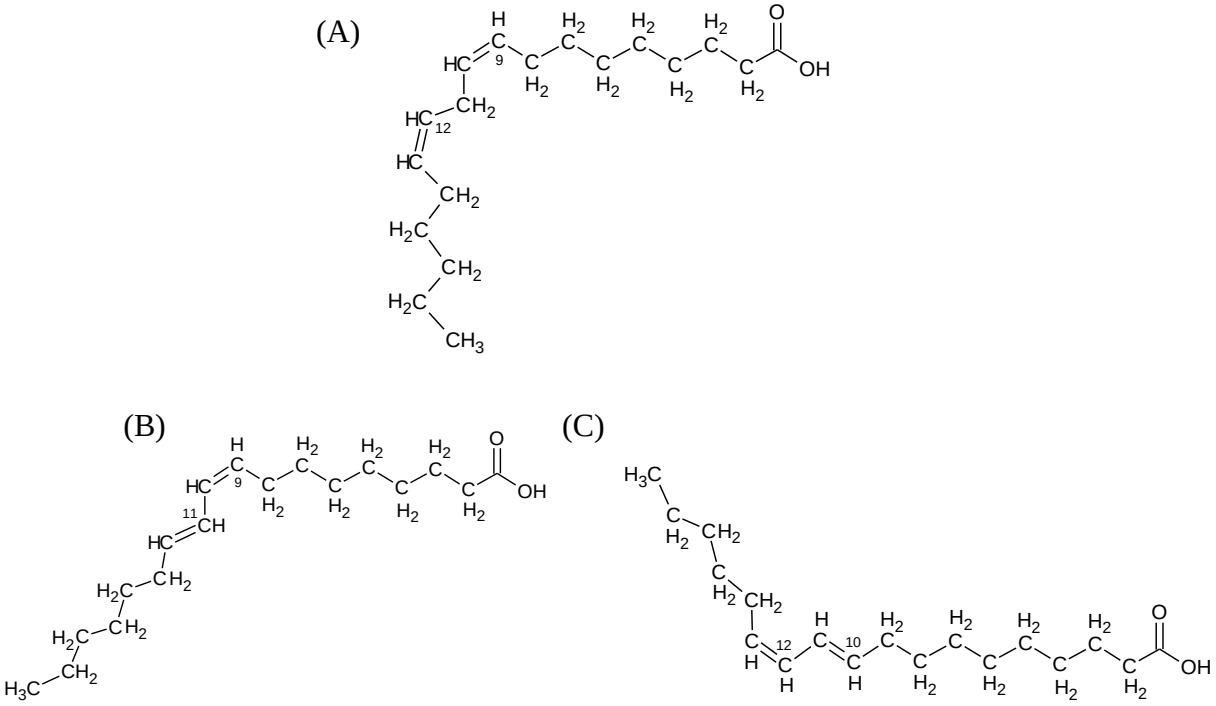

Figure 1. Chemical structure of (A) linoleic acid (*cis*-9,*cis*-12 $C_{18:2}$), (B) *cis*-9,*trans*-11 CLA and (C) *trans*-10,*cis*-12 CLA.

The richest natural dietary sources of CLA are meat and milk from ruminants (Griinari & Bauman 1999). The CLA content of ruminant meat ranges from 0.3-0.5 to 1 % of total fat (Griinari & Bauman 1999) and in milk fat of dairy cows it amounts to about 0.26 to 1.14 % of total fatty acids, varying dependent on the production system (Jahreis et al. 1997). At 80 to 90 %, *cis*-9,*trans*-11 CLA accounts for the largest share of total CLA in food products from

ruminants, whereas the proportion of *trans*-10,*cis*-12 CLA of total CLA is small, at 3-5 % (Khanal & Dhiman 2004).

The *cis*-9,*trans*-11 CLA isomer in fat of ruminants derives from two different sources: from ruminal biohydrogenation of linoleic acid or from endogenous synthesis in animal tissues (Bauman et al. 1999; Griinari & Bauman 1999; Khanal & Dhiman 2004). During ruminal biohydrogenation of linoleic acid (*cis*-9,*cis*-12 $C_{18:2}$) to stearic acid ($C_{18:0}$), *cis*-9,*trans*-11 CLA isomer arises as an intermediate of this process. It is formed by isomerization of linoleic acid, where the *cis*-12 double bond is transferred into the *trans*-11 double bond. The enzyme linoleate isomerase, originating from the bacteria *Butyrivibrio fibrisolvens* (Kepler & Tove 1967) and various other rumen bacteria species (Harfoot & Hazlewood 1988), is responsible for conjugation of the *cis*-9,*cis*-12 bonds. The *cis*-9,*trans*-11 CLA isomer is next hydrogenated to *trans*-vaccenic acid (*trans*-11 $C_{18:1}$), which is a precursor of CLA for endogenous synthesis and also occurs by ruminal biohydrogenation of linolenic acid (*cis*-9,*cis*-12,*cis*-15 $C_{18:3}$). In the final reaction of the biohydrogenation process, *trans*-vaccenic acid is hydrogenated to stearic acid ($C_{18:0}$). Due to the fact that the hydrogenation of *trans*-vaccenic acid is less rapid, its concentration increases in the rumen and is therefore more available for absorption. As a result, the c*is*-9,*trans*-11 CLA isomer is also synthesized endogenously through desaturation of *trans*-vaccenic acid (*trans*-11 $C_{18:1}$) by Δ^9-desaturase in different tissues, like mammary gland and adipose tissue (Bauman et al. 1999; Griinari & Bauman 1999; Khanal & Dhiman 2004). Over 80 % of the *cis*-9,*trans*-11 CLA in milk fat arises from desaturation of *trans*-vaccenic acid in mammary gland (Lock & Garnsworthy 2002) and Gillis et al. (2003) estimated that more than 86 % of this CLA isomer in beef fat originated also from endogenous synthesis in adipose tissue.

The second major CLA isomer, the *trans*-10,*cis*-12 CLA, can also be synthesized during the biohydrogenation process of linoleic acid to stearic acid in the rumen, albeit in varying extents in dependence on feeding (Bauman et al. 1999; Griinari & Bauman 1999; Khanal & Dhiman 2004). Feeding low-fibre/high-concentrate rations or diets containing lipid supplements rich in polyunsaturated fatty acids, especially linoleic acid, lead to increased ruminal synthesis and duodenal flow of the *trans*-10,*cis*-12 CLA isomer (Duckett et al. 2002; Flachowsky et al. 2006).

1.2 CLA modulates fat metabolism

Several physiological properties have been attributed to CLA, including for example anticarcinogenic, antidiabetic and immunomodulatory effects or prevention of cholesterol-

induced atherosclerosis (Belury 2002; Tanaka 2005). Moreover, already at the end of the 1990s, it could be shown that a mixture of CLA isomers, dietary supplemented or abomasally infused, induce a reduction in milk fat content and milk fat yield of lactating cows (Loor & Herbein 1998; Chouinard et al. 1999a; Chouinard et al. 1999b; Kraft et al. 2000). Baumgard et al. (2000) provided the evidence that especially the *trans*-10,*cis*-12 CLA isomer inhibits the milk fat synthesis and leads to a milk fat depression. After abomasal infusion of 10 g *trans*-10,*cis*-12 CLA per day into lactating dairy cows, Baumgard et al. (2000) observed a dramatic reduction in milk fat content and yield of approximately 40%, whereas the *cis*-9,*trans*-11 CLA isomer neither affected milk fat synthesis nor lipid metabolism of lactating cows (Baumgard et al. 2000; Baumgard et al. 2002a). Moreover, other CLA isomers like *trans*-7,*cis*-9 CLA, *trans*-9,*cis*-11 CLA or *cis*-10,*trans*-12 CLA have also milk fat reducing properties, albeit to a lesser extent than the *trans*-10,*cis*-12 CLA isomer (Saebo et al. 2005; Perfield et al. 2007; Kadegowda et al. 2008). For example, Perfield et al. (2007) described the effects of the *trans*-9,*cis*-11 CLA isomer in dairy cows. After abomasal infusion of 5 g/d *trans*-9,*cis*-11 CLA, a 15% milk fat yield reduction was achieved, whereas the *trans*-10,*cis*-12 CLA isomer in the same dose abomasally infused led to a 27 % reduction in milk fat yield. Therefore, the milk fat reducing properties of *trans*-9,*cis*-11 CLA represents only half as much as the *trans*-10,*cis*-12 CLA isomer. De Veth et al. (2004) summarized results from seven studies, in which lactating cows were abomasally infused with *trans*-10,*cis*-12 CLA. Results indicated a relative constant incorporation (22%) of the abomasally infused *trans*-10,*cis*-12 CLA isomer into milk fat. Moreover, a dose-dependent milk fat reduction through *trans*-10,*cis*-12 CLA infusion could be observed. The milk fat yield decreased sharply until a dosage of 6 g/d *trans*-10,*cis*-12 CLA, thereafter the additional reduction was lower and the maximum inhibition of the milk fat yield production ranged from 40 to 50%. These findings also clearly show that the *trans*-10,*cis*-12 CLA isomer is a potent inhibitor of milk fat synthesis in dairy cows. A decreased messenger ribonucleic acid (mRNA) expression for key enzymes involved in de novo fatty acid synthesis (acetyl CoA carboxylase [ACC] and fatty acid synthase [FAS]), uptake and transport of circulating fatty acids (lipoprotein lipase [LPL] and fatty acid binding protein [FABP]), desaturation of fatty acids (Δ^9-desaturase) and synthesis of triglycerides (glycerol phosphate acyltransferase [GPAT] and acylglycerol phosphate acyltransferase [AGPAT]) in mammary gland are described as the underlying mechanism by which *trans*-10,*cis*-12 CLA inhibits milk fat synthesis (Baumgard et al. 2002b; Bauman & Griinari 2003). Moreover, the expression of these lipogenetic enzymes is coordinately stimulated by transcription factors like peroxisome proliferator-activated

receptors (PPAR) and sterol regulatory element binding proteins (SREBP). It is supposed that CLA do not directly influence the lipogenic enzymes, but rather affect their transcriptions factors (Baumgard et al. 2002b; Bauman & Griinari 2003; Bauman et al. 2008). The transcription factor SREBP-1 seems to be of particular importance. All of the mentioned enzymes are SREBP-1 regulated (Bauman et al. 2008) and results from a study of Peterson et al. (2003) indicated that *trans*-10,*cis*-12 CLA application inhibits the proteolytic activation of SREBP-1 and reduces the transcriptional activation of the lipogenic genes.

In addition, on mice it could be observed that the body fat content was reduced after CLA supplementation (Park et al. 1997; West et al. 1998; Delany et al. 1999). Results indicate that CLA alters body composition and further studies provided the evidence that this effect is caused by the *trans*-10,*cis*-12 CLA isomer (Park et al. 1999; Loor et al. 2003). A CLA induced reduction of body fat content could also be observed in rats (Yamasaki et al. 2003) and pigs (Ostrowska et al. 1999). Moreover, it was shown that CLA reduced fat deposition, like in the inguinal, epididymal, retroperitoneal and mesenteric region, in mice (Delany et al. 1999; Park et al. 1999; West et al. 2000). Additionally, Tsuboyama-Kasaoka et al. (2000) reported about a CLA induced apoptosis of adipose cells and observed also that supplementation of 1% CLA-mix caused a redistribution of fat among adipose tissue and liver, leading to lower tissue and higher liver weights with an accompanying development of liver steatosis. Several potential mechanisms how CLA reduced body fat are discussed, including an increase of energy expenditure, alteration of adipocyte metabolism or an increase of fat oxidation (Wang & Jones 2004). The uncoupling protein 2 (UCP2) plays an important role in CLA regulation of energy expenditure. It is a member of the mitochondrial uncoupling protein family and uncouples the proton transfer over the inner mitochondrial membrane and thus the oxidative phosphorylation from adenosine triphosphate (ATP) synthesis, which results in a thermal dissipation on energy as heat instead of ATP (Belury 2002). In mice, CLA supplementation increased UCP2 mRNA in adipocytes and hence a higher energy expenditure appears to reduce body fat deposition (Tsuboyama-Kasaoka et al. 2000). Moreover as reviewed by Wang & Jones (2004), CLA supplementation was observed to increase the tumor necrosis factor-α (TNF-α) mRNA level in white adipose tissue. TNF-α has inhibitory effects on synthesis of LPL, ACC and FAS, indicating that supplemented CLA may alter the body fat disposition by reducing fatty acid uptake and transport as well as de novo fatty acid synthesis. In addition, the transcription factor PPARγ plays an important role in differentiation of preadipocytes into adipocytes via coordinating the expression of genes involved in maintaining and creating the phenotype of adipocytes. However, CLA suppressed gene

expression and activity of PPARγ, leading to inhibit the differentiation of preadipocytes into adipocytes. Furthermore, it could be observed that CLA supplementation led to an increasing activity of the rate-limiting enzyme for fatty acid β-oxidation, carnitine palmitoyltransferase (CPT), suggesting that CLA increases fat oxidation.

2 Physiological features of periparturient dairy cows and their relevance for metabolism and immune function

High-yielding dairy cows are metabolically challenged during transition period, which is defined as time between late pregnancy and early lactation from three weeks before to three weeks after parturition (Grummer 1995). This period is characterized by marked nutritional, metabolic, hormonal and immunological changes due to the fact that the events pregnancy, parturition and the beginning of milk production happen rapidly. Hence, the main challenge of the periparturient dairy cow is to adapt to these changes very quickly (Bell 1995; Grummer 1995; Goff & Horst 1997; Drackley 1999). Figure 2 presents the most important physiological changes of the dairy cow around parturition.

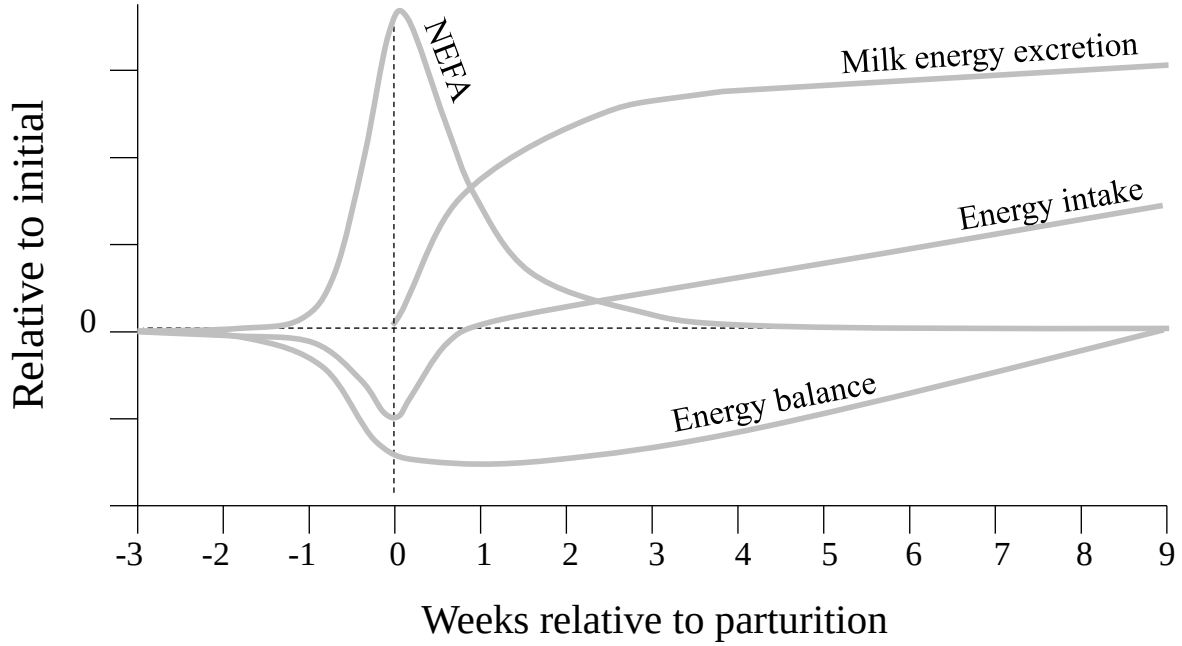

Figure 2. Physiological changes of the periparturient dairy cow relevant for metabolism and immune function. NEFA = Non-esterified fatty acids in blood.

The energy intake of cows is reduced in the time around parturition and increases only slowly after calving, as shown in Figure 2. The decrease in voluntary feed intake begins at three weeks prior to calving and accelerates during the last week a.p.. A reduction of 30 to 35 % is

usual during the last three weeks a.p. (Grummer 1993, 1995) and is less a result of a reduced rumen volume through the growing fetus, but rather caused by metabolic alterations due to pregnancy, changes in body composition and forthcoming lactation (Ingvartsen & Andersen 2000). This gradual decline in feed and thus energy intake around calving coincides with a considerable increase of nutrient demands for conceptus growth with progressing pregnancy and further for milk synthesis during early lactation. Therefore, dairy cows are often not able to meet their energy requirements for maintenance and pregnancy shortly a.p. as well as for maintenance and milk production at the onset of lactation (Bell 1995; Grummer 1995; Goff & Horst 1997). Drackley (1999) reported that at day 4 p.p. the requirements for net energy of lactation (NE_L) of healthy cows exceeded their intakes by 26 %. Moreover, 97 % of the energy intake is needed by the mammary gland for milk production. These pronounced energy demands for milk production induce a high energy deficit during early lactation, leading to an increase in body fat mobilization and thus blood NEFA concentrations to provide additional energy (Roberts et al. 1981; Drackley 1999). However, this status of negative energy balance and hence increased lipid mobilization is associated with a greater susceptibility for infections and metabolic diseases like ketosis or liver stress (Grummer 1995; Goff & Horst 1997; Drackley 1999) with a subsequent loss of performance and fertility problems (Butler et al. 1981; Beam & Butler 1999).

The liver is located at the crossroads of metabolism and plays a key role in coordinating metabolic processes such as gluconeogenesis, triglyceride synthesis and secretion, fatty acid oxidation as well as ketogenesis to ensure an adequate nutrient supply of the dairy cow. Hence, cows must be able to adapt quickly key metabolic pathways in the liver to pass early lactation without health problems (Drackley et al. 2001). Figure 3 shows schematically biochemical pathways of the lipid metabolism in liver, adipose tissue and mammary gland and their relation among each other during a reduced energy intake p.p..

As a result of a reduced feed intake, propionate is insufficiently available for the intermediate metabolism (Bell 1995; Drackley 1999; Drackley et al. 2001). This causes a lack of glucose due to the fact that propionate, which is a precursor of oxaloacetate, is the most important substrate for gluconeogenesis (Wiltrout & Satter 1972; Drackley et al. 2001). However, if a lack of glucose occurs, cows are able to supply energy via mobilization of body fat reserves (Grummer 1995; Goff & Horst 1997). In the adipose tissue, stored triglycerides are hydrolysed to glycerine and fatty acids (Bauman & Currie 1980), resulting in elevated blood NEFA concentrations (Grummer 1995; Goff & Horst 1997). This in turn increases the uptake

of NEFA by mammary glands and their utilization to synthesize milk fat triglycerides (Bell 1995).

NEFA can account for up to 40 % of milk fatty acids at day 4 p.p., whereas during established lactation nearly 50 % of the fatty acids in milk triglycerides originate from mammary de novo synthesis from acetate and beta-hydroxybutyrate (BHB) and approximately 50 % are derived preformed from plasma lipoprotein triglycerides (Bell 1995).

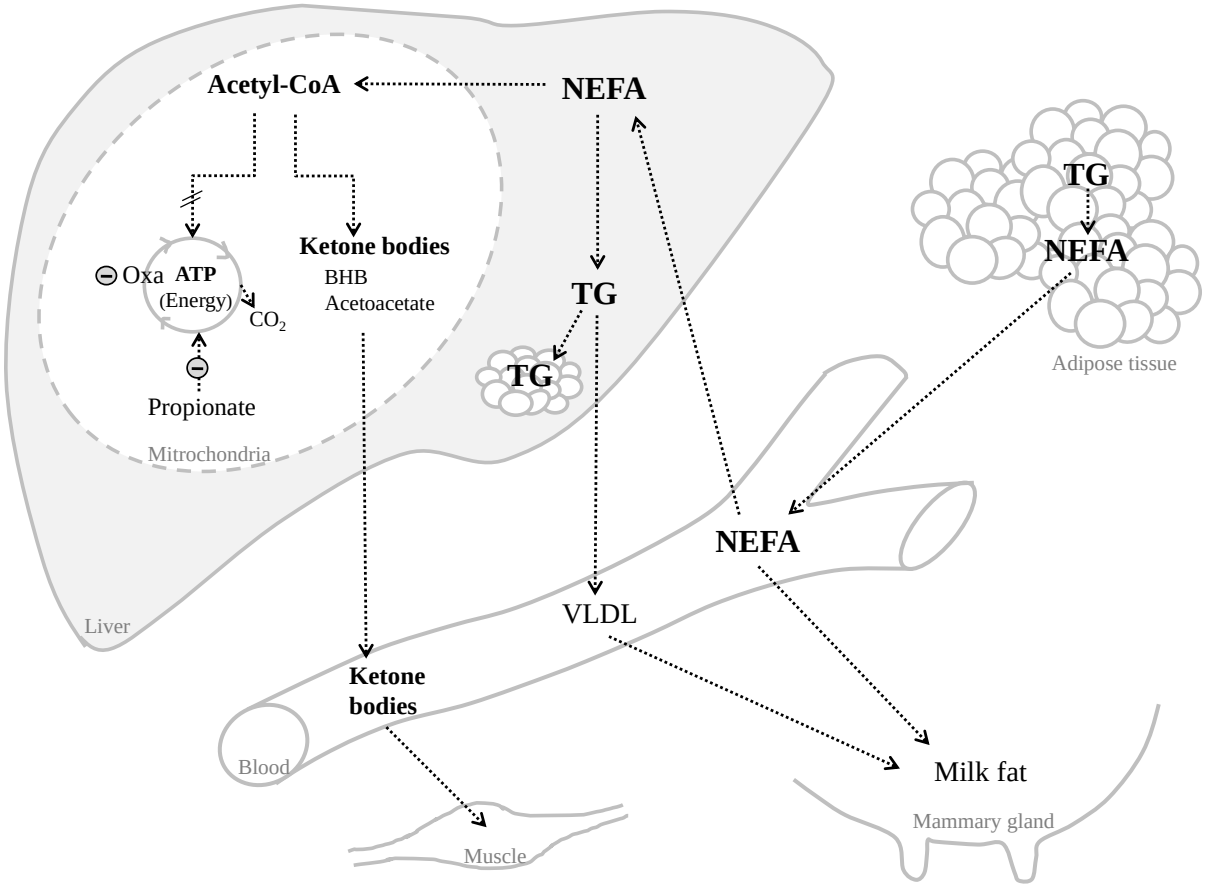

Figure 3. Relationships among lipid metabolism in adipose tissue, liver and mammary gland during times of energy deficits *postpartum*.
The symbol ⊖ indicates a reduced availability and // an inhibited biochemical pathway.
ATP = adenosine triphosphate; BHB = beta-hydroxybutyrate; NEFA = non-esterified fatty acids; Oxa = oxaloacetate; TG = triglyceride; VLDL = very low density lipoprotein.

In general, NEFA pass the β-oxidation and can enter the citrate acid cycle as acetyl-coA only via condensation with oxaloacetate in order to oxidize completely to CO_2. Therefore oxaloacetate is the limiting factor due to its need for both the gluconeogenesis and the utilization of fatty acids to provide energy. A lack of oxaloacetate leads to an accumulation of NEFA in the liver due to the fact that they cannot pass the citric acid cycle. As a result NEFA

are esterified to triglycerides or subjected to ketone body formation (Lehninger 1946; Wieland et al. 1964; Ballard et al. 1968; Goff & Horst 1997; Drackley et al. 2001). Ketone bodies, such as acetoacetate and BHB, are used alternatively as energy-providing substances in different tissues. Therefore the transformation of NEFA to ketone bodies helps sparing glucose during times of deficits (Drackley et al. 2001). Furthermore, an enhanced accumulation of triglycerides in the hepatic tissue occurs, if the rate of triglyceride hydrolysis and export as very low density lipoprotein (VLDL) falls below the level of triglyceride synthesis (Grummer 1993; Goff & Horst 1997; Drackley 1999; Drackley et al. 2001). Thus, a reinforced lipolysis and therefore an enhanced uptake of NEFA by the liver leads to an increase in the production of ketone bodies and an accumulation of triglycerides due to a deficit of oxaloacetate. As a result diseases such as ketosis or fatty liver may occur (Grummer 1993; Goff & Horst 1997).

Due to the fact that feed intake a.p. is positively correlated to feed intake p.p. (Grummer 1995), most approaches intend to increase the energy density in the a.p. diet using concentrates, oils or fat supplements in order to prevent a negative energy balance during early lactation (Grummer 1995; Drackley 1999). Even if they ensure a higher energy intake and thus counteract the reduced feed consumption, adverse effects may follow including overconditioning (Grummer 1995), the risk of developing rumen acidosis or displaced abomasum and thereby lead to a reduction in feed intake (Hayirli & Grummer 2004). This in turn increases the likelihood of both a decrease of liver functions and fatty liver development as a result of an increased lipolysis (Drackley 1999). Especially, overconditioning is negatively related to feed intake a.p. and the sluggish feed intake of obese cows continued during early lactation. Those cows are observed to have a reduced appetite and to lose more body weight at the onset of lactation. Due to an inadequate energy intake during early lactation, overconditioned cows are subjected to a more severe negative energy balance p.p. and an increased body fat mobilization, often in amounts greater than required. Hence, obese cows are more susceptible to metabolic diseases than thinner cows (Grummer 1995; Bobe et al. 2004; Hayirli & Grummer 2004).

Moreover, periparturient dairy cows are known to have a compromised immune system (Mallard et al. 1998), reflected in an impaired lymphocyte function (Franklin et al. 1991; Goff 2006) and further in a reduced response of peripheral blood mononuclear cells (PBMC) to mitogen-stimulation (Nonnecke et al. 2003; Loiselle et al. 2009). Immunosuppressed cows are more vulnerable to infection diseases like mastitis (Vangroenweghe et al. 2005; Goff 2006). However, the underlying reasons are not entirely clear. Goff (2006) assumed that metabolic

challenges, which occur during early lactation, may have an important influence on the immune function. Especially an increased lipolysis may adversely affect reproduction (Leroy et al. 2005) and immune functions (Lacetera et al. 2005) in dairy cows. Accordingly, Lacetera et al. (2004) observed in an *in vitro* study that the mitogen-stimulated proliferation of bovine PBMC was dose-dependently reduced by the incubation with increasing concentrations of NEFA. The authors concluded that increased plasma NEFA concentrations, as occurring through increased adipose tissue mobilization around parturition, have a direct influence on the function of PBMC and therefore may be responsible for the immunosuppression of the transition cow (Lacetera et al. 2004).

3 Possible impacts of dietary supplemented CLA on periparturient dairy cows

3.1 CLA effects on rumen metabolism

The impact of CLA on rumen metabolism has been rarely investigated. Huang et al. (2009) observed that dietary CLA supplementation did not influence the dry matter and crude protein digestibility in sheep. Additionally, supplemented CLA had no effect on ruminal volatile fatty acid (VFA) composition, but led to increased digestibility of lipids. Pappritz et al. (2011a) reported about a modified rumen fermentation pattern after supplementation of CLA to dairy cows, as indicated by an altered profile of short chain fatty acids. Moreover, dietary supplemented CLA increased apparent ruminal starch digestibility and decreased ruminal microbial protein synthesis.

3.2 CLA effects on energy and lipid metabolism

The increased energy demands of dairy cows during early lactation are mainly caused by the milk production, especially milk fat synthesis. Over 35 % of the net energy intake is needed for daily milk fat secretion at the onset of lactation (Bauman et al. 2008). Milk fat presents with approximately 50 % the largest proportion of total milk energy and is consequently the energetically most expensive milk component to synthesize (Tyrrell & Reid 1965). Especially, the *trans*-10,*cis*-12 CLA isomer is known to inhibit the milk fat synthesis (Baumgard et al. 2000). Due to a reduced fat and energy content of the milk, without any change in milk quantities, less energy is needed for milk production, which helps saving energy during times of energy deficits. Therefore, it is supposed that CLA supplements have the potential to counteract negative energy balances and thus reduce metabolic disturbances of early lactating cows due to a lesser extent of body fat mobilization.

Recently, a large number of studies were conducted to investigate the milk fat reducing properties of CLA on dairy cows during early (Liermann 2008; Hutchinson et al. 2011; Pappritz et al. 2011b; von Soosten et al. 2011) or established lactation (Giesy et al. 2002; Perfield et al. 2002; de Veth et al. 2005; Sippel et al. 2009). However, only a few trials exist, examining the mode of action of supplemented CLA on periparturient dairy cows. Table 1 presents an overview of previous CLA-studies with dairy cows during transition period.

Table 1. Effects of dietary supplemented conjugated linoleic acids (CLA) on selected parameters of periparturient dairy cows

Author	Time[1]	n[2]	CLA intake[3] [g/d]	Milk yield [kg/d]	Milk fat content [%]	Milk fat yield [kg/d]	Blood NEFA [µEq/l]	Energy balance [MJ NE_L/d]
Bernal-Santos et al. (2003)	-14 to 140	30	0	44.3	3.60[a]	1.57	436	-5.4
			9	47.1	3.15[b]	1.45	419	-2.5
Moore et al. (2004)	-10 to 21	19	0	33.4	4.57[a]	1.47[a]	362	-36.9
			5	33.7	3.97[ab]	1.29[ab]	498	-41.0
			10	35.5	3.32[b]	1.15[ab]	381	-21.6
			15	34.3	3.10[b]	1.03[b]	465	-25.8
Selberg et al. (2004)	-28 to 49	38	0	40.3	3.49[a]	1.38	Only figure[4]	Only figure[5]
			12	41.5	2.99[b]	1.19		
Castaneda-Gutierrez et al. (2005)	-14 to 63	48	0	43.4	3.82[a]	1.65[a]	393	-15.0
			9	43.8	3.43[b]	1.46[b]	305	-10.3
			18	43.8	3.08[c]	1.30[c]	349	-7.3
Kay et al. (2006)	-27±10 to 36±1	39	0	19.4[a]	5.12[a]	1.00[a]	810[a]	-20.5[a]
			21	21.1[b]	3.35[b]	0.74[b]	600[b]	3.9[b]
Odens et al. (2007)	-9±6 to 40	31	0	35.6	4.27[a]	1.41[a]	607[a]	-21.4
			33.6	38.2	3.16[b]	1.09[b]	502[ab]	-5.4
			33.6/11.2[6]	38.5	3.49[b]	1.17[b]	474[b]	-9.4
Sigl et al. (2010)	-14 to 28	10	0	24.5	6.10	1.49	483	-[8]
			3.75/10[7]	24.5	5.77	1.41	378	-[8]

NEFA = non-esterified fatty acids.

[1]Experimental time in days relative to calving.

[2]Number of animals.

[3]Daily Intake of the *trans*-10,*cis*-12 CLA isomer.

[4]Concentrations of NEFA were greater in CLA group compared to control group at one week *postpartum*.

[5]No significant differences.

[6]Until day 10 *postpartum* cows received 33.6 g CLA/d, thereafter 11.2 g CLA/d.

[7]Different intakes refer to *ante* and *postpartum.*

[8]Not stated.

[abc]Different superscript letters indicate significant differences between feeding groups ($p<0.05$).

Besides no differences in dry matter intakes (DMI), the experiments demonstrated that supplemented CLA led to a dose-dependent reduction in milk fat content (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Kay et al. 2006;

Odens et al. 2007). In addition, the milk fat yield was reduced in the studies of Moore et al. (2004), Castaneda-Gutierrez et al. (2005), Kay et al. (2006) and Odens et al. (2007). However, CLA supplementation resulted in a delayed milk fat response, when initiated before calving and continued p.p. (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005) or during early lactation (Liermann 2008; Pappritz et al. 2011b; von Soosten et al. 2011), becoming significant just after several weeks of lactation, whereas CLA supplementation or abomasal infusion during established lactation led to an immediate decrease in milk fat (Giesy et al. 2002; Perfield et al. 2002; de Veth et al. 2004; Sippel et al. 2009). The reason for this insensitivity is widely unknown. However, higher CLA dosages may lead to reduced milk fat content and yield within one week of lactation (Kay et al. 2006; Odens et al. 2007). The milk yield remained almost unchanged by CLA supplementation. However, Kay et al. (2006) observed a significantly higher milk yield in the CLA group compared to control group and a numerical increase can also be seen in the studies of Bernal-Santos et al. (2003) and Odens et al. (2007). Hammon et al. (2011) discussed that the CLA supplementation may lead to a glucose-sparing effect. Due to using less glucose for milk fat synthesis, more glucose for lactose and protein synthesis is available, resulting in an increased milk yield (Medeiros et al. 2010; Hammon et al. 2011). Hence, the energy-saving effect through low-fat milk could also result in greater liver stress due to an increased gluconeogenesis for lactose synthesis. Moreover, CLA supplementation is known to alter body fat composition (Wang & Jones 2004) and increased the risk for developing liver steatosis (Tsuboyama-Kasaoka et al. 2000) in mice. Hence, an increased gluconeogenesis may be led to an additional burden for liver. In accordance with a higher milk yield, Kay et al. (2006) recorded an increased milk lactose content during early lactation, whereas milk protein and lactose remained uninfluenced by CLA treatment in the other studies. In contrast to results in mice, liver weight (von Soosten et al. 2011) and hepatic triglyceride concentration (Bernal-Santos et al. 2003; Castaneda-Gutierrez et al. 2005) were not affected by CLA supplementation in early lactating cows.

However, only Kay et al. (2006) observed a significant improvement of the calculated energy balance p.p. due to CLA supplementation, whereby in three other studies an apparent slight amelioration can be seen (Bernal-Santos et al. 2003; Castaneda-Gutierrez et al. 2005; Odens et al. 2007). CLA does not seem to have an effect on body fat mobilization during early lactation and thus metabolic state of cows due to the fact that calculated energy balance was mainly uninfluenced. However, high blood NEFA concentrations, indicating an energy deficit and a subsequent lipid mobilization (Dirksen et al. 2006), were inconsistently affected due to

CLA supplementation. Besides no change, Kay et al. (2006) and Odens et al. (2007) found significantly reduced NEFA blood values, associated with a lesser extent of lipolysis and thus reduced metabolic disturbances. Moreover, von Soosten et al. (2011) observed a reduced mobilization of retroperitoneal fat depot during the first 42 weeks of lactation, which may indicate an energy sparing effect due to CLA supplementation. In contrast, Selberg et al. (2004) observed that NEFA blood concentrations were significantly higher through CLA supplementation at one week of lactation, indicating a greater lipid mobilization, which may have adverse effects on animal health. Overall, results from previous studies regarding energy and lipid metabolism were inconsistent and further research is necessary.

3.3 CLA effects on milk fat after terminated supplementation

Some studies dealt with the question, how milk fat behaves after the termination of a CLA supplementation. Liermann (2008) observed an approximation of the milk fat content among the groups within 4 weeks after completion of supplemented CLA. It is supposed that CLA were stored in the adipose tissue during treatment period. With termination of supplementation, CLA were gradually metabolized and thus the inhibitory effect on milk fat synthesis is preserved. However, other authors observed that the CLA effect on milk fat was reversible, because treated groups returned to levels similar to control groups within 14 days after completed CLA supplementation (Castaneda-Gutierrez et al. 2005; Pappritz et al. 2011b) or 4 days after terminated infusion (Baumgard et al. 2000).

3.4 CLA effects on bovine immune function

Only few studies are available, which investigated the influence of CLA on the bovine immune system. As mentioned in Chapter 2, dairy cows are known to be immune-compromised around parturition (Mallard et al. 1998) and Lacetera et al. (2004) demonstrated that the bovine immune system is sensitive to fatty acids. Principally, it is possible that fatty acids like CLA influence the function of immune cells by changing the phospholipid fatty acid composition of the membrane and therefore affect their physical properties. Furthermore fatty acids are able to affect the cell signaling pathways as well as alter the pattern of lipid mediators (Calder 2008). Regarding the influence of CLA on the immune system it could be observed that the mitogen-induced activation of human T-lymphocytes was dose-dependently decreased, whereby a simultaneous increase of the two main CLA-isomers, *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA, in cell lipids were measured (Tricon et al. 2004). Furthermore Kelley et al. (2001) reported that supplemented CLA are able to increase the CLA

concentration in lipids of human PBMC, but did not affect their function. These results are in accordance with the observation of Renner et al. (2012a). The authors found an alteration in the fatty acid profile of bovine PBMC through CLA supplementation during lactation, whereas the mitogen-induced proliferation of these PBMC was not affected. Renner et al. (2012b) observed in a further study that besides an unaffected function of PBMC, the mitogen-induced activation of splenocytes was reduced due to dietary CLA supplementation p.p..

3.5 Maternal CLA supplementation and its effects on their calves

The bovine fetus grows about 75 % during the last two month of pregnancy, whereby maternal nutrition during late pregnancy plays an indispensable role in fully development and growth of the fetus (Funston et al. 2010). However, little is known about the effect of maternal CLA supplementation on their offspring. Previous studies with non-ruminants demonstrated that CLA fed to pregnant humans or rats were transferred from maternal to fetal blood and the authors suggested possible CLA effects on metabolic functions of the fetuses (Ringseis et al. 2004; Müller et al. 2007). In a study of Corino et al. (2009), sows were fed a 0.5 % CLA supplemented diet from 7 days before until 7 days after parturition and piglets of sows fed CLA were heavier and had a higher serum Immunoglobulin G titer than controls. Based on these results, the authors concluded that a CLA supplementation around parturition has positive effects on the immune components in piglets and hence may reduce the susceptibility to diseases. Dänicke et al. (2012) observed after feeding either 4 g or 8 g *trans*-10,*cis*-12 CLA per day to early pregnant cows an altered fatty acid profile of erythrocyte lipids in calves. Additionally, CLA supplementation during preceding lactation and gestation period influenced the stimulation ability of PBMC of cows after the following parturition and of calves. Based on these results, Dänicke et al. (2012) assumed long-term effects of CLA feeding on cows and their offspring. Moreover, preceding studies with dairy cows showed that *trans*-10,*cis*-12 CLA was consistently transferred into milk fat during treatment period and that its proportion in milk fat was dose-dependently increased (Moore et al. 2004; Pappritz et al. 2011b), indicating that colostrum from cows fed CLA could also have important effects on calves.

SCOPE OF THE THESIS

Based on the current literature a large number of studies exist, examining the milk fat reducing properties of CLA and its consequences on energy and lipid metabolism of early lactating cows. However, CLA effects on energy balance and lipid metabolism of dairy cows were contradictory and the impact of CLA addition before calving and after terminated supplementation has been rarely investigated. There is also a lack of information about the influence of supplemented CLA on the bovine immune system during transition period and on rumen metabolism. Moreover, little is known about the effects of maternal CLA supplementation on their offspring.

Based on these gaps in knowledge the following hypotheses were deduced:

1. CLA reduce the milk energy output and thus minimize the energy deficit and extent of adipose tissue mobilization of early lactating cows when dietary supplemented during late pregnancy. Consequently, supplemented CLA improve the metabolic and immunological status of cows at the onset of lactation.

2. The CLA effects are more pronounced in cows energetically overfed during late pregnancy.

3. Post-treatment effects can be observed after CLA supplementation.

4. CLA supplementation influences rumen fermentation as indicated by rumen temperature (RT) and rumen pH (RpH).

5. The intrauterine exposure to CLA during late pregnancy and the intake of CLA enriched colostrum affects calf metabolism and development.

For investigation of these hypotheses an experiment with 64 pregnant German Holstein cows was carried out. The experiment started three weeks prior to calving and was terminated on day 60 p.p.. During this period cows had *ad libitum* access to partial mixed rations consisting of concentrate and roughage. A.p., cows received a control fat (CON) or a CLA supplement, either in a low (20 %, CON-20, CLA-20) or high-concentrate diet (60 %, CON-60, CLA-60). Compared to a feeding adjusted to the requirements, the high concentrate level was fed to

induce a ketogenic metabolic situation of cows p.p. for a better examination of the supposed lipid metabolism modifying properties of added CLA. After calving, the concentrate proportion was adjusted to 50 % in all groups while the fat supplementation continued. The animals of CLA-groups consumed approximately 8 g/d of *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA, respectively. Cows of CON-groups received a control fat supplement, where CLA isomers were substituted by stearic acid.

The results of the experiment are presented in four papers. CLA effects on performance, milk yield, milk composition, estimated energy balance and serum levels of NEFA and BHB as parameters meaningful for energy and lipid metabolism were evaluated in **Paper I**. Furthermore, blood samples for isolation of PBMC and for blood chemistry were taken over the entire experimental period to elucidate how CLA influences bovine metabolism and immune function during transition period **(Paper II)**. Possible post-treatment effects of supplemented CLA were determined by a group-specific completion of the CLA addition after day 32 p.p. **(Paper I and II)**. To investigate the influence of supplemented CLA and dietary concentrate proportion around parturition a part of the animals were equipped with rumen probes for continuous RpH and RT measurements **(Paper III)**. Moreover, CLA impacts on the metabolism of the newborn calves were examined before and after colostrum intake by means of blood analyses **(Paper IV)**. The results of these investigations are presented in the following four publications and are discussed comprehensively in the general discussion.

PAPER I

Effects of conjugated linoleic acids and dietary concentrate proportion on performance, milk composition, milk yield and metabolic parameters of periparturient dairy cows

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Abstract

The study aimed to examine effects of supplemented conjugated linoleic acids (CLA) to periparturient cows receiving different concentrate proportions *ante partum* (a.p.) to investigate CLA effects on lipid mobilization and metabolism. Compared to adapted feeding, a high concentrate diet a.p. should induce a ketogenic metabolic situation *post partum* (p.p.) to better understand how CLA works. Sixty-four pregnant German Holstein cows had *ad libitum* access to partial mixed rations 3 weeks prior to calving until day 60 p.p.. *Ante partum*, cows received control fat (CON) or CLA supplement at 100 g/d, either in a low-concentrate (CON-20, CLA-20) or high-concentrate diet (CON-60, CLA-60). *Post partum*, concentrate proportion was adjusted, while fat supplementation continued. After day 32 p.p., half of the animals of CLA-groups changed to CON supplementation (CLA-20-CON, CLA-60-CON). A ketogenic metabolic situation p.p. was not achieved and therefore impacts of CLA could not be examined. Live weight, milk yield and composition, blood parameters remained unaffected by the treatments. Only slightly reduced milk fat yield (non-significant) was recorded for Group CLA-20. The proportion of *trans*-10,*cis*-12 (*t*10,*c*12) CLA in milk fat was significantly increased in CLA-groups compared to CON-groups. With the exception of a reversible CLA effect on milk fat in Group CLA-20, no post-treatment effects occurred. Dry matter intake (DMI) of Group CLA-60 was highest before calving, resulting in a significantly improved estimated energy balance after calving. *Ante partum*, net energy intakes were significantly increased in high-concentrate groups. Overall, supplemented CLA preparation did not relieve metabolism and lipid mobilization of early lactating cows. But feeding CLA in a high-concentrate diet a.p. seems to increase DMI and thereby improve the energy balance of cows immediately after calving.

Keywords: concentrates; conjugated linoleic acid; dairy cows; energy balance; lipid metabolism; milk yield

1. Introduction

In early lactation cows are unable to meet their energy requirements for both maintenance and milk production (Bell 1995; Grummer 1995), leading to a high energy deficit, so cows are dependent on the use of their body fat reserves (Drackley 1999). This in turn may result in the occurrence of metabolic disorders, like ketosis or liver stress (Grummer 1995; Drackley 1999) with a subsequent loss of performance and fertility problems (Butler et al. 1981; Beam & Butler 1999). Conjugated linoleic acids (CLA) are a group of positional and geometric

isomers of linoleic acid, marked by having conjugated linoleic double bonds. Especially, the *trans*-10,*cis*-12 (*t*10,*c*12) CLA isomer is often added to cow diets because of its milk fat depression properties (Baumgard et al. 2000; Bauman & Griinari 2003). Consequently, CLA supplements have the potential to relieve a negative energy balance (Selberg et al. 2004; Odens et al. 2007) and thus reduce metabolic disturbances of cows during early lactation. However, previous studies investigating possible impacts of CLA on lipid metabolism and energy balance during transition period presented contradictory results (Bernal-Santos et al. 2003; Castaneda-Gutierrez et al. 2005; Odens et al. 2007) and CLA may also affect the mentioned parameters after completion of CLA supplementation (Liermann 2008). Therefore, more information about effects of CLA addition on energy supply and metabolism of cows during the transition period is needed. The study aimed to examine the effects of supplemented CLA on cows fed various concentrate proportions in the ration *ante partum* (a.p.). It was expected that CLA reduce milk energy output and thus improve the energy deficit and metabolic situation of early lactation cows due to decreased adipose tissue mobilization. Influencing the metabolic situation of cows immediately before calving is a possibility to investigate and better understand how CLA affects metabolism. Therefore, compared to an adapted feeding a.p,, the high-concentrate diet was fed to cows three weeks prior to calving to induce a ketogenic metabolic situation *post partum* (p.p.) and thus an increased lipolysis after calving. Effects on performance, milk yield and composition and metabolic parameters around calving were studied in order to evaluate CLA effects on lipid mobilization, metabolism and energy balance. A group-specific termination of CLA supplementation in the p.p. period was performed to determine possible post-supplementation effects.

2. Materials and methods

2.1. Animals, treatments and experimental design

The study was conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany, in compliance with the European Union Guidelines concerning the protection of experimental animals and was approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Oldenburg, Germany. Sixty-four pregnant German Holstein cows (48 pluriparous cows, 762 ± 14 kg and 16 heifers, 640 ± 11 kg) were assigned to one of the four dietary treatments according to the mean number of lactation (1.7 ± 0.2) and fat-corrected milk yield (FCM) of previous lactation (5938 ± 160 kg, 200 d milk yield). It was ensured that the number of primiparous cows was

randomly assigned to the treatments. The experiment was divided into three periods and started on day 21 a.p. and was terminated on day 60 p.p.. The experimental design is shown in Table 1. Three weeks prior to calving (Period 1), Groups CON-20 (n=16) and CLA-20 (n=16) received 100 g/d control fat (CON) or CLA supplement (containing 10% *t*10,*c*12 CLA and 10% *cis*-9,*trans*-11 [*c*9,*t*11] CLA, respectively) in a low-concentrate diet (20%). Groups CON-60 (n=16) and CLA-60 (n=16) were fed 100 g/d control fat or CLA in a high-concentrate diet (60%). Control fat and CLA supplement were added in a rumen-protected form. Period 2 started after calving and lasted for 31 days. The concentrate proportion in the feeding groups was adjusted to 50% while the fat supplementation continued. In Period 3, day 32-60 p.p., the Groups CLA-20 and CLA-60 were divided equally into two groups. The cows of Groups CLA-20-CON (n=8) and CLA-60-CON (n=8) received CON instead of the CLA supplement while in Groups CLA-20 (n=8) and CLA-60 (n=8) the CLA supplementation continued. The feeding in Group CON-20 and CON-60 was not changed.

Diets were formulated to meet the nutritional requirements of the cows stated by the Society of Nutrition Physiology (GfE 2001). During Period 1, cows had *ad libitum* access to partial mixed rations (PMR) based on 20% or 60% concentrate and 80% or 40% roughage (60% corn silage and 40% grass silage on dry matter [DM] -basis), respectively. After calving, the cows were fed a PMR for *ad libitum* consumption consisting of 50% concentrate and 50% roughage (60% corn silage and 40% grass silage on DM-basis). In addition, each cow received 2 kg concentrate per day in pelleted form from a concentrate station (Insentec, B.V., Marknesse, the Netherlands) including either 100 g control fat or 100 g CLA as supplement. The composition of the concentrates and the PMR are presented in Table 2. Two different PMR-concentrates were used during the treatment periods, the first for the a.p. period and the second one for lactating cows. These concentrates were without any fatty acid supplements. The rumen-protected CLA supplement (lipid encapsulation technique) added to the CLA-concentrate was a commercial CLA preparation (Lutrell® Pure, BASF SE, Ludwigshafen, Germany). It contained approximately 10% of each of the predominant CLA isomers *t*10,*c*12 CLA and *c*9,*t*11 CLA. The CLA, in form of fatty acid methyl esters (FAME), was coated with hydrogenated vegetable fats comprising palmitic and stearic acid linked to glycerine. The CLA-concentrate contained 4.6 g *t*10,*c*12 CLA/kg DM and 4.4 g *c*9,*t*11 CLA/kg DM (Table 2). This implies that the cows of the CLA-groups received approximately 8 g/d of the respective CLA isomer. The CON-concentrate included a rumen-protected fat supplement (Silafat®, BASF SE, Ludwigshafen, Germany) containing stearic acid instead of

Table 1. Experimental design.

Period 1 (day 21-1 a.p.)			Period 2 (day 1-31 p.p.)			Period 3 (day 32-60 p.p.)		
Experimental groups	Fat (100 g/d)	Concentrate level [%]	Experimental groups	Fat (100 g/d)	Concentrate level [%]	Experimental groups	Fat (100 g/d)	Concentrate level [%]
CON-20 (*n* =16)	Control	20	CON-20 (*n* =16)	Control	50	CON-20 (*n* = 16)	Control	50
CON-60 (*n* =15)	Control	60	CON-60 (*n* =15)	Control	50	CON-60 (*n* = 15)	Control	50
CLA-20 (*n* =16)	CLA	20	CLA-20 (*n* =16)	CLA	50	CLA-20 (*n* = 8)	CLA	50
						CLA-20-CON (*n* = 8)	Control	50
CLA-60 (*n* =16)	CLA	60	CLA-60 (*n* =16)	CLA	50	CLA-60 (*n* = 8)	CLA	50
						CLA-60-CON (*n* = 8)	Control	50

the CLA. The complete fatty acid profile of the CLA- and CON-supplement of a previous batch is reported by Pappritz et al. (2011a).

During experiment, cows were kept in two group pens in a free stall barn according to their diet a.p.. The PMR were offered in self-feeding stations (type RIC, Insentec, B.V.). Cows had *ad libitum* access to water.

Table 2. Components and chemical composition of concentrates and partial mixed rations (PMR).

Variable	Concentrate				Partial mixed ration		
	CON[‖]	CLA[‡]	PMR-20/60	PMR-50	PMR-20*	PMR-60[§]	PMR-50[&]
Components [%]							
Wheat	41.0	41.0	41.0	41.0			
Dried sugar beet pulp	25.5	25.5	30.5	30.3			
Rapeseed meal	20.0	20.0	20.0	20.0			
Soybean meal	6.5	6.5	6.5	6.5			
Vitamin/mineral premix[$]	2.0	2.0	2.0	-			
Vitamin/mineral premix[†]	-	-	-	2.0			
Calcium carbonate	-	-	-	0.2			
CLA supplement	-	5.0	-	-			
Control fat supplement	5.0	-	-	-			
Analysed chemical profile							
Dry matter [g/kg]	886	886	876	879	335	450	447
Nutrient [g/kg DM]							
Crude ash	58	65	64	67	67	65	66
Crude protein	188	187	195	192	109	136	138
Ether extract	80	64	25	29	35	32	35
Crude fibre	94	94	97	103	207	174	172
Acid detergent fibre	134	126	130	136	231	202	200
Neutral detergent fibre	274	259	265	275	436	392	399
Energy[Ŧ] [MJ /kg DM]							
ME	13.9	13.8	13.5	13.6	10.5	12.1	11.7
NE_L	8.8	8.7	8.6	8.7	6.3	7.5	7.3
CLA[¥] [g/kg DM]							
C18:2 *t*10,*c*12	0.0	4.6	0.0	0.0	0.0	0.0	0.0
C18:2 *c*9,*t*11	0.0	4.4	0.0	0.0	0.0	0.0	0.0

Notes: *Fed in Period 1 (*ante partum*), containing 20% concentrate on DM basis; [§]Fed in Period 1 (*ante partum*), containing 60% concentrate on DM-basis; [&]Fed in Periods 2 and 3 (*post partum),* containing 50% concentrate on DM basis; [$]For dry cows. Ingredients per kg mineral feed: 60 g Ca, 105 g Na, 80 g P, 50 g Mg, 7000 mg Zn, 4800 mg Mn, 1250 mg Cu, 100 mg I, 40 mg Se, 30 mg Co, 800,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E; [†]For lactating dairy cows. Ingredients per kg mineral feed: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6000 mg Zn, 5400 mg Mn, 1000 mg Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E; [Ŧ]Calculation based on nutrient digestibilities measured with wethers (GfE 1991); [‡]CLA, Conjugated linoleic acid; [‖]CON, control fat. [¥]Calculation based on analysed concentrates and silage; Means.

2.2. Sample collection and preparation

All cows were equipped with an ear transponder, which ensured that the daily individual feed and water intake was recorded continuously during experimental period. Representative samples of the different PMR were taken daily and pooled over approximately 4 weeks.

Concentrate samples were collected once, samples of grass and corn silage were taken twice a week and pooled monthly. Following the standard procedure described by GfE (1991), balance studies with four wethers each were performed for the four given concentrates (CON, CLA, PMR 20/60, PMR 50) and for corn and grass silage to determine the energy content of the feedstuffs. In the p.p. period, cows were milked twice daily at 05:30 and 15:30. The individual milk yield was recorded automatically by the milking system. Milk samples for analysis of milk composition were taken twice a week during the morning and afternoon milking. Milk samples were conserved with bronopol and stored at 8 °C until analysis. Milk samples (200 ml) for the analysis of the fatty acid profile in milk fat were collected two times at day 21 p.p. and stored at -20 °C. Live weight (LW) was recorded once a.p. and automatically daily p.p. Blood samples were obtained from each cow by jugular venipuncture on days -21, -14, -7, -3, 1, 3, 7, 14, 21, 28, 42 and 56 relative to calving. Blood was centrifuged at 2000 x g and 15 °C for 15 minutes after incubating 30 minutes by 30 °C. Blood serum was stored at -20 °C until clinical chemical analysis. Sixty-three cows completed the whole study, because one animal (Group CON-60) was excluded from the experiment due to serious milk fever problems.

2.3. Analyses

The chemical composition of the feed was analysed according to the methods of Verband Deutsche Landwirtschaftliche Untersuchungs- und Forschungsanstalten (VDLUFA) (Naumann & Bassler 1976). Milk samples were analysed for fat, protein, lactose and somatic cell count (SCC) by infrared milk analyser (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark) combined with a flow cytometric measurement (Fossomatic 500, Hillerød, Denmark). Before the fatty acid profile in milk fat was analysed, milk samples were heated to 40 °C and homogenised using an Ultra Turrax (T25, Janke & Kunkel, IKA®-Labortechnik, Germany). Milk samples were freeze dried according to their milk yields after mixing the morning and evening milk samples. The fat extraction of freeze-dried milk was performed according to Soxhlet listed in VDLUFA (Naumann & Bassler 1976). Subsequently, total milk fat was converted into FAME using sodium methoxide as catalyst. Lipids were extracted from feed samples according to Folch et al. (1957). Afterwards, boron trifluoride (BF_3) was added to produce FAME by a *trans*-esterification, which were purified by thin-layer chromatography (SIL G-25 UV_{254}, Machery-Nagel, Germany). The FAME extracts of all samples were analysed by gas chromatography (GC; GC-17A Version 3, Schimadzu, Japan) equipped with an auto sampler and flame ionisation detector. GC procedures are described in

detail by Degen et al. (2011). The final results were given as percentage values of total FAME. Serum concentrations of beta-hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA) were determined photometrically by an automatic clinical chemistry analyser (Quinlab Diagnostik GbR, Martinsried, Germany).

2.4. Calculations

FCM was calculated according to Gaines (1928) as follows:

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

where milk fat is given in percentage and milk yield in kilogram per day.

Milk fat, protein and lactose concentrations were calculated as a weighted mean corresponding to the milk yield. The metabolisable energy (ME) and net energy lactation (NE_L) of the experimental feed were calculated according to GfE (1991) by using the nutrient digestibilities from studies with wethers. Before calving (Period 1), the energy balance was calculated by subtracting the requirement of maintenance (GfE 2001) and for pregnancy (GfE 2001) from daily net energy intake. The energy balance during the Periods 2 and 3 was calculated by subtracting maintenance requirement (GfE 2001) and daily requirement for milk production (GfE 2001) from daily net energy intake.

$$\text{Maintenance requirement [MJ } NE_L\text{/d]} = 0.293 \cdot \text{Metabolic LW [kg}^{0.75}\text{]} \quad (2)$$

$$\text{Milk energy concentration [MJ/kg]} = (0.95 + 0.38 \cdot \text{Milk fat} + 0.21 \cdot \text{Milk protein}) + 0.086 \quad (3)$$

where milk fat and milk protein is given in percentage.

$$\text{Requirement for milk production / Milk energy output [MJ/d]} = \text{Milk energy concentration} \cdot \text{Milk yield} \quad (4)$$

where milk energy concentration is given in MJ/kg and milk yield in kg/d.

Daily NE_L intake was calculated by multiplying daily dry matter intake (DMI) [kg] and NE_L [MJ/kg] of the respective feedstuff. LW, daily DMI and milk yield values were condensed to weekly means before data analysis. Accordingly, weekly means were used for

calculations. In evaluating the data, proportions of the fatty acid profile in milk fat, fat supplements and feed stuff, which were below the detection limits, were set to zero.

2.5. Statistical analyses

The software package SAS (Version 9.2, SAS Institute, Cary, NC, USA) was used for all statistical analysis. Data were processed using a random regressions model with fixed regression coefficients for the groups and random regression coefficients to take into consideration repeated measures per animal. The nonlinear relationships between the traits under investigation and the experimental day relative to calving were modelled by splines. This model enabled a better description of the kinetics of investigated parameters over several experimental periods. The model contained the date of sampling day and group-specific regression coefficients, respectively, as fixed factors. The regression coefficients of each animal within the respective group and rest effects were considered in the model as random effect. An average estimated value of the respective period for each group was calculated. Results are shown as least square means (LSMeans) and standard errors (SE). Using Tukey's multiple range test, differences with $p < 0.05$ were considered to be significant and a tendency was declared when $p < 0.1$. The generated values for each period for the respective groups are shown in Tables 3 and 4. These are used for general statistical assessment and consider longer time periods (Period 1 day 21-1 a.p., Period 2 day 1-31 p.p. and Period 3 day 32-60 p.p.). The weekly dissolved values for each group for milk fat yield, DMI, NE_L intake and estimated energy balance are presented as progressive graphs in figures (Figure 1-4). The graphs are used to get an impression of the time-dependent course and, additionally, for the evaluation of the development of differences between treatment groups. Changes of the milk fatty acid profile on day 21 p.p. were analysed by using analysis of variance (ANOVA) according to a one factorial design followed by the Tukey-test.

3. Results

During experimental periods LW, milk yield, milk protein and lactose were not influenced by the treatments (Table 3 and 4). When comparing the averaged estimated values for each period, FCM, milk fat, milk energy concentration and output remained unaffected by the treatments, whereas differences between the groups occurred when comparing the weekly dissolved values for each group over the whole experimental time. Three weeks p.p., the milk fat content of Group CLA-20 was significantly reduced compared to Groups CON-20 and CON-60 (data not shown). A significant reduction by 20% of the milk fat yield of Group

CLA-20 compared to Group CON-60 was observed around the third week p.p. Furthermore, a significantly reduced milk fat yield (-14% to -25%) of Group CLA-20 compared to Group CLA-20-CON was recorded from the sixth week p.p. onwards (Figure 1), reflected also in FCM yield and milk energy output. A significantly higher FCM yield and milk energy output were found for Group CLA-20-CON compared to Group CLA-20 from the seventh week p.p. (data not shown). The proportion of *c*9,*t*11 CLA in milk fat remained unaffected ($p=0.057$) on day 21 p.p., whereas the proportion of *t*10,*c*12 CLA was significantly increased ($p<0.001$) in CLA-groups (CLA-20 and CLA-60, 0.024% and 0.021% of total FAME) compared to CON-groups (CON-20 and CON-60, 0.001% and 0.003 % of total FAME). For Group CLA-20, a significantly increased SCC was observed compared to Groups CON-20 and CLA-60 in the third week p.p. and to Groups CON-20, CON-60 and CLA-60 in the following week (data not shown). Evaluating the respective periods, the SCC remained unaffected by the treatments in Period 2 (Table 3). In Period 3, the SCC of Group CLA-20-CON tended to be higher ($p=0.071$) than the SCC of Group CLA-60, CON-20 and CLA-60-CON (Table 4). DMI decreased slightly with progressing pregnancy and increased consistently after calving (Figure 2). In Period 1, Group CLA-60 consumed significantly more total DM (24%) compared to the low-concentrate Groups CON-20 and CLA-20 ($p<0.001$, Table 3). A significantly higher consumption of DM was observed for Group CLA-60 compared to Group CLA-20 in Period 1 and the first 2 weeks p.p. (Figure 2). After a slight decline in a.p., NE_L intakes increased p.p. (Figure 3). In Period 1, the NE_L intakes in the high-concentrate groups were significantly higher ($p<0.001$) than in the low-concentrate groups (Table 3). Within the high-concentrate groups, CON-60 consumed significantly less NE_L than CLA-60 around calving (Figure 3). In Period 2, Group CLA-20 tended to have a lower NE_L intake (-12%) than Group CLA-60 within the CLA-groups ($p=0.078$, Table 3). A significantly higher net energy intake for Group CLA-60 was observed compared to all other groups 1 week after calving as well as to Group CLA-20 in the second week p.p. Positive estimated energy balances were recorded for each group a.p., whereas significantly higher (71-74%) estimated energy balances were found for the high-concentrate groups than for the low-concentrate groups ($p<0.001$, Table 3). Negative estimated energy balances were observed p.p. One week after calving, a significantly less negative energy balance was recorded for Group CLA-60 in contrast to groups fed a low-concentrate diet a.p. (Figure 4). Group CLA-60 had a less negative estimated energy balance than Group CON-60 within the second week p.p. Cows returned to positive estimated energy balances in Period 3. Blood NEFA and BHB concentrations were not affected by the treatments (Table 3 and 4). Concentrations of NEFA increased sharply in Period 1. One week

before parturition, NEFA were observed to exceed the reference value (Dirksen et al. 2006) by 0.35 mmol/l in all groups with highest values shortly after calving followed by a marked decrease (progressing graph not shown). Serum BHB concentrations decreased slightly a.p. with considerably higher values after calving. Though not significantly different, Groups CLA-60 and CON-60 exceeded temporarily the reference value (Dirksen et al. 2006) by 1.20 mmol/l in Period 2 (progressing graph not shown).

Table 3. Performance, milk composition, energetic variables and blood parameters of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat in Period 1 (day 21-1 a.p.) and Period 2 (day 1-31 p.p.) (LSMeans±SE).

	Control fat (100 g/d)		CLA (100 g/d)		*p*-value
	CON-20[*] (*n* = 16)	CON-60[§] (*n* = 16)	CLA-20[*] (*n* = 16)	CLA-60[§] (*n* = 16)	
Period 1					
Live weight [kg]	714 ±38	713 ±38	710 ±36	708 ±36	0.510
DMI[†] [kg/d]	12.5[a] ±1.1	14.3[ab] ±1.1	12.3[a] ±1.0	16.1[b] ±1.0	<0.001
Net energy intake [MJ/d]	84.4[a] ±7.9	112.7[b] ±8.1	85.1[a] ±7.6	126.8[b] ±7.7	<0.001
Estimated energy balance [MJ NE_L/d]	13.8[a] ±11.3	51.8[b] ±12.4	15.1[a] ±10.6	53.3[b] ±9.9	<0.001
Blood parameter					
BHB[ǀ] [mmol/l]	0.71 ±0.14	0.66 ±0.14	0.76 ±0.13	0.66 ±0.14	0.787
NEFA[ø] [mmol/l]	0.23 ±0.05	0.26 ±0.05	0.28 ±0.05	0.22 ±0.05	0.210
Period 2[&]					
Live weight [kg]	636 ±26	628 ±27	626 ±24	638 ±25	0.975
DMI[†] [kg/d]	17.4 ±0.7	17.5 ±0.8	17.0 ±0.7	18.9 ±0.7	0.177
Milk yield [kg/d]	34.6 ±2.5	36.1 ±2.6	33.9 ±2.4	34.9 ±2.4	0.887
Milk fat [%]	5.51 ±0.22	5.50 ±0.26	5.26 ±0.22	5.31 ±0.2	0.694
Milk fat [kg/d]	1.88 ±0.15	1.85 ±0.22	1.69 ±0.2	1.81 ±0.17	0.943
FCM[Ŧ] [kg/d]	40.7 ±3.1	41.9 ±3.7	37.0 ±3.1	40.6 ±3.0	0.587
Milk protein[%]	3.34 ±0.07	3.28 ±0.07	3.34 ±0.07	3.39 ±0.07	0.485
Milk protein[kg/d]	1.16 ±0.08	1.20 ±0.08	1.13 ±0.08	1.17 ±0.08	0.866
Milk lactose [%]	4.65 ±0.05	4.68 ±0.06	4.66 ±0.05	4.71 ±0.05	0.692
Milk lactose [kg/d]	1.64 ±0.14	1.72 ±0.14	1.58 ±0.13	1.66 ±0.13	0.807
SCC[‡] [log10/ml]	4.80 ±0.14	4.89 ±0.14	5.12 ±0.13	4.89 ±0.13	0.163
Milk energy concentration [MJ/kg]	3.72 ±0.08	3.76 ±0.09	3.68 ±0.08	3.68 ±0.08	0.805
Milk energy output [MJ/d]	131.4 ±9.4	133.9 ±11.5	123.8 ±9.1	126.5 ±8.8	0.808
Net energy intake [MJ/d]	135.5[ab] ±5.8	139.1[ab] ±6.0	131.2[a] ±5.7	149.4[b] ±5.8	0.078
Estimated energy balance [MJ NE_L/d]	-26.0 ±6.0	-32.7 ±6.7	-24.9 ±5.7	-14.4 ±5.9	0.527
Blood parameter					
BHB[ǀ] [mmol/l]	1.00 ±0.11	1.15 ±0.11	0.97 ±0.14	1.17 ±0.15	0.512
NEFA[ø] [mmol/l]	0.59 ±0.06	0.63 ±0.05	0.58 ±0.06	0.60 ±0.06	0.908

Notes: [*]Groups CON-20 and CLA-20 received a low-concentrate diet *ante partum*; [§]Groups CON-60 and CLA-60 were fed a high-concentrate diet *ante partum*; [&]*Post partum*, concentrate proportion was adjusted to 50% while fat supplementation continued; [†]DMI, Dry matter intake; [Ŧ]FCM, 4% fat corrected milk; [‡]SCC, Somatic cell count; [ǀ]BHB, Beta-hydroxybutyrate; [ø]NEFA, nonesterified fatty acids ;[ab] Different superscripts within a period indicate significantly differences between the groups (p<0.05).

Table 4. Performance, milk composition, energetic variables and blood parameters of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat in Period 3 (day 32-60 p.p.)[$] (LSMeans ± SE).

	Control fat (100 g/d)		CLA (100 g/d)		Control fat (100 g/d)		p-value
	CON-20[*] (*n* = 16)	CON-60[§] (*n* = 15)	CLA-20[*] (*n* = 9)	CLA-60[§] (*n* = 8)	CLA-20-CON (*n* = 7)	CLA-60-CON (*n* = 8)	
Live weight [kg]	628 ±26	617 ±26	618 ±27	628 ±27	613 ±28	630 ±27	0.994
DMI[†] [kg/d]	21.5 ±1	21.6 ±1	21.5 ±1.1	22.1 ±1.2	21.4 ±1.2	22.3 ±1.2	0.986
Milk yield [kg/d]	36.0 ±2.2	36.7 ±2.2	35.0 ±2.4	36.7 ±2.4	36.0 ±2.4	37.7 ±2.4	0.902
Milk fat [%]	4.36 ±0.16	4.02 ±0.17	3.98 ±0.19	4.03 ±0.2	4.31 ±0.21	4.04 ±0.21	0.327
Milk fat [kg/d]	1.59 ±0.11	1.54 ±0.12	1.39 ±0.13	1.58 ±0.13	1.68 ±0.14	1.55 ±0.13	0.453
FCM[₸] [kg/d]	39.1 ±2.5	38.2 ±2.6	35.5 ±2.8	39.0 ±2.8	40.6 ±2.9	38.2 ±2.8	0.603
Milk protein[%]	3.12 ±0.06	3.09 ±0.06	3.06 ±0.07	3.06 ±0.07	3.11 ±0.07	3.10 ±0.07	0.944
Milk protein [kg/d]	1.12 ±0.06	1.14 ±0.06	1.06 ±0.07	1.15 ±0.07	1.13 ±0.07	1.15 ±0.07	0.820
Milk lactose [%]	4.8 ±0.04	4.81 ±0.04	4.77 ±0.04	4.85 ±0.05	4.81 ±0.05	4.86 ±0.05	0.662
Milk lactose [kg/d]	1.74 ±0.12	1.79 ±0.13	1.66 ±0.14	1.82 ±0.14	1.78 ±0.14	1.85 ±0.14	0.751
SCC[‡] [log10/ml]	4.65^{a} ±0.11	4.84^{ab} ±0.11	4.91^{ab} ±0.13	4.61^{a} ±0.13	5.11^{b} ±0.14	4.71^{a} ±0.14	0.071
Milk energy concentration [MJ/kg]	3.3 ±0.06	3.16 ±0.07	3.18 ±0.08	3.17 ±0.08	3.31 ±0.08	3.16 ±0.08	0.239
Milk energy output [MJ/d]	118.8 ±7.1	118.5 ±7.3	109.8 ±7.9	115.7 ±8	122.2 ±8.3	122.9 ±8.1	0.315
Net energy intake [MJ /d]	155.6 ±7.3	155.6 ±7.4	156.1 ±8.3	159.9 ±8.5	156 ±8.9	160.3 ±8.5	0.994
Estimated energy balance [MJ/NE_L/d]	-15.9 ±7.1	-17.4 ±8.7	-8.5 ±7.8	-12.5 ±8.3	-18.3 ±8.3	-5.5 ±8.2	0.731
Blood parameter							
BHB[ǀ] [mmol/l]	0.62 ±0.19	0.85 ±0.2	0.60 ±1.22	0.75 ±1.24	0.50 ±1.22	0.28 ±1.24	0.531
NEFA[ø] [mmol/l]	0.39 ±0.07	0.42 ±0.07	0.50 ±0.09	0.42 ±0.09	0.47 ±0.1	0.49 ±0.1	0.737

Notes: [*]Groups CON-20 and CLA-20 received a low-concentrate diet *ante partum;* [§]Groups CON-60 and CLA-60 were fed a high-concentrate diet *ante partum*; [$]In Period 3, half of the animals of CLA-groups changes to control fat supplementation; [†]DMI, Dry matter intake; [₸]FCM, 4% fat corrected milk; [‡]SCC, Somatic cell count; [ǀ]BHB, Beta-hydroxybutyrate; [ø]NEFA, nonesterified fatty acids ; [ab] Different superscripts within a period indicate significantly differences between the groups ($p < 0.05$).

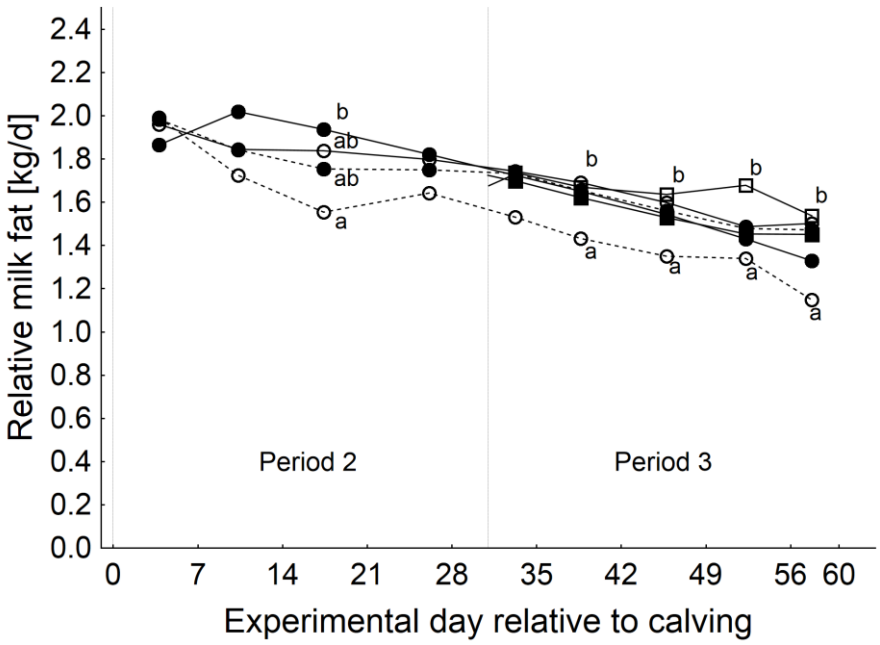

Figure 1. Development of relative milk fat yield
Notes: *Ante partum* (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Post partum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). [ab]Only significantly different LSMeans between the groups are marked with superscripts ($p < 0.05$).

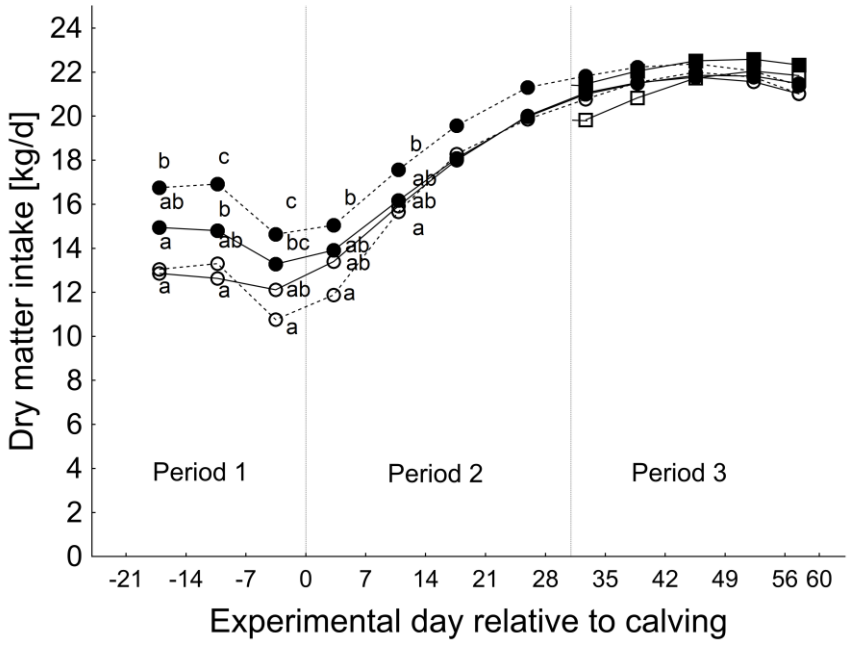

Figure 2. Development of dry matter intake
Notes: *Ante partum* (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Post partum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). [abc]Only significantly different LSMeans between the groups are marked with superscripts ($p < 0.05$).

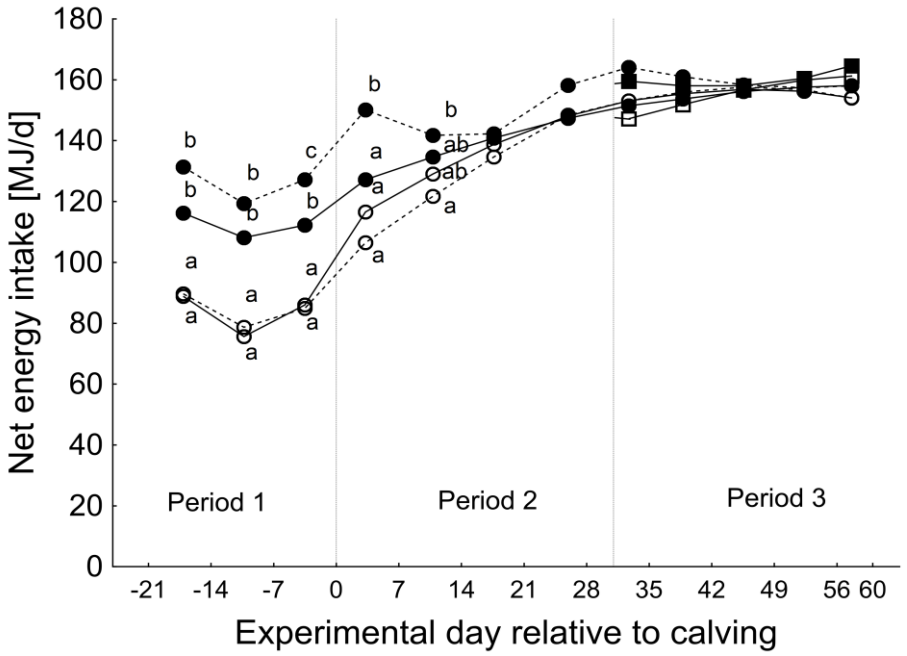

Figure 3. Development of net energy intake
Notes: *Ante partum* (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Post partum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). [ab]Only significantly different LSMeans between the groups are marked with superscripts ($p < 0.05$).

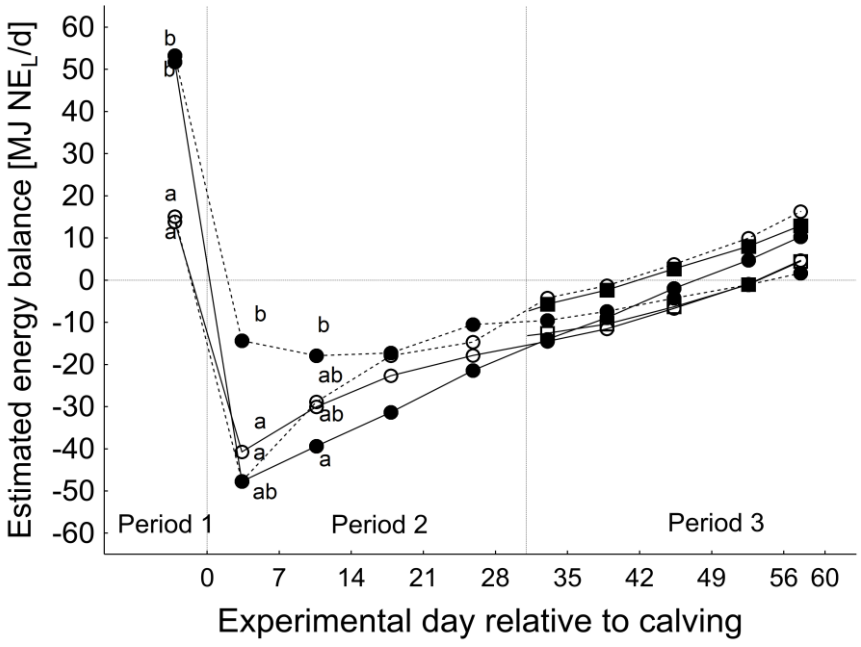

Figure 4. Development of estimated energy balance
Notes: *Ante partum* (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Post partum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). [ab]Only significantly different LSMeans between the groups are marked with superscripts ($p < 0.05$).

4. Discussion

CLA supplementation was initiated approximately 21 days before anticipated calving and continued until day 60 p.p. to record the entire metabolic changes around parturition. A group-specific completion of CLA addition after day 32 p.p. was conducted to determine possible post-treatment effects. To elucidate how CLA works and affects metabolism, cows received diets with either low or high-concentrate proportion during late pregnancy. The high-concentrate level a.p. was fed to induce a ketogenic metabolic situation of cows p.p. Overconditioning is negatively related to DMI a.p. and obese cows are observed to have reduced appetite and lose more body weight in early lactation. Due to inadequate DMI p.p., overconditioned cows are subjected to increased body fat mobilization and are therefore more susceptible to metabolic diseases, like ketosis (Grummer 1995; Hayirli & Grummer 2004). However, differently as expected, no decline in DMI in Groups CLA-60 and CON-60 fed a high-concentrate diet a.p. was observed during early lactation. Accordingly, LW and blood NEFA and BHB concentrations did not differ between the respective groups during the entire experimental period, even if NEFA serum concentrations increased shortly after parturition in all groups, indicating an energy deficit and subsequent lipid mobilization (Dirksen et al. 2006) and serum BHB concentrations let suggest that cows of Groups CLA-60 and CON-60 may be subjected to subclinical ketosis in early lactation (Dirksen et al. 2006). It seems that the attempt to induce a ketogenic metabolic situation p.p. by feeding a high-concentrate level a.p. failed. Hence, the possibility to examine the mode of action of CLA under such a metabolic state was not given. A more successful approach to induce a ketotic state p.p. might have been to restrict or deprive feed intake during early lactation, as realized in other studies (Loor et al. 2007; Kuhla et al. 2009).

Nevertheless, CLA was supplemented as an approach to induce milk fat depression and hence relieve the energy deficit and metabolism of cows p.p. Cows consumed approximately 8 g/d of *t*10,*c*12 CLA and *c*9,*t*11 CLA, respectively. The *c*9,*t*11 CLA isomer neither affects milk fat synthesis nor lipid metabolism of lactating cows (Baumgard et al. 2000; Baumgard et al. 2002), but is an unavoidable component of CLA preparation during manufacturing process. However, CLA supplementation did not affect milk yield, milk protein or lactose and, unexpectedly, milk fat composition. Only Group CLA-20 showed slightly reduced milk fat values, being significant in the third week p.p. compared to Group CON-60. This differs from other studies. Abomasal infusion (Baumgard et al. 2000) or supplementation of CLA (Perfield et al. 2002) led to decreased milk fat immediately during established lactation, whereas CLA addition, when initiated several weeks before parturition and continued p.p.

resulted in a delayed milk fat response, becoming significant after 2 or 3 weeks in milk and lasted during treatment period (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005). A reason for this lack of CLA response in milk fat in early lactation is widely unknown (Bernal-Santos et al. 2003). It is possible that genes associated with milk fat synthesis are resistant to manipulation at this time (Moore et al. 2004). However, differences in the uptake of *t*10,*c*12 CLA by mammary glands can be excluded (Bernal-Santos et al. 2003) because of its continuous transfer into milk fat during supplementation (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005). This could be confirmed by this study, observing significantly increased proportions of *t*10,*c*12 CLA in milk fat of CLA-groups compared to CON-groups on day 21 p.p. Another possible explanation for insensitivity may be the differing sources of milk fatty acids for milk fat synthesis during early lactation (Bernal-Santos et al. 2003). Cows are often characterized by a negative energy balance several weeks p.p., resulting in high circulating levels of NEFA due to increased body fat mobilization (Drackley 1999). This in turn increases NEFA uptake by mammary glands and their utilisation to synthesize milk fat triglycerides (Bell 1995). Possibly, adequate epithelial CLA uptake is prevented by the competitive binding and cellular incorporation of NEFA (Moore et al. 2004) and may explain the slightly decreased milk fat of Group CLA-20. The mammary uptake of CLA may be increased due to the fact that thin cows have a reduced lipolysis-reaction p.p. and thus lower concentrations of NEFA compared to overconditioned cows, resulting in a decrease of milk fat (Holter et al. 1990). However, differences in LW or NEFA serum concentrations were not observed between groups. Moreover, for unknown reasons and contrary to other studies with comparable CLA doses (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005), a CLA effect on milk fat after several weeks p.p. was not observed. However, since proportions of *t*10,*c*12 CLA in milk fat of CLA-groups increased on day 21 p.p., it can be excluded that supplemented CLA was not absorbed in the intestine. Pappritz et al. (2011a) investigated the duodenal availability of a lipid-encapsulated CLA preparation in cows, which was also used in this trial. The authors observed, based on intakes of 8 g/d *t*10,*c*12 CLA, that only 5% of CLA were available in duodenum, indicating unexpectedly low rumen-protection. Assuming the same conditions in this study, only 0.4 g of 8 g consumed *t*10,*c*12 CLA/d would have reached the duodenum. Perhaps, this insufficient CLA rumen protection against microbial degradation may have led to this very slight and nonsignificant effect on milk fat composition due to a relatively low amount of available CLA in the duodenum. Moreover, the milk yield level of cows used in this trial was relatively low compared to high-yielding cows, which could also cause a lack of

response in milk fat. However, Group CLA-20-CON, receiving CON instead of CLA supplement in Period 3, showed a significantly increased milk fat yield compared to Group CLA-20 approximately 1 week upon completion of CLA supplementation, which was also reflected in FCM and milk energy output. This observation agrees with results reported by Castaneda-Gutierrez et al. (2005) and Baumgard et al. (2000), who observed that the CLA effect on milk fat was reversible as treated groups returned to levels similar to control groups within 14 days after termination of CLA supplementation (Castaneda-Gutierrez et al. 2005) or 4 days after termination of infusion (Baumgard et al. 2000). A higher occurrence of temporary mastitis of CLA-groups was observed after calving (Groups CON-20, CON-60, CLA-20, CLA-60 with 3, 2, 9 and 6 cows with mastitis, respectively). These findings are reflected in significantly increased SCC of Group CLA-20 in Period 2 and Group CLA-20-CON in Period 3. Unlike the present findings, previous studies observed unaltered SCC during CLA supplementation (Bernal-Santos et al. 2003; Odens et al. 2007). Based on the previous findings, it can be assumed that supplemented CLA preparation does not have the potential to counteract an energy deficit and to relieve the metabolic situation of early lactating cows by inducing a milk fat depression. However, cows of Group CLA-60 consumed significantly more DM compared to CON-20 and CLA-20 during late pregnancy, which may be a result of a pronounced concentrate effect on total DM a.p. (Friggens et al. 1998; Khorasani & Kennelly 2001). It is well known that DMI increases with an increase of digestibility in the diet (NRC 1988). In contrast, the rumen volume is the limiting factor for DMI when high amounts of roughage were fed to ruminants (NRC 1988). Comparable results were found for NE_L intakes a.p. Depending on DMI and energy content of feedstuff, Groups CLA-60 and CON-60 consumed significantly more NE_L than Groups CLA-20 and CON-20 before calving, leading to a marked energy surplus in those groups a.p. Even if the concentrate proportion in the diet was adjusted to 50% in all groups after calving and thus the energy content of the feed was comparable for each group, NE_L intake of Group CLA-60 was highest during the first 2 weeks p.p. and significant compared to Group CLA-20. A reason for these significances may be a better adaption of Group CLA-60 to concentrate level in the p.p. diet. Grummer (1995) assumed that cows fed high amounts of concentrate a.p. may adapt rumen microbes to lactation rations, support development of ruminal papillae and thus enhance absorbency of rumen epithelium. However, DMI and NE_L intake of Group CON-60 were not increased in this time, indicating that CLA supplementation may affect feed intake. Most of previous CLA studies during transition period observed unchanged DMI (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005), which is in accordance with present findings,

but only after the second week p.p. Nevertheless, besides a reduced consumption of DM (Pappritz et al. 2011b), no study could be found where CLA supplementation increased DMI. In this case, a CLA effect cannot be excluded. For unknown reasons, present data show that the combination of supplemented CLA in a high concentrate diet a.p. lead to increase DMI and hence NE_L intake in the first 2 weeks after calving. These findings may present an opportunity to relieve energy deficit and metabolism of cows in this time. In the first weeks of lactation all cows were in a negative estimated energy balance, with those of Group CON-60 being more severe affected. McNamara et al. (2003) also observed that cows fed high energy density before calving had a pronounced energy surplus a.p. and a greater degree of negative energy balance p.p. Despite the fact that Group CON-60 consumed significantly more NE_L compared to CON-20 during late pregnancy, LW, milk yield, milk composition, milk energy output or estimated energy balance remained unchanged between these groups p.p. This result was unexpected and may be caused by a reduced energy utilisation, an increased passage rate or higher activity of Group CON-60. However, its counterpart Group CLA-60 had indeed a significantly less negative estimated energy balance within the first 2 weeks in lactation, indicating an improved energy utilisation due to CLA supplementation. This variation can be directly attributed to significantly increased DMI and NE_L intake, as there was no difference in LW, milk yield, milk composition or milk energy output. Furthermore, serum NEFA and BHB concentrations were increased but did not differ between Groups CLA-60 and CON-60 in this time, indicating that in both groups body fat was equally mobilized and hence CLA supplementation did not alter metabolism of early lactating cows. This improvement of estimated energy balance and unaltered metabolic situation of Group CLA-60 was unexpected and cannot be completely clarified on the basis of investigated parameters. Further studies are necessary to get more information about the influence of supplemented CLA and high-concentrate proportion a.p. on energy metabolism of cows around calving. Based on the presented parameters, there is no evidence that CLA may reduce body fat mobilization and thus influence lipid metabolism or improve the metabolic situation of early lactating cows, which is in accordance with previous studies (Baumgard et al. 2000; Bernal-Santos et al. 2003; Moore et al. 2004). However, data show that CLA fed in a high-concentrate a.p. has the potential to ameliorate an energy deficit of early lactating cows.

5. Conclusion

In the present study, a ketogenic metabolic situation p.p. was not achieved and respective impacts of CLA could not be examined. It seems that supplemented CLA preparation had a

low rumen-protection. Most of the observed effects were related to variations in DMI resulting from different concentrate proportion a.p. and CLA addition. The study demonstrates that CLA supplementation in a high-concentrate diet a.p. causes an improvement in estimated energy balance during early lactation due to increasing DM and NE_L intakes, whereas milk yield, milk composition, LW as well as NEFA and BHB serum levels remained unaffected. However, CLA supplementation has clearly been established by significantly increased proportions of *t*10,*c*12 CLA in milk fatty acid profile. But administered amounts reduced milk fat yields only slightly in Group CLA-20, which questions the efficiency of this CLA supplement. However, supplemented CLA preparation could not improve the metabolic situation, especially the lipid mobilisation, of transition cows. Further studies are necessary in order to examine the underlying metabolic mechanism of the observed effects.

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References

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

Baumgard LH, Corl BA, Dwyer DA, Bauman DE. 2002. Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. J Anim Sci. 80:1285-1293.

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

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

Bell AW. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J Anim Sci. 73:2804-2819.

Bernal-Santos G, Perfield JW, 2nd, Barbano DM, Bauman DE, Overton TR. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J Dairy Sci. 86:3218-3228.

Butler WR, Everett RW, Coppock CE. 1981. The relationships between energy-balance, milk production and ovulation in postpartum holstein cows. J Anim Sci. 53:742-748.

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

Degen C, Lochner A, Keller S, Kuhnt K, Dänicke S, Jahreis G. 2011. 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.

Dirksen G, Gründer HD, Stöber MH. 2006. Innere Medizin und Chirugie des Rindes [Internal medicine and surgery of boovine animals], Parey in MVS Medizinverlage Stuttgart GmbH & Co. KG Stuttgart, Germany.

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

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

Friggens NC, Emmans GC, Kyriazakis I, Oldham JD, Lewis M. 1998. Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. J Dairy Sci. 81:2228-2239.

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

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

GfE. 2001. (Society of Nutrition Physiolgy). 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.

Grummer RR. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J Anim Sci. 73:2820-2833.

Hayirli A, Grummer RR. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Can J Anim Sci. 84:337-347.

Holter JB, Slotnick MJ, Hayes HH, Bozak CK, Urban WE, Mcgilliard ML. 1990. Effect of Prepartum Dietary Energy on Condition Score, Postpartum Energy, Nitrogen Partitions, and Lactation Production Responses. J Dairy Sci. 73:3502-3511.

Khorasani GR, Kennelly JJ. 2001. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in late-lactation Holstein cows. J Dairy Sci. 84:1707-1716.

Kuhla B, Albrecht D, Kuhla S, Metges CC. 2009. Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid, and glycogen metabolism. Physiol Genomics. 37:88-98.

Liermann T. 2008. Einfluss einer Zulage von pansengeschützter konjugierter Linolsäure(CLA) in Kombination mit Propylenglykol oder pansengeschütztem Fett auf Leistungsmerkmale, Stoffwechselparameter und den Energiestatus frischlaktierender Milchkühe [Effects of feeding rumenprotected conjugated linoleic acids (CLA) alone or in combination with propylene glycol or rumenprotected fat on performance and metabolic parameters and energy status of early lactation dairy cows], Dissertation. Technische Universität München. Germany. 188 pages.

Loor JJ, Everts RE, Bionaz M, Dann HM, Morin DE, Oliveira R, Rodriguez-Zas SL, Drackley JK, Lewin HA. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiol Genomics. 32:105-116.

McNamara S, Murphy JJ, Rath M, O'Mara FP. 2003. Effects of different transition diets on energy balance, blood metabolites and reproductive performance in dairy cows. Livest Prod Sci. 84:195-206.

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

Naumann C, Bassler R. 1976. Die chemische Untersuchung von Futtermitteln [The chemical evaluation of animal feed], VDLUFA-Verlag, Darmstadt, Germany.

NRC. 1988. (National Research Council). Subcommittee on Dairy Cattle Nutrition. Nutrient Requirements of Dairy Cattle, National Academy Press, Washington, D.C.

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

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

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

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

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

PAPER II

Impacts of conjugated linoleic acids and dietary concentrate proportion on blood metabolite concentration and proliferation of peripheral blood mononuclear cells of periparturient dairy cows

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Animal, resubmitted after revision

Abstract

The study aimed to examine effects of supplemented conjugated linoleic acids (CLA) to periparturient dairy cows receiving different concentrate proportions *antepartum* (a.p.) to investigate CLA effects on metabolism and immune function. Compared to adapted feeding, high-concentrate diet a.p. should induce a ketogenic metabolic situation *postpartum* (p.p.) to better understand how CLA works. 64 pregnant German Holstein cows had *ad libitum* access to partial mixed rations based on concentrate and roughage 3 weeks prior to calving until day 60 p.p.. A.p., cows received 100 g/d control fat (CON) or a CLA supplement, either in a low-concentrate (20%, CON-20, CLA-20) or high-concentrate diet (60%, CON-60, CLA-60). P.p., concentrate proportion was adjusted to 50% while fat supplementation continued. After day 32 p.p., half of the animals of CLA-groups changed to CON supplementation (CLA-20-CON, CLA-60-CON). A ketogenic metabolic state p.p. was not achieved and respective impacts of CLA could not be examined. Blood samples for isolation of peripheral blood mononuclear cells (PBMC) were collected on day -21, 7, 28 and 56 relative to calving. Blood chemistry samples were taken over the entire experimental period. Mitogen-stimulated proliferation of PBMC remained unaffected. Besides serum concentrations of triglycerides, total bilirubin, total protein, albumin and Insulin-like growth factor 1, clinical-chemical serum characteristics remained uninfluenced by treatments. No post-supplementation effect could be observed. Measured blood metabolites and mitogen-stimulated proliferation of PBMC indicate that all groups had an increased metabolic stress around calving, whereby group CLA-20 was affected more severely. Overall, supplemented CLA did not positively affect metabolism or immune function of periparturient dairy cows. But feeding CLA in a low-concentrate diet a.p. seems to increase liver stress around calving via reduced DMI.

Keywords: conjugated linoleic acid, concentrates, cows, blood metabolites, peripheral blood mononuclear cells

Implications

Trans-10,cis-12 CLA isomer is known to have milk fat reducing properties. Hence, CLA may have the potential to counteract negative energy balances and further may reduce metabolic disorders of early lactating cows. However, possible CLA impacts on metabolism and immune function have not been sufficiently investigated during transition period. Effects on blood metabolite concentration and proliferation of PBMC of periparturient cows receiving different concentrate feed proportions a.p. were studied to evaluate possible impacts of CLA

on metabolism and immune function. Compared to adapted feeding, high-concentrate diet a.p. should induce a ketogenic metabolic situation p.p. to better understand how CLA works.

Introduction

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of linoleic acid characterized by conjugated double bonds. Especially, *trans*-10,*cis*-12 CLA isomer is frequently added to dairy cow diets, because of its milk fat reducing properties (Baumgard *et al.*, 2000) and their potential to counteract a negative energy balance during early lactation (Odens *et al.*, 2007). Consequently, dietary CLA supplementation may induce a reduction of metabolic disorders of early lactating cows, which may be reflected in altered blood metabolite concentrations. Furthermore, little is known about CLA impacts on the function of bovine peripheral blood mononuclear cells (PBMC) during transition period, which is influenced by strong fluctuations of blood non-esterified fatty acid (NEFA) concentrations. Dairy cows are known to have a suppressed immune system during transition period (Vangroenweghe *et al.*, 2005), reflected in a reduced response of PBMCs to mitogen-stimulation (Nonnecke *et al.*, 2003, Loiselle *et al.*, 2009). The underlying reasons are not entirely clear, but Lacetera *et al.* (2004) assumed that increased blood NEFA concentrations, as occurring through increased body fat mobilization around parturition, have a negative influence on the function of PBMCs and thus may be responsible for the immunosuppression of transition cows. For this purposes, the study aimed to investigate effects of supplemented CLA on cows fed various concentrate proportions in the ration *antepartum* (a.p.). It was expected that dietary supplemented CLA reduce metabolic disturbances of early lactating cows due to a decreased milk energy output and thus improved negative energy balance in this time. Blood NEFA concentration might be reduced via decreased adipose tissue mobilization, which in turn might ameliorate effects of immunosuppression. Furthermore, lower blood NEFA concentrations might reduce liver stress at the onset of lactation, may reflecting in blood metabolites especially activities of liver enzymes. Influencing the metabolic situation of cows immediately before calving is a possibility to examine and to better understand how CLA affects metabolism. Therefore, compared to an adapted feeding a.p., the high concentrate diet was fed 3 weeks prior to calving to induce a ketogenic metabolic situation and hence an increased lipolysis *postpartum* (p.p.). A group-specific termination of CLA addition in the p.p. period was performed to determine possible post-treatment effects, since CLA may also affect the mentioned parameters after completion of CLA supplementation (Liermann, 2008). Overall, effects on blood metabolite concentration

and proliferation of PBMCs of periparturient dairy cows were studied to evaluate possible impacts of CLA on metabolism and immune function.

Material and methods

Experimental design, animals and feeding

The study was performed at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany, according to European Community regulations concerning the protection of experimental animals. The present trial was part of a comprehensive feeding study, which was previously described in detail by Petzold *et al.* (2013). Briefly, 64 pregnant German Holstein cows (48 pluriparous, 762 ± 14 kg and 16 primiparous, 640 ± 11 kg) were assigned to one of four dietary treatments according to mean number of lactation (1.7 ± 0.2) and fat-corrected milk yield of previous lactation (5938 ± 160 kg, 200 d milk yield). It was ensured that the number of primiparous cows was randomly assigned to treatments. The trial was divided into three periods and started on day 21 a.p. and was terminated on day 60 p.p.. Three weeks prior to calving (Period 1), group CON-20 (n=16) and CLA-20 (n=16) received 100 g/d control fat (CON) or CLA supplement in a low (20%) concentrate diet, whereas group CON-60 (n=16) and CLA-60 (n=16) were fed 100 g/d CON or CLA supplement in a high (60%) concentrate diet. Cows had *ad libitum* access to partial mixed rations (PMRs) consisting of 20% or 60% concentrate and 80% or 40% roughage (60% corn silage and 40% grass silage on dry matter [DM] -basis), respectively. PMRs were offered in self-feeding stations (type RIC, manufacturer Insentec, B.V., Marknesse, The Netherlands). The fat supplements were included into 2 kg concentrate supplied via computerized concentrate feeding stations (manufacturer Insentec, B.V., Marknesse, The Netherlands). A commercial rumen-protected CLA preparation (Lutrell® Pure, BASF SE, Ludwigshafen, Germany), containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA, and a rumen-protected CON preparation (Silafat®, BASF SE, Ludwigshafen, Germany), containing stearic acid instead of conjugated linoleic acids, were used as CLA and CON supplements, respectively. Period 2 started after calving and lasted for 31 days. Cows were fed a PMR for *ad libitum* consumption based on 50% concentrate and 50% roughage (60% corn silage and 40% grass silage on DM-basis) while fat supplementation continued. In Period 3, day 32-60 p.p., half of the animals of CLA-groups changed to CON supplementation (CLA-20-CON and CLA-60-CON), while in groups CLA-20 and CLA-60 the CLA supplementation continued. The feeding in groups CON-20 and CON-60 was not changed. The composition of concentrates and PMRs are presented in Table

1. All diets were formulated to meet the nutritional requirements of cows stated by the Society of Nutrition Physiology (GfE, 2001). Cows had *ad libitum* access to water.

Table 1 Components and chemical composition of concentrates and partial mixed rations (PMR); Means.

Variable	Concentrate				Partial mixed ration		
	CON	CLA	PMR-20/60	PMR-50	PMR-20[1]	PMR-60[2]	PMR-50[3]
Components [%]							
Wheat	41.0	41.0	41.0	41.0			
Dried sugar beet pulp	25.5	25.5	30.5	30.3			
Rapeseed meal	20.0	20.0	20.0	20.0			
Soybean meal	6.5	6.5	6.5	6.5			
Vitamin/mineral premix[4]	2.0	2.0	2.0	-			
Vitamin/mineral premix[5]	-	-	-	2.0			
Calcium carbonate	-	-	-	0.2			
CLA supplement	-	5.0	-	-			
Control fat supplement	5.0	-	-	-			
Analysed chemical profile							
Dry matter [g/kg]	886	886	876	879	335	450	447
Nutrient [g/kg DM]							
Crude ash	58	65	64	67	67	65	66
Crude protein	188	187	195	192	109	136	138
Ether extract	80	64	25	29	35	32	35
Crude fibre	94	94	97	103	207	174	172
Acid detergent fibre	134	126	130	136	231	202	200
Neutral detergent fibre	274	259	265	275	436	392	399
Energy[6] [MJ /kg DM]							
ME	13.9	13.8	13.5	13.6	10.5	12.1	11.7
NE_L	8.8	8.7	8.6	8.7	6.3	7.5	7.3
CLA[7] [g/kg DM]							
C18:2 *t*10,*c*12	0.0	4.6	0.0	0.0	0.0	0.0	0.0
C18:2 *c*9,*t*11	0.0	4.4	0.0	0.0	0.0	0.0	0.0

CON = Control fat; CLA = Conjugated linoleic acid; PMR= Partial mixed ration
[1]*Antepartum* PMR containing 20% concentrate on DM basis.
[2]*Antepartum* PMR containing 60% concentrate on DM-basis.
[3]*Postpartum* PMR containing 50% concentrate on DM basis.
[4]For dry cows. Ingredients per kg mineral feed: 60 g Ca, 105 g Na, 80 g P, 50 g Mg, 7000 mg Zn, 4800 mg Mn, 1250 mg Cu, 100 mg I, 40 mg Se, 30 mg Co, 800,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
[5]For lactating dairy cows. Ingredients per kg mineral feed: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6000 mg Zn, 5400 mg Mn, 1000 mg Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
[6]Calculation based on nutrient digestibilities measured with wethers (GfE, 1991).
[7]Calculation based on analysed concentrates and silage.

Sample collection and analyses

Blood samples were obtained from *Vena jugularis externa* on day -21, -14, -7, -3, 1, 3, 7, 14, 21, 28, 42 and 56 relative to calving. Blood was centrifuged at 2000 x g and 15 °C for 15 minutes after incubating 30 minutes by 30 °C. Concentrations of albumin, aspartate amino-tranferase (ASAT), γ-glutamyl-transferase (GGT), glutamate dehydrogenase (GLDH),

glucose, triglycerides, total bilirubin (Bili), total cholesterol (Chol) and total protein (Prot) in blood serum were determined photometrically by an automatic clinical chemistry analyser (Eurolyser, Qinlab Diagnostic GbR, Martinsried, Germany). Insulin-like growth factor 1 (IGF-1) concentration in blood serum was analyzed at the Department of Obstetrics and Reproduction, Faculty of Veterinary Science of the Szent Istvan University in Budapest, Hungary. Briefly, IGF-1 blood serum concentration was measured with a ^{125}I-labelled IGF-1-RIA CT kit developed for human samples, including a preceding extraction of IGF-1 with an ethanolic HCl solution and a before-assay neutralization of extracts (Cisbio Bioassays Codolet/ France; sensitivity: 0.85 ng/ml; intra- and inter-assay CV: from 3.4 to 6.6 and ≤ 7.0%, respectively). The assay was adapted and validated for bovine plasma samples, described by Balogh *et al.* (2012).

On day -21, 7, 28 and 56 relative to calving, blood was taken by jugular venipuncture into heparinized vacutainer tubes for isolation and proliferation of bovine PBMC. Both procedures were carried out according to Renner *et al.* (2011). Briefly, PBMC were separated from heparinized, diluted blood by density-gradient centrifugation. After resuspension in a freezing medium based on foetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO), PBMC were frozen and stored at -80 °C until beginning of proliferation assay. Ten replications of thawed and washed PBMC were seeded into 96-well plates and five of them were stimulated with ConcanavalinA (ConA, Sigma-Aldrich, Steinheim, Germany, C5275) for proliferation test. A MTT-assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to test the metabolic activity of proliferating cells. The optical density (OD) of incubated PBMC was measured at a wavelength of 570 nm and was corrected by a blank value. The stimulation index (SI) was calculated by the ratio between OD of CON A stimulated and non-stimulated PBMC.

Statistical analyses

The software package SAS (Version 9.2, SAS Institute, Cary, NC, USA) was used for all statistical evaluation. Data were processed using a random-regressions model with fixed regression coefficients for groups and random regression coefficients to take into consideration repeated measures per animal. The non-linear relationships between the traits under investigation and the experimental day relative to calving were modeled by splines with two or three nodal points according to the parameters development during the experimental time to generate group-specific progressive graphs. This model enabled a better description of kinetics of investigated parameters over several experimental periods. The model contained

date of measuring day and group-specific regression coefficients, respectively, as fixed factors. The regression coefficient of each animal within the respective group and the rest effects were considered in the model as random effect. In order to generate least square means (LSMeans) for each period for the respective groups, the data were processed using the same model approach. An average estimated value of the respective period for each group was calculated and the statistical analysis was performed using the F-test and Tukey-test. The F-test between the LSMeans of each group for the respective periods was calculated by the CONTRAST-option using the PROC MIXED procedure. In the case of significances between the LSMeans of the F-test, the Tukey's multiple range test was carried out. Differences with $p < 0.05$ were considered to be significant and a tendency was declared when $p < 0.1$. A *p*-value for the F-test between the LSMeans is provided. Results are shown as LSMeans and root mean square errors (RMSE). The generated values for each group for the respective groups are shown in Table 3 and 4. The weekly dissolved values for each group for serum triglyceride, Bili and IGF-1 are presented as progressive graph in figures (Figure 1-3) and used to get an impression of the time-dependent course and additionally for evaluation of the development of differences between the groups.

Results

Based on analyses of concentrates and silages (Table 1), group CLA-20 and CLA-60 received in total approximately 8 g/d *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA, respectively. Dry matter intake (DMI) decreased slightly with progressing pregnancy and increased consistently p.p.. In Period 1, Group CLA-60 consumed significantly more DM compared to low-concentrate groups (Table 2). More data concerning the performance of cows used in the present study are reported by Petzold *et al.* (2013). Measured serum metabolite concentrations and SI of PBMC changed in the course of the trial (Table 3, 4 and Figure 1-3). Serum concentrations of ASAT, GGT, GLDH, Chol, Bili and Prot increased with processing experimental time (ASAT from 74 to 98 to 85 IU/l, GGT from 18 to 22 to 31 IU/l, GLDH from 8 to 16 to 21 IU/l, Chol from 65 to 100 to 181 mg/dl, Bili from 2 to 3 mg/l and Prot from 73 to 79 to 85 g/l), whereby serum glucose showed reduced concentrations in Period 2 (from 60 to 55 to 60 mg/dl). For serum albumin similar concentrations could be observed. Serum triglyceride and IGF-1 concentrations decreased after calving, followed by a slight increase in Period 3 (triglyceride from 18 to 12 to 14, IGF-1 from 206 to 110 to 131 ng/ml). SI of PBMC was reduced around calving and increased slightly p.p. (SI of PBMCs from 7 to 8 to 8). With exception of serum triglyceride, Bili and IGF-1 in Period 1, measured blood metabolites and SI of PBMC were

Table 2 Dry matter intakes of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) in Period 1 (day 21-1 a.p.), Period 2 (day 1-31 p.p.) and Period 3 (day 32-60 p.p.); LSMeans; (Petzold et al., 2013).

	Control fat (100 g/d)		CLA (100 g/d)		Control fat (100 g/d)			
	CON-20[1]	**CON-60**[2]	**CLA-20**[1]	**CLA-60**[2]	**CLA-20-CON**	**CLA-60-CON**	RMSE[5]	*p-value*[6]
	(n=16)	*(n=15)*	*(n=16)*	*(n=16)*				
Period 1	12.5[a]	14.3[ab]	12.3[a]	16.1[b]			1.53	<0.001
Period 2[3]	17.4	17.5	17.0	18.9			1.53	0.177
	(n=16)	*(n=15)*	*(n=9)*	*(n=8)*	*(n=7)*	*(n=8)*		
Period 3[4]	21.5	21.6	21.5	22.1	21.4	22.3	1.53	0.986

RMSE = root mean square error.
[1]Group CON-20 and CLA-20 received a low concentrate diet *antepartum*.
[2]Group CON-60 and CLA-60 received a high concentrate diet *antepartum*.
[3]*Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued.
[4]Half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON and CLA-60-CON).
[5]Depending on the statistical model, only one RMSE per trait exist for the entire experimental.
[6]Data were processed using a random-regressions model with fixed regression coefficients for groups and random regression coefficients to take into consideration repeated measures per animal, as described in detail in Statistical analysis.
[a,b]Values within a row with different superscripts differ significantly at $p<0.05$.

not influenced by the treatments during experimental periods (Table 3, 4). Group CLA-20 showed significantly increased serum triglyceride concentrations compared to group CON-60 and CLA-60 in Period 1 (Table 3) and to group CON-20 on day 14 and 7 a.p. when comparing weekly dissolved values (Figure 1). Serum Bili concentration of group CLA-20 was significantly increased compared to group CLA-60 before calving (Table 3) and to all remaining groups on day 1 and 3 p.p. (Figure 2). Reduced IGF-1 concentrations were observed for group CLA-20 compared to CON-60 in Period 1 (Table 3). When comparing weekly dissolved values, group CLA-20 had lower IGF-1 concentrations than high-concentrate groups before and shortly after calving (Figure 3). Even if serum Prot and albumin concentration remained unaffected by treatments (Table 3), differences between the groups occurred when comparing weekly dissolved values for each group over the whole experiment. Significantly reduced Prot serum concentrations were observed for group CLA-20 compared to CLA-60 one week before and shortly after calving. Additionally, serum albumin concentration was significantly reduced compared to high-concentrate groups within the first week p.p. and to all groups in week 2 and 3 p.p. (data not shown). Blood metabolites were within the reference range according to Kraft and Dürr (2005), besides lower serum concentrations of Chol and albumin in Period 1, increased serum Bili concentrations around calving (Figure 2) and reduced triglyceride and higher ASAT serum concentrations in Period 2.

Table 3 Blood metabolites and stimulation index of peripheral blood mononuclear cells of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) in Period 1 (day 21 to 1 a.p.) and Period 2 (day 1 to 31 p.p.); LSMeans.

	Control fat (100 g/d)		CLA (100 g/d)			
	CON-20[1] *(n=16)*	**Con-60**[2] *(n=15)*	**CLA-20**[1] *(n=16)*	**CLA-60**[2] *(n=16)*	RMSE[5]	p-value
Period 1						
Glu [mg/dl]	60.8	59.3	60.2	60.1	7.83	0.937
TG [mg/dl][4]	17.8^{ab}	17.0^{a}	19.6^{b}	15.4^{a}	3.88	<0.001
ASAT [IU/l][4]	68.8	72.2	62.6	90.3	33.66	0.454
GGT [IU/l][4]	17.1	17.0	19.4	19.2	4.87	0.483
GLDH [IU/l][4]	6.9	7.6	7.7	8.8	10.40	0.910
Chol [mg/dl][4]	64.3	65.0	69.3	62.4	14.68	0.721
Bili [mg/l][4]	2.2^{ab}	1.9^{ab}	2.4^{b}	1.6^{a}	1.07	0.018
Prot [g/l][4]	73.2	73.9	70.6	74.8	6.38	0.349
Alb [g/l][4]	24.7	25.1	24.4	25.6	2.30	0.178
IGF-1 [ng/ml]	202.8^{ab}	233.3^{b}	173.9^{a}	215.6^{ab}	33.33	0.019
SI of PBMCs	7.2	5.9	7.1	6.5	2.48	0.614
Period 2[3]						
Glu [mg/dl]	53.9	53.7	56.3	55.2	7.83	0.712
TG[mg/dl][4]	12.3	12.8	12.3	12.3	3.88	0.950
ASAT [IU/l][4]	89.0	101.2	94.7	108.7	33.66	0.427
GGT [IU/l][4]	22.3	22.4	21.7	21.3	4.87	0.982
GLDH [IU/l][4]	13.5	19.2	13.4	16.5	10.40	0.697
Chol [mg/dl][4]	102.5	104.5	87.8	104.1	14.68	0.125
Bili [mg/l][4]	2.8	2.9	2.9	2.7	1.07	0.816
Prot [g/l][4]	78.5	81.1	75.9	79.3	6.38	0.204
Alb [g/l][4]	24.7	24.8	24.8	25.9	2.30	0.380
IGF-1 [ng/ml]	101.9	111.8	106.1	118.7	33.33	0.727
SI of PBMCs	7.4	8.3	8.0	7.9	2.48	0.804

RMSE = root mean square error; Gluc = Glucose; TG = Triglyceride; ASAT = aspartate amino-transferase; GGT = γ-glutamyl-transferase; GLDH = glutamate dehydrogenase; Chol = Total cholesterol; Bili = Total bilirubin; Prot = Total protein; Alb = Albumin; IGF-1 = Insulin-like growth factor 1; SI of PBMCs = Stimulation index of peripheral blood mononuclear cells.

[1]*Antepartum*, Group CON-20 and CLA-20 received a low concentrate diet.

[2]*Antepartum*, Group CON-60 and CLA-60 received a high concentrate diet.

[3]*Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued.

[4]Reference values according to Kraft and Dürr (2005): TG: 15-45 mg/dl; ASAT: <80 IU/l; GGT: <50 IU/l; GLDH: <30 IU/l; Chol: >75 mg/dl; Bili: <3 mg/dl; Prot: 60-80 g/l; Alb: 30-42 g/l.

[5]Depending on the statistical model, only one RMSE per trait exist for the entire experimental period.

[a,b]Values within a row with different superscripts differ significantly at $p<0.05$.

Table 4 Blood metabolites and stimulation index of peripheral blood mononuclear cells of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) in Period 3 (day 32 to 60 p.p)[3]; LSMeans.

	Control fat (100 g/d)		CLA (100 g/d)		Control fat (100 g/d)			
	CON-20[1] *(n=16)*	**Con-60**[2] *(n=15)*	**CLA-20**[1] *(n=9)*	**CLA-60**[2] *(n=8)*	**CLA-20-CON** *(n=7)*	**CLA-60-CON** *(n=8)*	RMSE[5]	*p*-value
Gluc [mg/dl]	59.4	55.3	58.9	63.1	62.0	61.7	7.83	0.300
TG [mg/dl][4]	13.9	14.6	12.3	13.0	15.0	12.6	3.88	0.588
ASAT [IU/l][4]	78.2	93.9	81.0	89.8	83.4	86.5	33.66	0.685
GGT [IU/l][4]	31.3	35.1	31.3	27.1	28.4	31.7	4.87	0.611
GLDH [IU/l][4]	17.4	30.3	18.8	19.0	19.6	21.3	10.40	0.510
Chol [mg/dl][4]	185.3	196.6	161.8	182.7	168.1	189.8	14.68	0.290
Prot [g/l][4]	82.6	88.1	86.2	85.1	89.1	80.3	1.07	0.204
Alb [g/l][4]	30.9	31.2	31.4	33.4	33.1	30.8	6.38	0.516
IGF-1 [ng/ml]	140.2	147.6	126.9	125.6	117.0	125.7	2.30	0.661
SI of PBMCs	8.0	7.7	8.0	7.6	9.3	8.0	33.33	0.844

RMSE = root mean square error; Gluc = Glucose; TG = Triglyceride; ASAT = aspartate amino-transferase; GGT = γ-glutamyl-transferase; GLDH = glutamate dehydrogenase; Chol = Total cholesterol; Prot = Total protein; Alb = Albumin; IGF-1 = Insulin-like growth factor 1; SI of PBMCs = Stimulation index of peripheral blood mononuclear cells.

[1]*Antepartum*, Group CON-20 and CLA-20 received a low concentrate diet.

[2]*Antepartum*, Group CON-60 and CLA-60 received a high concentrate diet.

[3]*Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON and CLA-60-CON).

[4]Reference values according to Kraft and Dürr (2005): TG: 15-45 mg/dl; ASAT: <80 IU/l; GGT: <50 IU/l; GLDH: <30 IU/l; Chol: >75 mg/dl; Prot: 60-80 g/l; Alb: 30-42 g/l.

[5]Depending on the statistical model, only one RMSE per trait exist for the entire experimental period.

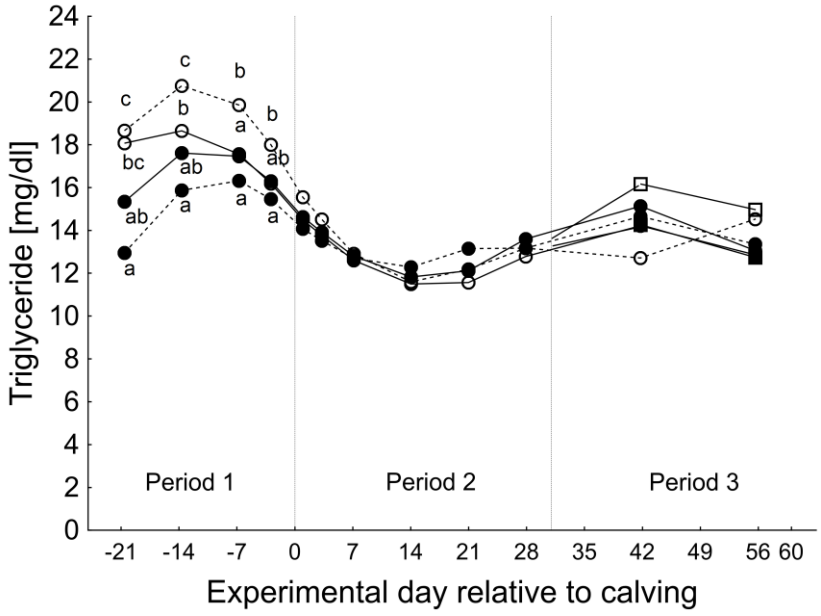

Figure 1 Development of triglycerides in serum

Antepartum (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. In Period 3 half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-

CON [■]). [a,b,c]Only significantly different LSMeans between the groups are marked with superscripts (P<0.05).

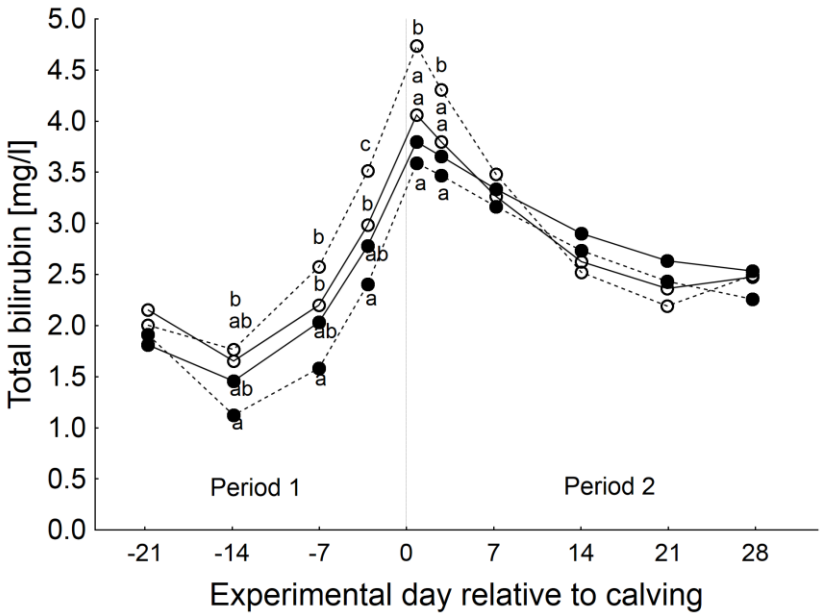

Figure 2 Development of total bilirubin in serum
Antepartum (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [a,b]Only significantly different LSMeans between the groups are marked with superscripts (P<0.05).

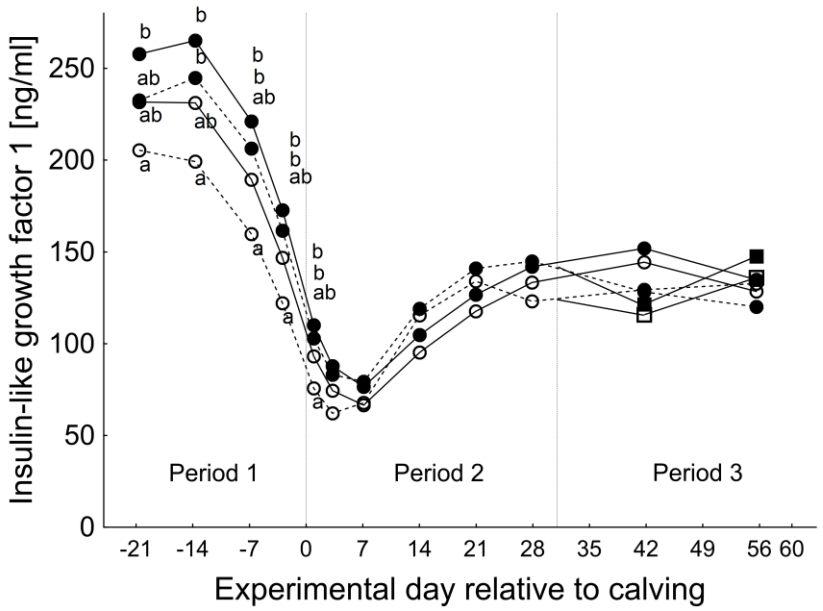

Figure 3 Development of Insulin-like growth factor 1in serum
Antepartum (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. In Period 3 half of

the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). [a,b]Only significantly different LSMeans between the groups are marked with superscripts ($P<0.05$).

Discussion

The study aimed to investigate effects of CLA supplementation and various concentrate proportion in diets during late pregnancy on blood metabolites and SI of PBMC of periparturient dairy cows to evaluate possible CLA impacts on metabolism and immune function. CLA supplementation was initiated 21 days before anticipated calving and continued until day 60 p.p., whereby a group-specific termination of CLA supplementation after day 32 p.p. was conducted to determine possible post-treatment effects. Cows received diets with either a low or high concentrate diet a.p., whereby the high concentrate proportion was fed to induce a ketogenic metabolic situation of cows p.p. for a better examination of the supposed lipid metabolism modifying properties of added CLA. Overconditioning is negatively related to DMI a.p. and obese cows are observed to have a reduced appetite and lose more body weight in early lactation. Due to inadequate DMI p.p., overconditioned cows are subjected to increased body fat mobilization and are therefore more susceptible to metabolic diseases, like ketosis (Grummer, 1995, Hayirli and Grummer, 2004). However, differently as expected, such a metabolic state was not achieved and hence respective impacts of CLA could not be examined. DMI of group CLA-60 and CON-60 fed a high concentrate proportion was not observed to be reduced during early lactation. Moreover, live weight and blood NEFA and BHB concentrations, which are generally used to assess the body fat mobilization and energy deficit in early lactating cows, remained also unaffected during the p.p. period between the groups (Petzold *et al.*, 2013). Perhaps, feed intake restriction or deprivation during early lactation, as realized in other trials (Loor *et al.*, 2007, Kuhla *et al.*, 2009), might have been a more successful approach to induce a ketotic state p.p..

CLA effects on blood metabolites

CLA was supplemented as an approach to induce a milk fat reduction and hence decrease the energy deficit and thus metabolic disorders of early lactating cows. However, only CLA supplementation in a high concentrate diet a.p. caused an improvement in estimated energy balance p.p. through increasing DMI, whereas milk composition and milk yield of cows used in the present study remained unchanged by the treatments. (Petzold *et al.*, 2013). Moreover, serum NEFA and BHB concentrations (Petzold *et al.*, 2013) and nearly all investigated blood metabolites remained unaffected by CLA supplementation and dietary concentrate proportion. Additionally, no CLA post-supplementation effect could be observed. Findings indicate that CLA supplements did not have the potential to reduce metabolic disorders of periparturient

dairy cows, even if CLA supplementation has clearly been established by significantly increased proportions of *trans*-10,*cis*-12 CLA in milk fat of CLA-groups compared to CON-groups on day 21 p.p. (Petzold *et al.*, 2013), revealing that CLA was absorbed in the intestine. However, Pappritz *et al.* (2011) examined the duodenal availability of a lipid-encapsulated CLA preparation in cows, which was also used in the present study. Authors observed, based on intakes of 8 g/d *trans*-10,*cis*-12 CLA, that only 5% of CLA was available in duodenum. Assuming the same conditions in this trial, only 0.4 g of 8 g *trans*-10,*cis*-12 CLA/d would have reached the duodenum. Perhaps, this insufficient CLA rumen protection against microbial degradation and hence relatively low amounts of available CLA in the duodenum could have led to a lack of CLA effects.

Most of the observed effects on blood metabolite concentrations were related to variations in DMI resulting from different concentrate proportion a.p. and CLA addition. DMI were reduced around calving and coincides with elevated NEFA concentrations, indicating an energy deficit and a subsequent lipid mobilization (Dirksen et al. 2006). Accordingly, cows were characterized by low serum glucose levels around parturition. However, no treatment effect could be observed, revealing that CLA did not alter gluconeogenesis, which is in accordance with other CLA-studies (Bernal-Santos *et al.*, 2003, Castaneda-Gutierrez *et al.*, 2005). Additionally, blood triglyceride concentration is mainly dependent on feed intake and also on hepatic synthesis (Kraft and Dürr, 2005). Consequently, observed differences among the groups a.p. may indicate that cows of Group CLA-60 and CON-60 may have had a slightly impaired liver function a.p. through a higher DMI a.p and thus an increased hepatic infiltration of fatty acids. A CLA effect can be excluded since Bernal-Santos *et al.* (2003) and Selberg *et al.* (2004) observed that dietary supplemented CLA does not have an influence on triglyceride concentrations in liver. Moreover, reduced DMI may have led to a general decline in serum triglyceride concentration after calving in all groups. However, it should be noted that a reinforced lipolysis and thus enhanced uptake of NEFA by hepatic tissue leads to increased triglyceride accumulation, resulting in diseases like fatty liver (Grummer, 1993, Goff and Horst, 1997). Hence, the occurrence of fatty liver or an impaired liver function could have further intensified the decline of serum triglycerides p.p. through reduced hepatic synthesis. However, cell and liver damage indicating enzymes like GGT or GLDH remained unaffected during experimental periods. Only the enzyme ASAT showed increased activities in Period 2. Bostedt (1974) discussed that elevated ASAT activities are not necessarily pathological and assumed that liver cells need to adapt to a reinforced turnover rate through increased metabolic requirements during early lactation. Therefore, it can be assumed that

hepatic tissue was not pathological altered around parturition. Reduced serum triglyceride concentrations were in accordance with low Chol and high Bili concentrations around calving. Low Chol levels are associated with disorders of digestion and liver diseases like fatty liver (Kraft and Dürr, 2005). Accordingly, Bili concentrations were highest shortly p.p., which might be due to hepatopathies, cholestasis or increased disintegration of erythrocytes (Kraft and Dürr, 2005). Chol and Bili concentrations were observed to increase or decrease, respectively, with progressing lactation parallel to DMI p.p., which may strengthen the hypothesis that liver needed to adapt to nutritional and metabolic changes around parturition. However, Group CLA-20 showed increased Bili values compared to group CLA-60 before calving and to all remaining groups on day 1 and 3 p.p.. Liermann (2008) observed that CLA supplementation does not have an influence on blood Bili concentrations. A reason for elevated Bili concentrations of Group CLA-20 might be its significantly reduced DMI in this time, especially compared to Group CLA-60. Hence, NEFA serum concentrations of Group CLA-20 were expected to be increased around parturition compared to the other groups due to supply more energy via body fat mobilization. As a consequence, Bili serum concentrations of Group CLA-20 may be increased because NEFA compete with Bili for the same transport proteins in hepatocytes (Kraft and Dürr, 2005). However, NEFA serum concentrations remained unaffected, but Group CLA-20 showed significantly reduced Prot serum concentrations compared to Group CLA-60 around calving, indicating malnutrition or an impaired hepatic protein synthesis (Kraft and Dürr, 2005). Bogin *et al.* (1988) reported that a hepatic failure to synthesize proteins due to liver diseases like fatty liver are associated with reduced albumin concentrations. Accordingly, low serum albumin levels were observed in Period 2 for Group CLA-20 and let suggest that Group CLA-20 may have developed hepathopaties through its significantly reduced DMI before and after parturition. Moreover, Lucy (2000) reported that diseased or mal-nourished animals generally have lower blood IGF-1 concentrations than well-nourished and healthy animals, indicating a compromised state of tissue, organ and cell function. All groups were characterized by a decrease in serum IGF-1 concentration around calving, but Group CLA-20 showed significantly reduced IGF-1 serum concentrations before and shortly after calving compared to Group CON-60 and CLA-60, which may confirm the suspicion about its more negatively affected liver function. However, as discussed above, there was no indication that liver cells were damaged at this time. Compared to the other groups, Group CLA-20 may have had a less optimal energy and nutrient supply via reduced DMI and thus a severely impaired metabolism around calving. Moreover, energy deficits leading to a time delay of first ovulation p.p. and it is known that

IGF-1 blood levels depending on cows' energy state, whereby rising levels are related with earlier resumption of ovulation (Butler, 2000). Previous studies (Bernal-Santos *et al.*, 2003, Castaneda-Gutierrez *et al.*, 2005) reported that dietary supplemented CLA tended to reduce days until first ovulation p.p. and increase pregnancy rates in cows. Castaneda-Gutierrez *et al.* (2007) assumed that these observations are possibly related to increased IGF-1 blood levels, caused by CLA supplementation. However, IGF-1 serum concentrations remained unaffected by CLA-supplementation in the present study, which could also be observed by von Soosten *et al.* (2012). Moreover, Moallem *et al.* (2010) could also found no evidence that supplemented CLA have a direct influence on reproduction.

CLA effects on immune function

No differences in mitogen-stimulated proliferation of PBMC could be observed among the groups during experimental periods, which might be due to unaffected milk fat yield and serum NEFA concentrations (Petzold *et al.*, 2013). However, with the beginning of feed intake reduction a.p., NEFA serum concentrations exhibited a sharp increase with highest values shortly p.p. (Petzold et al. 2013), which were in accordance with reduced mitogen-stimulated proliferations of PBMC in all groups. This observation may reflect a compromised immune function (Nonnecke *et al.*, 2003, Loiselle *et al.*, 2009) of cows around parturition and may confirm the inverse correlation between the function of PBMC and blood NEFA concentration (Lacetera *et al.*, 2004), but indicate also that supplemented CLA did not have the potential to ameliorate the effects of an immunosuppression.

Conclusion

A ketogenic metabolic state p.p. was not achieved and respective impacts of CLA could not be examined. The evaluation of blood data and mitogen-stimulated proliferation of bovine PBMC *ex vivo* indicate that dietary supplemented CLA did not have positive effects on metabolism and immune function of periparturient dairy cows. No post-supplementation effects could be observed. Measured blood metabolites were mainly affected by differences in DMI, resulting from different concentrate proportions a.p. and CLA addition. All cows were subjected to metabolic challenges around calving through limited DMI potential and tended to develop liver diseases in this time, whereby cows of Group CLA-20 were affected more severely. It seems that feeding CLA in a low concentrate diet prepartum leads to increase liver stress around calving via reduced DMI. Further studies are necessary to examine the underlying metabolic mechanism of observed effects.

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References

Balogh OG, Febel H, Huszenicza G, Kulcsar M, Abonyi-Toth Z, Endrodi T and Gabor G 2012. Seasonal fertility differences in synchronised dairy cows: ultrasonic, metabolic and endocrine findings. Acta Veterinaria Hungarica 60, 131-143.

Baumgard LH, Corl BA, Dwyer DA, Saebo A and Bauman DE 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. American Journal of Physiology, Regulatory, Integrative and comparative Physiology 278, R179-R184.

Bernal-Santos G, Perfield JW, 2nd, Barbano DM, Bauman DE and Overton TR 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. Journal of Dairy Science 86, 3218-3228.

Bogin E, Avidar Y, Merom M, Soback S and Brenner G 1988. Biochemical changes associated with the fatty liver syndrome in cows. Journal of Comparative Pathology 98, 337-347.

Bostedt H 1974. Enzyme activity in the blood serum of cows during the period before and after parturition. Berliner und Muenchener Tieraerztliche Wochenschrift 87, 365-371.

Butler WR 2000. Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduction Science 60, 449-457.

Castaneda-Gutierrez E, Overton TR, Butler WR and Bauman DE 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. Journal of Dairy Science 88, 1078-1089.

Castaneda-Gutierrez E, Benefield BC, de Veth MJ, Santos NR, Gilbert RO, Butler WR and Bauman DE 2007. Evaluation of the mechanism of action of conjugated linoleic acid isomers on reproduction in dairy Cows(1). Journal of Dairy Science 90, 4253-4264.

GfE 1991. (Society of Nutrition Physiolgy). Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern (Guidelines for determining the digestibility of crude ruminants). Journal of Animal Physiology and Animal Nutrition 65, 229-234.

GfE 2001. (Society of Nutrition Physiolgy). 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.

Goff JP and Horst RL 1997. Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science 80, 1260-1268.

Grummer RR 1993. Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy-Cows. Journal of Dairy Science 76, 3882-3896.

Grummer RR 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science 73, 2820-2833.

Hayirli A and Grummer RR 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Canadian Journal of Animal Science 84, 337-347.

Kraft W and Dürr UM 2005. Klinische Labordiagnostik in der Tiermedizin [Clinical laboratory diagnostics in veterinary medicine]. Schattauer Verlag, Stuttgart, Germany.

Kuhla B, Albrecht D, Kuhla S and Metges CC 2009. Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid, and glycogen metabolism. Physiological Genomics 37, 88-98.

Lacetera N, Scalia D, Franci O, Bernabucci U, Ronchi B and Nardone A 2004. Short communication: Effects of nonesterified fatty acids on lymphocyte function in dairy heifers. Journal of Dairy Science 87, 1012-1014.

Liermann T 2008. Einfluss einer Zulage von pansengeschützter konjugierter Linolsäure(CLA) in Kombination mit Propylenglykol oder pansengeschütztem Fett auf Leistungsmerkmale, Stoffwechselparameter und den Energiestatus frischlaktierender Milchkühe [Effects of feeding rumenprotected conjugated linoleic acids (CLA) alone or in combination with propylene glycol or rumenprotected fat on performance and metabolic parameters and energy status of early lactation dairy cows]. Dissertation, Technische Universität München, München, Germany, 188 pages.

Loiselle MC, Ster C, Talbot BG, Zhao X, Wagner GF, Boisclair YR and Lacasse P 2009. Impact of postpartum milking frequency on the immune system and the blood metabolite concentration of dairy cows. Journal of Dairy Science 92, 1900-1912.

Loor JJ, Everts RE, Bionaz M, Dann HM, Morin DE, Oliveira R, Rodriguez-Zas SL, Drackley JK and Lewin HA 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiological Genomics 32, 105-116.

Lucy MC 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. Journal of Dairy Science 83, 1635-1647.

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

Nonnecke BJ, Kimura K, Goff JP and Kehrli ME 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. Journal of Dairy Science 86, 2359-2368.

Odens LJ, Burgos R, Innocenti M, VanBaale MJ and Baumgard LH 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. Journal of Dairy Science 90, 293-305.

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

Petzold M, Meyer U, Kersten S, Spilke J, Kramer R, Jahreis G and Dänicke S 2013. Effects of conjugated linoleic acids and dietary concentrate proportion on performance, milk composition, milk yield and metabolic parameters of periparturient dairy cows. Archives of Animal Nutrition 67, 185-201.

Renner L, Schwabe A, Döll S, Holtershinken M and Dänicke S 2011. Effect of rare earth elements on beef cattle growth performance, blood clinical chemical parameters and mitogen stimulated proliferation of bovine peripheral blood mononuclear cells in vitro and ex vivo. Toxicol Lett 201, 277-284.

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

Vangroenweghe F, Lamote I and Burvenich C 2005. Physiology of the periparturient period and its relation to severity of clinical mastitis. Domestic Animal Endocrinology 29, 283-293.

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

PAPER III

Using rumen probes to examine effects of conjugated linoleic acids and dietary concentrate proportion on rumen pH and rumen temperature of periparturient dairy cows

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Summary

The study aimed to examine the influence of supplemented conjugated linoleic acids (CLA) to periparturient cows receiving different concentrate proportions *antepartum* on rumen pH (RpH) and rumen temperature (RT). Twenty pregnant German Holstein cows were equipped with rumen probes for continuous RpH and RT measurement in a frequency of 15 min to investigate effects of dietary concentrate and CLA around parturition and the impact of parturition itself on RpH and RT. Cows had *ad libitum* access to partial mixed rations, 3 weeks prior to calving until days 7 *postpartum*. *Antepartum*, cows received 100 g/day control fat (CON) or CLA supplement, either in low (20%; CON-20, CLA-20) or high concentrate diet (60%; CON-60, CLA-60). *Postpartum*, concentrate proportion was adjusted to 50 % while fat supplementation continued. Compared with adapted feeding, high concentrate proportions *antepartum* tended to increase DMI and reduced RpH. Groups CON-60 and CLA-60 spent more than 4 hours per day below RpH 5.6 during late pregnancy, indicating the presence of sub-acute rumen acidosis (SARA). The RT remained unaffected *antepartum*. Before calving, cows spent less time below RpH 5.6 and SARA could be detected in each group *postpartum*. Mean RpH increased slightly *antepartum*, whereas few hours before parturition a sharp decrease of RpH could be observed, accompanied with increased RT. Overall, it seems that CLA supplementation influences RpH and RT. Bearing in mind that rumen parameters fluctuate during day and herd level must be known, rumen probes for continuous RpH and RT measurement could be a useful management tool for animal health surveillance and may also help to predict parturition.

Keywords: rumen probes, conjugated linoleic acid, concentrate, parturition, rumen parameter, transition cow

Introduction

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of linoleic acid characterized by conjugated double bonds. It has been reported that CLA exert several positive physiological functions like anticarinogenic, anti-atherogenic and immunomodulatory effects or growth and lean body mass promotion (Tanaka 2005). In addition, especially the *trans*-10,*cis*-12 CLA isomer is often added to dairy cow diets, because of its milk fat reducing properties (Baumgard et al. 2000) and thus its potential to counteract a negative energy balance during early lactation (Kay et al. 2006; Odens et al. 2007). However, less is known about the impact of CLA on rumen metabolism. Conjugated linoleic acids have

been associated with a modified rumen fermentation pattern as indicated by an altered profile of short chain fatty acids. In addition, dietary supplemented CLA increased starch degradation and reduced rumen microbial protein synthesis (Pappritz et al. 2011). Consequently, CLA may influence rumen parameters like rumen pH (RpH) and rumen temperature (RT). An increased starch digestion may be led to a drop in RpH due to the fact that a rapid rumen fermentation raises the production of short chain fatty acids and further lactate (reviewed by Kafil et al. (2011)). Accordingly, RT may be increased because of the negative relationship between RpH and RT (AlZahal et al. 2008; Lohölter et al. 2013a) and a supposed higher rumen fermentation. In contrast to balance experiments and spot sampling of rumen fluid from cows fed at steady state, so-called rumen probes offer the opportunity for a continuous recording of both RpH and RT and can therefore provide useful additional information on the effects of CLA on general rumen fermentation in *ad libitum*-fed and free ranging dairy cows. Additionally, detailed data regarding the development of RpH and RT around calving under the influence of different feeding strategies during late pregnancy are less available. Moreover, there is a lack of information about the effect of parturition itself on RpH and RT.
Hence, an experiment with dairy cows during the transition period was used to study the dynamics of RpH and RT via continuous measuring under the use of rumen probes. The experiment was previously described in detail by Petzold et al. (2013). The objective of this trial was to examine the influence of supplemented CLA on rumen parameters of cows fed low and high concentrate proportions in the diet *antepartum* (ap). For the present investigation a part of the animals of the four experimental groups were equipped with rumen probes, which enabled to study the effects of dietary concentrate feed proportion and CLA supplementation around parturition and the impact of parturition itself on RpH and RT. Compared with a adapted feeding, the rumen fermentation profile was supposed to be shifted towards a lowered RpH, eventually to a sub-acute rumen acidosis (SARA) and an elevated RT due to the high concentrate level. Moreover, it was hypothesized that CLA addition leads also in a drop of RpH and an increase in RT.

Material and methods

Experimental design, animals and feeding

The study was performed at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Brunswick, Germany, in compliance with the European Union Guidelines concerning the protection of experimental animals. The present trial was part of a more comprehensive feeding study (Petzold et al. 2013).

A part of, in total, 64 pregnant German Holstein [all 16 heifers and 4 pluriparous cows with a mean live weight (LW) of 664 ± 20 kg] were assigned to one of four dietary treatment groups. The trial started on day 21 ap and continued until days 7 *postpartum* (pp) and was divided into two main periods, which refer to the time frames during late pregnancy (days 21 to 8 ap) and around calving (days 7 ap to 7 pp). *Antepartum*, groups CON-20 (*n*=5) and CLA-20 (*n*=5) received 100 g/day control fat (CON) or CLA supplement in a low (20%) concentrate diet, whereas groups CON-60 (*n*=5) and CLA-60 (*n*=5) were fed 100 g/day CON or CLA supplement in a high (60%) concentrate diet. After calving, the concentrate proportion in the feeding groups was adjusted to 50% while fat supplementation continued.

All diets were formulated to meet the nutritional requirements of cows stated by the Society of Nutrition Physiology (GfE 2001). Before calving, cows had *ad libitum* access to partial mixed rations (PMR) consisting of 20% or 60% concentrate and 80% or 40% roughage (60% corn silage and 40% grass silage on dry matter [DM] -basis), respectively. *Postpartum*, cows were fed a PMR for *ad libitum* consumption based on 50% concentrate and 50% roughage (60% corn silage and 40% grass silage on DM-basis). Partial mixed rations were offered in self-feeding stations (type RIC, manufacturer Insentec, B.V., Marknesse, The Netherlands), which were refilled daily at approximately 10.00 h. The fat supplements were included into 2 kg concentrate supplied via computerized concentrate feeding station (manufacturer Insentec, B.V., Marknesse, The Netherlands). The composition of the concentrates and PMR are presented in Table 1. A commercial rumen-protected CLA preparation (Lutrell® Pure, BASF SE, Ludwigshafen, Germany), containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA, and a rumen-protected control fat preparation (Silafat®, BASF SE, Ludwigshafen, Germany), containing stearic acid instead of the conjugated linoleic acids, were used as CLA and CON supplements, respectively. During experimental period cows were kept in two group pens in a free stall barn according to their diet ap. Cows had *ad libitum* access to water.

Sample collection and analyses

Each cow was equipped with an ear transponder to record daily individual feed and water intake. Representative PMR samples were taken daily, samples of concentrates were collected once, and samples of corn and grass silage were taken twice a week and pooled monthly. Feed samples were dried at 60°C for 72 h and were ground to a size of maximum 1mm using a Retsch mill (SM 1, Retsch, Haan, Germany) before analysing. The chemical composition of the feed [DM, crude ash (CA), crude protein (CP), crude fibre (CF), ether extract (EE), acid detergent fibre (ADF) and neutral detergent fibre (NDF)] were analysed according to the

Table 1 Components and chemical composition of concentrates and partial mixed rations (PMR); Means.

Variable	Concentrate				Partial mixed ration		
	CON	CLA	PMR-20/60	PMR-50	PMR-20*	PMR-60†	PMR-50‡
Components [%]							
Wheat	41.0	41.0	41.0	41.0			
Dried sugar beet pulp	25.5	25.5	30.5	30.3			
Rapeseed meal	20.0	20.0	20.0	20.0			
Soybean meal	6.5	6.5	6.5	6.5			
Vitamin/mineral premix§	2.0	2.0	2.0	-			
Vitamin/mineral premix¶	-	-	-	2.0			
Calcium carbonate	-	-	-	0.2			
CLA supplement	-	5.0	-	-			
Control fat supplement	5.0	-	-	-			
Analysed chemical profile							
Dry matter [g/kg]	886	886	876	879	335	450	447
Nutrient [g/kg DM]							
Crude ash	58	65	64	67	67	65	66
Crude protein	188	187	195	192	109	136	138
Ether extract	80	64	25	29	35	32	35
Crude fibre	94	94	97	103	207	174	172
Acid detergent fibre	134	126	130	136	231	202	200
Neutral detergent fibre	274	259	265	275	436	392	399
Non-fibre carbohydrates	401	424	451	437	353	375	363
*Energy*** [MJ /kg DM]							
ME	13.9	13.8	13.5	13.6	10.5	12.1	11.7
NE_L	8.8	8.7	8.6	8.7	6.3	7.5	7.3
CLA†† [g/kg DM]							
C18:2 *t*10,*c*12	0.0	4.6	0.0	0.0	0.0	0.0	0.0
C18:2 *c*9,*t*11	0.0	4.4	0.0	0.0	0.0	0.0	0.0

CON, control fat; CLA, Conjugated linoleic acid.
*_Antepartum_ PMR containing 20% concentrate on DM basis.
†_Antepartum_ PMR containing 60% concentrate on DM-basis.
‡_Postpartum_ PMR containing 50% concentrate on DM basis.
§For dry cows. Ingredients per kg mineral feed: 60 g Ca, 105 g Na, 80 g P, 50 g Mg, 7000 mg Zn, 4800 mg Mn, 1250 mg Cu, 100 mg I, 40 mg Se, 30 mg Co, 800,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
¶For lactating dairy cows. Ingredients per kg mineral feed: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6000 mg Zn, 5400 mg Mn, 1000 mg Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
**Calculation based on nutrient digestibilities measured with wethers (GfE 1991).
††Calculation based on analysed concentrates and silage.
‡‡ Non-fibre carbohydrates = 1000 – (crude ash + crude protein + ether extract + neutral detergent fibre)

protocols of the Verband Deutsche landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA 2007), whereas acid and NDF were expressed without residual ash. Non-fibre carbohydrates (NFC) were calculated as follows:

NFC= 1000 – (CA + CP + EE + NDF).

According to Folch et al. (1957), lipids were extracted from feed samples for analysis of the CLA content in feed stuff. Afterwards, boron trifluoride (BF_3) was added to produce fatty acid methyl esters (FAME) by a *trans*-esterification, which was purified by thin-layer

chromatography (SIL G-25 UV_{254}, Machery-Nagel, Germany). The FAME extracts of all samples were analysed by gas chromatography (GC; GC-17A Version 3, Schimadzu, Japan) equipped with an auto sampler and flame ionisation detector. GC procedures are described in detail by Degen et al. (2011). *Postpartum*, cows were milked twice daily at 05.30 and 15.30. LW was recorded once ap and automatically daily pp.

Rumen probes

Before the beginning of the trial, a part of the animals of the 4 experimental groups were equipped with a probe (KB 3/04 bolus, Kahne Limited, New Zealand) developed for adjustable continuous intrarumen measurements of RpH and RT. As described in detail by Lohölter et al. (2013b), probes were built as a copolymer barrel of 145 mm length and 27 mm diameter with wings of 185 mm attached to tapered top. A temperature sensor was incorporated in probe enclosure, while in its bottom a glass membrane pH sensor was integrated. The pH sensors pre-use storage was implemented as recommended in a three molar potassium chloride solution and was calibrated, as described elsewhere (Kaur et al. 2010). For bolus calibration, download and export of measured data a transceiver (Kahne KR 2001, Kahne Limited, New Zealand) was connected to a computer and associated software (Kahne Data Processing System V 5.1) was used. The boluses were set to measure every 15 min, before inserted via balling gun through oesophagus and persisted in the reticulorumen of all examined cows throughout the experiment. Generated data were stored in a probe integrated memory card. Bolus data transmission was initiated via handheld trigger device with a frequency of 134.2 kHz (Kahne Wand KW1, Kahne Limited, New Zealand) once in a week and captured by a receiver with antenna and frequency of 433.9 MHz (KR2002, Kahne Limited, New Zealand). Transmission took only a few minutes.

Calculations

Due to a previously observed pH sensor drift, RpH data were corrected according to Lohölter et al. (2013a):

RpH = Measured RpH – (0.0042 x Respective number of days after bolus insertion).

As described by Lohölter et al. (2013a), minutes per day of RpH below 5.6, 5.8 and 6.0, respectively, were calculated by multiplying individual daily percentage of values below the respective threshold with the total number of minutes per day to exclude data falsification by potentially missing values. Thresholds (RpH 5.6, 5.8 and 6.0) were selected according previous studies to draw direct comparisons (Gozho et al. 2005; AlZahal et al. 2008; Lohölter

et al. 2013a). Values of RpH and RT around calving (days 7 ap to 7 pp) were condensed to daily means before statistical analysis.

Statistical analyses

The software package SAS (Version 9.2, SAS Institute, Cary, NC, USA) was used for all statistical evaluation. Two different models were applied for statistical analyses of dry matter intake (DMI), NDF, NFC, RpH and RT data. All results of both models are shown as least square means (LSMeans) and standard errors (SE). Using Tukey's multiple range test, differences with p <0.05 were considered to be significant and a tendency was declared when p <0.1. Bolus data were only used when available for complete days to consider potential diurnal variation.

Model 1 – Period: During late pregnancy (days 21 to 8 ap)

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \beta_1 \cdot DMI_{ijk} + \beta_2 \cdot DT_{ijk} + c_{ijk} + e_{ijk}$$

with

y_{ijk} = observation of trait y on animal k, treated with CLA and concentrate

α_i = fixed effect of CLA i (i = 0,1)

β_j = fixed effect of concentrate j (j = 20,60)

$(\alpha\beta)_{ij}$ = interaction effects of CLA and concentrate

c_{ijk} = random effect of animal k within i and j ($\sum_{i,j} k_{ij} = 20$)

β_1, β_2 = regression coefficient of dry matter intake (DMI), daytime (DT) on y

e_{ijk} = residual effect

Changes of DMI, NDF, NFC, RpH and RT were analysed by using the PROC MIXED procedure according to a two factorial design, containing the dietary concentrate proportion, CLA supplementation and the interaction between both factors as fixed factors. The DMI was considered as covariate for analyses of the time per day below RpH 5.6, 5.8 and 6.0. For evaluation of mean RpH and RT, time of day was included as an additional covariate. To account frequent measurements of each cow within treatments, the repeated statement was used. Among covariance structures tested for the model compound symmetry (CS) was found to be the most appropriate according to Akaike information criterion.

Model 2 – Period: Around calving (days 7 ap to 7 pp)

$$y_{ijk}(t) = \mu + \sum_{r=0}^{2}(\beta_{ir} + a_{kr}) \cdot x_r(t) + \sum_{s=1}^{2}(\beta^*_{i,2+s} + a^*_{k,2+s}) \cdot x_{2+s}(t) + TT_j + e_{ijk}(t)$$

with

$y_{ijk}(t)$ = observation of trait y on animal k, group i, testday j and experimental day t

β_{ir}, a_{ijkr} = fixed/random regressions coefficients of group i and animal k, respectively

$\beta^*_{i,2+s}, a^*_{k,2+s}$ = fixed/random regression coefficent of the spline function with degree 2 of group i and animal k, respectively

TT_j = fixed effect of testday j residual effect

$e_{ijk}(t)$ = residual effect

Covariables are defined as follows:

$x_0(t) = 1; x_1(t) = t; x_2(t) = t^2; x_3(t) = ((t > p_1) \cdot (t - p_1))^2; x_4(t) = ((t > p_2) \cdot (t - p_2))^2,$

where

p_1, p_2 are the spline knots.

Data were processed using a random regressions model with fixed regression coefficients for the groups and random regression coefficients to take into consideration repeated measures per animal. The nonlinear relationships between the traits under investigation and the experimental day relative to calving were modelled by splines with two nodal points. This model enabled a better description of the kinetics of the investigated parameters before and after calving. The model contained the date of measuring day and group-specific regression coefficients, respectively, as fixed factors. The regression coefficient of each animal within the respective group and the rest effects were considered in the model as random effect. An average estimated value of the time before (days 7 to 1 ap) and after (days 1 to 7 pp) calving was calculated for each group. In order to generate LSMeans for each period for the respective groups, the data were processed using the same model approach. An average estimated value of the respective period for each group was calculated and the statistical analysis was performed using the F-test and Tukey's test. The F-test between the LSMeans of each group for the respective periods was calculated by the CONTRAST option using the PROC MIXED procedure. In the case of significances between the LSMeans of the F-test, the Tukey's multiple range test was carried out. The daily dissolved values for each group for DMI, NDF, NFC, RpH and RT are presented as progressive graphs in Figures (Figs 2-6). The graphs are used to get an impression of the time-dependent course and additionally for evaluation of the development of differences between treatment groups.

Results

Based on the analyses of concentrates and silages (Table 1), groups CLA-20 and CLA-60 received in total approximately 8 g/day *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA respectively. Technical disturbances caused losses of bolus data, resulted in varying numbers of animals in the groups between the respective experimental periods.

Period: During late pregnancy (days 21 to 8 ap)

The DMI tended to be higher in groups fed on high concentrate ($p=0.052$). The intake of NDF tended to be greater ($p=0.091$) in groups fed on low concentrate, whereby their intakes of NFC were reduced ($p=0.001$) compared with high-concentrate groups. The RpH was influenced ($p<0.001$) by dietary concentrate proportion, whereas RT remained unaffected by the treatments (Table 2). Time below rumen pH 5.6 (time-RpH<5.6), 5.8 (time-RpH<5.8) and 6.0 (time-RpH<6.0), expressed in minutes per day, were affected ($p<0.001$) by dietary concentrate proportion (Table 2). However, CLA supplementation and interaction between CLA and concentrate proportion tended to influence time-RpH<5.6 and affected time-RpH<5.8 ($p=0.018$, $p=0.021$), respectively. The time-RpH<5.8 was increased ($p<0.001$) in groups CON-60 (428 min/day) and CLA-60 (427 min/day) compared with groups CON-20 (218 min/day) and CLA-20 (107 min/day). Within groups fed a low concentrate diet ap, time-RpH<5.8 was reduced ($p=0.027$) in group CLA-20 compared with group CON-20. The patterns of diurnal variation in RpH during late pregnancy are presented in Fig. 1 and showed differences among the feeding groups. The RpH of groups CON-20 and CLA-20 remained largely constant throughout the day and fluctuated mainly from pH 6.0 to 6.6. The RpH of cows fed on high concentrate increased consistently in the early morning until a peak at approximately 10.00 h, the time of PMR refilling, followed by a gradual decrease till the evening hours. Compared with groups CON-20 and CLA-20, a lower RpH from approximately 5.4 to 6.0 from 0.00 to 6.00 h and 15.00 to 24.00 h were observed in these groups.

Table 2 Rumen pH, rumen temperature and dry matter intake (DMI) of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) during late pregnancy (days 21 to 8 ap); LSMeans±SE.

	Control fat (100 g/day)		CLA‡ (100 g/day)		p-value		
	CON-20*	**CON-60†**	**CLA-20***	**CLA-60†**	CLA	Conc	CLA x Conc
	(n=4)	*(n=4)*	*(n=4)*	*(n=5)*			
Rumen pH	6.23 ±0.04	6.06 ±0.04	6.32 ±0.04	6.08 ±0.03	0.142	<0.001	0.320
pH<5.6 [min/day]	96 ±16	274 ±13	34 ±18	273 ±14	0.061	<0.001	0.075
pH<5.8 [min/day]	218[b] ±20	428[c] ±16	107[a] ±23	427[c] ±17	0.018	<0.001	0.021
pH<6.0 [min/day]	381 ±43	618 ±40	257 ±46	607 ±38	0.134	<0.001	0.204
Rumen temperature [°C]	39.7 ±0.2	40.0 ±0.2	39.8 ±0.2	39.8 ±0.1	0.582	0.274	0.434
DMI [kg/day]	12.1 ±0.7	13.2 ±0.7	11.8 ±0.7	13.6 ±0.6	0.961	0.052	0.615
NDF intake [kg/day]	4.9 ±0.2	4.4 ±0.2	4.8 ±0.3	4.5 ±0.2	0.998	0.091	0.662
NFC intake [kg/day]	4.5 ±0.3	5.5 ±0.3	4.5 ±0.3	5.7 ±0.3	0.795	0.001	0.683

NDF = Neutral detergent fibre; NFC = Non-fibre carbohydrates; Conc = Concentrate.
*Group CON-20 and CLA-20 received a low concentrate diet *antepartum*.
†Group CON-60 and CLA-60 were fed a high concentrate diet *antepartum*.
‡CLA supplement contained approximately 8 g *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA, respectively.
[abc] Different superscripts within a period indicate significantly differences between the groups (p<0.05).

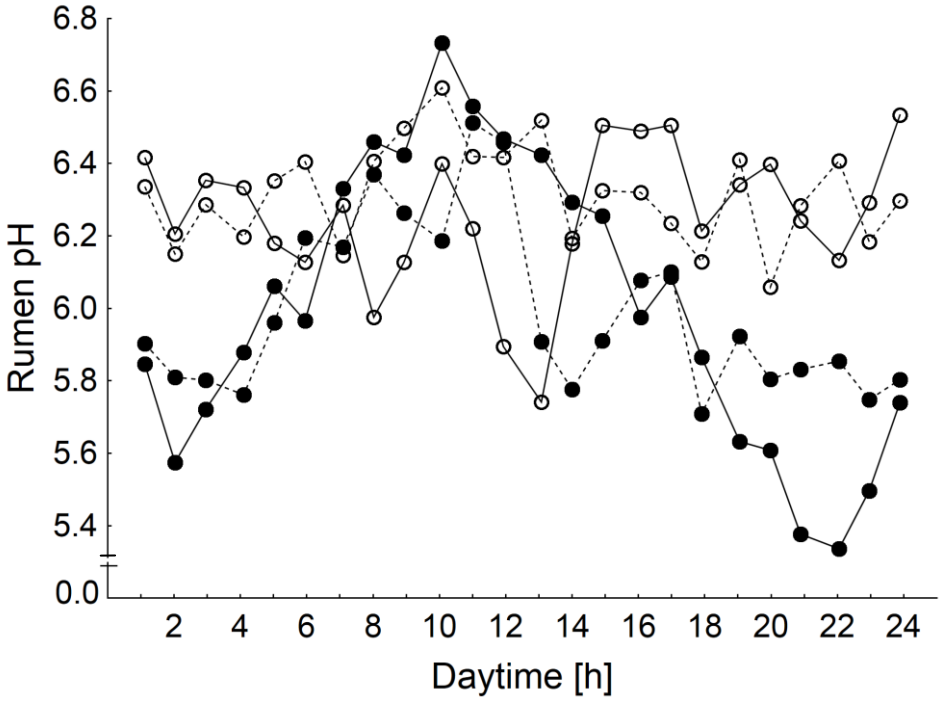

Fig. 1 Diet effects on diurnal variation in rumen pH of cows during late pregnancy.
Group CON-20 and CLA-20 received control fat (—) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. Values presented as means per hour.

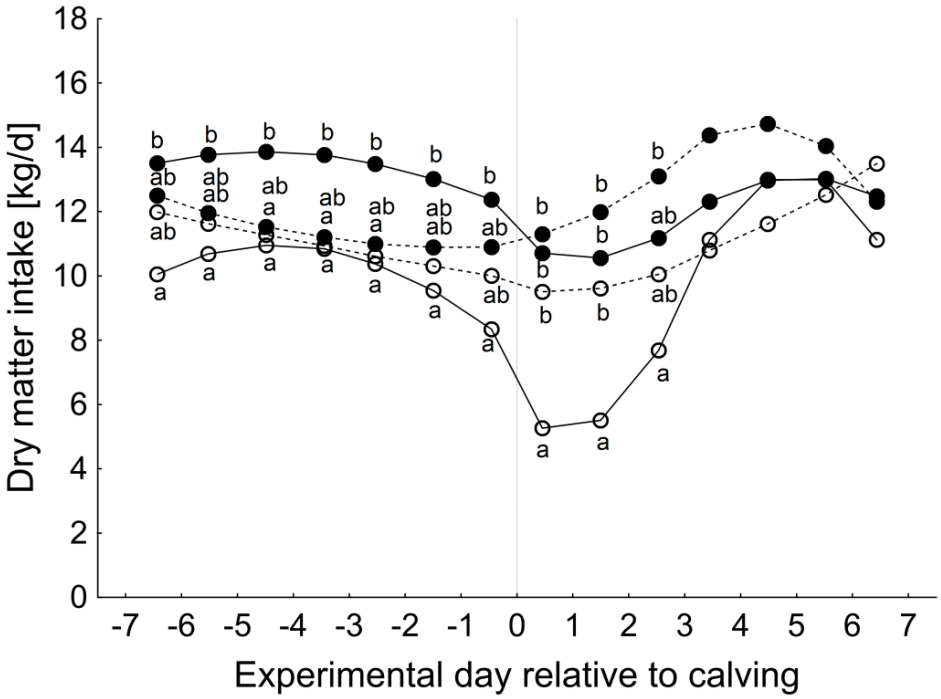

Fig. 2 Development of dry matter intake around calving.
Group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [ab] Only significantly different LSMeans between the groups are marked with superscripts (p<0.05).

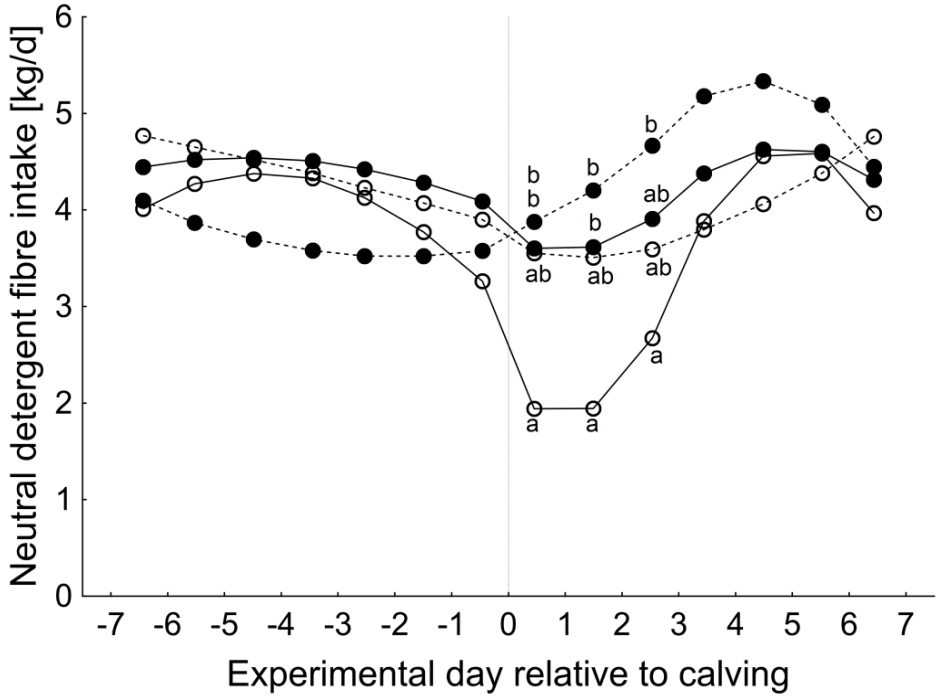

Fig. 3 Development of neutral detergent fibre intake around calving.
Group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [ab] Only significantly different LSMeans between the groups are marked with superscripts (p<0.05).

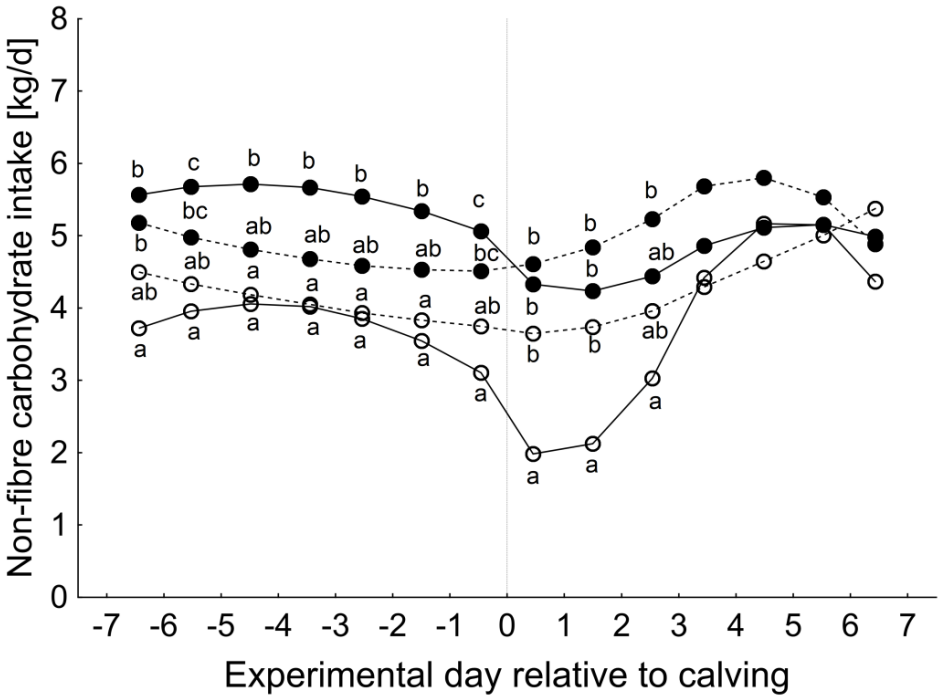

Fig. 4 Development non-fibre carbohydrate intake of around calving.
Group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [abc] Only significantly different LSMeans between the groups are marked with superscripts (p<0.05).

Period: Around calving (days 7 ap to 7 pp)

As shown in Figs 2-4, DMI, NDF and NFC intake decreased before calving (days 7 to 1 ap) and increases slightly pp. Before calving, group CON-60 consumed more (p=0.032) total DM than its counterpart group CON-20, whereas the intake of NDF remained unaffected. Group CON-60 consumed more (p=0.001) NFC than the groups CLA-20 and CON-20. After calving (days 1 to 7 pp), no differences between the groups in DMI, NDF and NFC intake could be observed in the averaged estimated values (Table 3). However, when comparing weekly dissolved values, Group CON-20 consumed less (p<0.02) DMI, NDF and NFC shortly pp than the remaining groups (Figs 2 to 4). The RpH and RT as well as time-RpH<5.6, 5.8 and 6.0 remained unaffected by the treatments (Table 3), whereby it seems that the high-concentrate groups spent more time and group CON-20 spent least time below the respective thresholds before calving. When comparing the weekly dissolved values for RpH and RT for each group over the experimental time, differences between the groups occurred (Figs 5 and 6). Seven days before anticipated calving, the RpH for group CON-60 was reduced (p=0.039) compared to group CON-20 (Fig. 5). As presented in Fig. 5, the RpH increased slightly in all groups ap, followed by a decrease shortly after calving. Values of RT were almost consistent in the time periods before and after calving (Table 3). An increased RT was observed for

CON-60 compared with CON-20 ($p<0.04$) from days 5 ap to 4 pp and to all remaining groups ($p<0.02$) from days 1 to 4 pp (Fig. 6). An example of variations in RpH and RT of one dairy cow at the time of calving is given in Fig. 7. In general, the RpH and RT were characterized by short-term fluctuations, whereby RT was subjected to abrupt declines several times. Especially in the last 6 h ap, the RpH decreased sharply, whereas RT showed a steep increase. Shortly after calving, both parameters returned back to their initial level and showed almost constant values.

Table 3 Rumen pH, rumen temperature and dry matter intake (DMI) of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) before and after calving (days 7 to 1 ap and days 1 to 7 pp); LSMeans±SE.

	Control fat (100 g/day)		CLA[§] (100 g/day)		Group
	CON-20[*]	**CON-60**[†]	**CLA-20**[*]	**CLA-60**[†]	*p-value*
	(n=3)	*(n=5)*	*(n=5)*	*(n=3)*	
Before calving					
Rumen pH	6.40 ±0.09	6.24 ±0.08	6.35 ±0.09	6.25 ±0.11	0.424
pH<5.6 [min/day]	13 ±72	172 ±68	64 ±79	151 ±89	0.290
pH<5.8 [min/day]	69 ±94	299 ±86	163 ±102	249 ±116	0.244
pH<6.0 [min/day]	180 ±119	459 ±103	274 ±126	400 ±147	0.282
Rumen temperature [°C]	39.7 ±0.2	40.4 ±0.2	39.9 ±0.2	40.0 ±0.3	0.113
DMI [kg/day]	10.1[a] ±0.9	13.4[b] ±0.8	11.0[ab] ±0.9	11.4[ab] ±1.1	0.032
NDF intake [kg/day]	4.0 ±0.3	4.4 ±0.2	4.4 ±0.3	3.7 ±0.4	0.297
NFC intake [kg/day]	3.8[a] ±0.3	5.5[b] ±0.3	4.1[a] ±0.4	4.8[ab] ±0.4	0.001
After calving [‡]					
Rumen pH	6.13 ±0.10	5.98 ±0.12	6.06 ±0.07	6.13 ±0.12	0.763
pH<5.6 [min/day]	259 ±110	349 ±123	318 ±76	230 ±128	0.884
pH<5.8 [min/day]	410 ±148	549 ±165	490 ±100	364 ±170	0.849
pH<6.0 [min/day]	556 ±146	770 ±163	641 ±102	555 ±164	0.752
Rumen temperature [°C]	39.9 ±0.2	40.6 ±0.2	39.9 ±0.1	39.8 ±0.2	0.140
DMI [kg/day]	9.5 ±1.2	11.9 ±1.4	11.1 ±0.9	13.1 ±1.4	0.273
NDF intake [kg/day]	3.4 ±0.4	4.1 ±0.5	4.0 ±0.3	4.7 0.5	0.288
NFC intake [kg/day]	3.7 ±0.5	4.7 ±0.5	4.4 ±0.3	5.2 ±0.6	0.244

NDF = Neutral detergent fibre; NFC = Non-fibre carbohydrates.
[*]Group CON-20 and CLA-20 received a low concentrate diet *antepartum*.
[†]Group CON-60 and CLA-60 were fed a high concentrate diet *antepartum*.
[‡]*Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued.
[§]CLA supplement contained approximately 8 g *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA, respectively.
[ab] Different superscripts within a period indicate significantly differences between the groups ($p<0.05$).

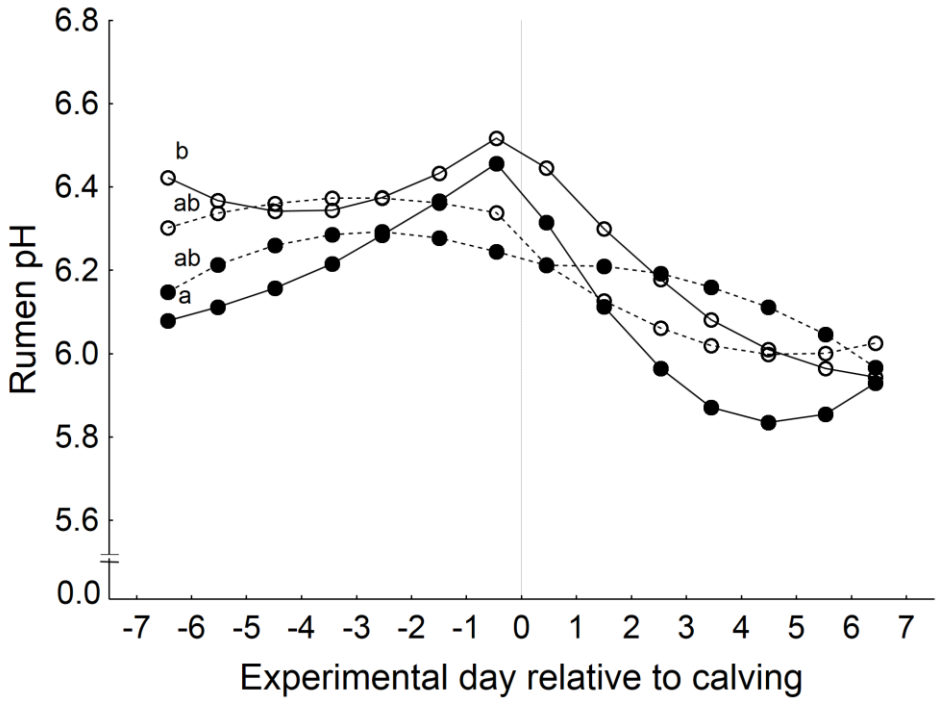

Fig. 5 Development of rumen pH around calving.
Group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [ab] Only significantly different LSMeans between the groups are marked with superscripts (p<0.05).

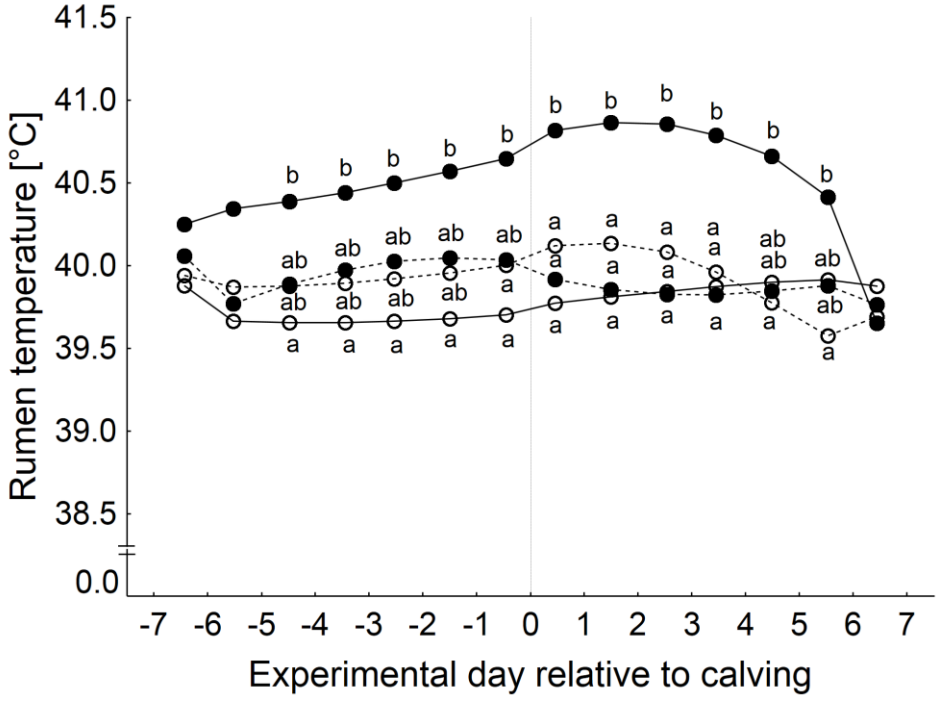

Fig. 6 Development of rumen temperature around calving.
Group CON-20 and CLA-20 received control fat (——) or CLA (- - -) supplement in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet *antepartum*. *Postpartum*, concentrate proportion was adjusted to 50% while fat supplementation continued. [ab] Only significantly different LSMeans between the groups are marked with superscripts (p<0.05).

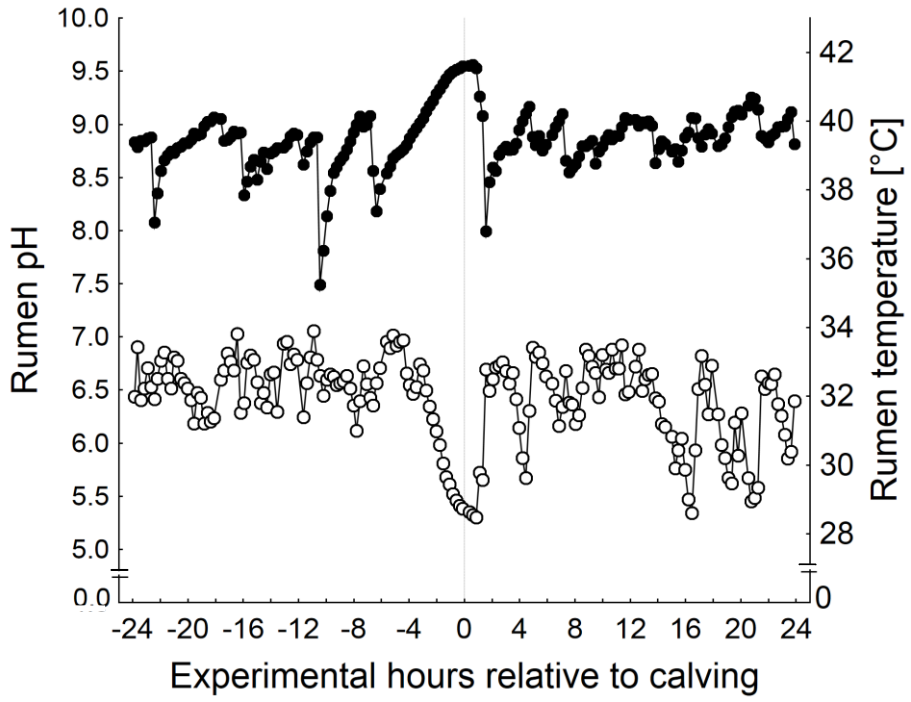

Fig. 7 Example of variations in rumen pH (○) and temperature (●) of one dairy cow at the time of calving.

Discussion

The study aimed to examine the influence of supplemented CLA on rumen parameters of cows fed low or high concentrate proportions in the diet ap. Supplementation of CLA was initiated 21 days before anticipated calving and continued until days 7 pp. The high concentrate level was fed to induce a ketogenic metabolic situation of cows pp for a better examination of the supposed lipid metabolism modifying properties of added CLA. At the same time, the rumen fermentation profile was supposed to be shifted towards a lowered RpH and eventually to a SARA. A SARA is a rumen disorder, which caused by feeding diets low in fibre and rich in highly fermentable carbohydrates. An accumulation of short chain fatty acids within the rumen will occur, resulting in a reduced RpH (Kleen et al. 2003). The onset of SARA is characterized by a depressed RpH below 5.6 for at least 180 min per day (Gozho et al. 2005) and influences health and productivity of dairy cows negatively. Furthermore, the impact of parturition itself on RpH and RT should be investigated. Thus, rumen probes were used in the present investigations to monitor these rumen adaptations continuously.

Effects of supplemented CLA and dietary concentrate feed proportion on RpH and RT

During late pregnancy (days 21 to 8 ap), cows of groups CON-60 and CLA-60 tended to consume more DM compared with groups CON-20 and CLA-20, which may be a result of a pronounced concentrate effect on total DM ap (Friggens et al. 1998). Differences in NDF and

NFC intake among the groups were caused by the various concentrate-roughage ratios. Unlike the present findings, feeding a high concentrate proportion was accompanied with an elevation in RT in previous studies (AlZahal et al. 2008; AlZahal et al. 2009; Lohölter et al. 2013a). The unaffected RT may be caused by a lower proportion of NFC (38%) in the high concentrate diet and thus a reduced extent of rumen fermentation compared to studies mentioned, which fed cows high-concentrate diets containing 45-50% NFC. However, cows fed on high concentrate had a reduced mean RpH compared with cows fed on low concentrate during late pregnancy, which is in agreement with results reported in the literature (Mishra et al. 1970; Kennelly et al. 1999; Lohölter et al. 2013a). An increased intake of NFC and thus easily digestible carbohydrates has been associated with a reduced RpH, as can clearly be seen in the diurnal variation in RpH of the groups (Fig. 1), because of its rapidly bacterial rumen fermentation and thus rising production of short chain fatty acids and lactate (Kafil et al. 2011). Accordingly, RpH of groups CON-60 and CLA-60 decreased immediately after feeding till the evening hours, as observed also by Gasteiner et al. (2009). In contrast, an increased intake of NDF led to increase the chewing time of cows due to the particle size of the feed must be reduced, resulting in a greater amount of salvia per unit of DM (Maekawa et al. 2002). Hence, RpH of cows fed on low concentrate remained widely unchanged throughout the day, except for group CON-20 within 2 h after PMR refilling. The reduced RpH values of groups CON-60, CLA-60 and CON-20 directly after PMR refilling may be depend on immediate intake of substantial amounts of feed and in particular on the NDF and NFC intakes. The consistent increase of RpH of groups CON-60 and CLA-60 before PMR refilling may be a result of enhanced salivation (Gasteiner et al. 2009). Moreover, prolonged periods of reduced RpH may indicate SARA in cattle, whereby different thresholds have been discussed in the literature to detect its presence (Kleen et al. 2003; Kafil et al. 2011). According to Gozho et al. (2005) a RpH below 5.6 for at least 180 min per day may identify the occurrence of SARA. In the present study, RpH were observed to be below 5.6 for more than 4 h per day in cows fed 60% concentrate and in contrast for 96 or 34 minutes per day in cows fed 20% concentrate with either a CON or CLA preparation during late pregnancy. These findings may be indicating the presence of SARA in groups CON-60 and CLA-60 due to an increased NFC and a lower NDF intake. This is in agreement with previous studies (AlZahal et al. 2008), the authors observed that cows fed on high concentrate spent more than 5 h per day below RpH 5.6 and concluded the presence of SARA compared with control groups. However, clinical signs regularly associated with SARA such as loss of condition, laminitis or diarrhea (Kleen et al. 2003; Kafil et al. 2011; Lohölter et al. 2013a) were not

recorded to occur in the present study. Moreover, CLA supplementation might have influenced RpH in cows fed low concentrate since time-RpH<5.8 was significantly reduced in group CLA-20 compared with CON-20, whereby DM, NDF and NFC intake were the same. It seems that CLA slightly increased RpH in these groups contrary to RpH in cows fed on high concentrate. Pappritz et al. (2011) investigated the duodenal availability of a lipid-encapsulated CLA preparation, which was also used in the present trial. The authors observed, based on intakes of 8 g/day *trans*-10,*cis*-12 CLA, that only 5% of CLA were available in the duodenum, indicating an unexpectedly low rumen-protection. Assuming the same conditions in the present study, only approximately 0.4 g of 8 g consumed *trans*-10,*cis*-12 CLA/day reached the duodenum. Perhaps, this insufficient CLA rumen protection may have negatively affected rumen microbes and thus may have inhibited rumen fermentation. This theory could also be explain the observed differences in diurnal variation in RpH between groups CON-20 and CLA-20 immediately after PMR refilling (Fig. 1). Pappritz et al. (2011) observed modified molar proportions of volatile fatty acids (VFA) through CLA supplementation, but supposed that CLA effects on rumen fermentation were negligible due to observing also an unaffected rumen concentration of total VFA and NH_3 and unchanged acetic acid-to-propionic acid ratio. Furthermore, no differences in DMI between groups CLA-20 and CON-20 could be observed. Hence, cows of group CLA-20 may have had an enhanced salivation via increased ruminating activity leading to a marginal rise in RpH. However, reasons for this observation cannot completely be explained under the conditions of the present study.

Furthermore, changes in RpH and RT around and at the time of calving (days 7 ap to 7 pp) were evaluated (Figs 5 and 6). Compared to DMI during late pregnancy, DMI was slightly reduced in groups CON-20, CLA-20 and CLA-60 before calving. Group CON-60 consumed significantly more total DM and NFC than group CON-20, which may depend on the pronounced concentrate effect on total DM ap (Friggens et al. 1998) and further resulting in a significantly reduced RpH on day 7 ap. In accordance with results reported by Gasteiner et al. (2012), RpH increased slightly before calving in each group, which may result from the observed and generally depressed feed intake in this time (Grummer 1995). Consequently, cows spent less time below RpH-5.6 than during late pregnancy, indicating that cows may be not subjected to SARA shortly before calving. Nevertheless, although not significantly different, it also seemed that group CON-20 spent least time below the respective thresholds, especially compared with groups fed on high concentrate may be caused by lower DM and NFC intake. The RpH decreased immediately after calving. An increased DMI and further elevated energy density in diet pp may be responsible for reduced RpH values (Gasteiner et

al. 2012). Sato et al. (2012) observed also a higher frequency of RpH below 5.5 within 3 and 4 days after calving, corresponding with increased concentrate intakes. Time-RpH<5.6, 5.8 and 6.0 was increased in the present study, and cows spent 4-6 h below RpH 5.6, which may indicate the presence of SARA shortly pp in all groups. This is in accordance with the literature (Goff and Horst 1997); fresh lactating cows are susceptible to rumen acidosis, especially if the energy density switched abruptly in the pp diet. Due to a rapidly response of the lactate producers to the higher NFC and lower NDF amounts, the lactate production is increased, resulting in an RpH depression (Goff and Horst 1997). Despite to the fact that each group received the same ration, group CON-20 consumed less DM immediately pp. Moreover, no differences in time<RpH-5.6 among the groups were observed. This may be a caused by a reduced rumen adaption on the high NFC and low NDF proportion in the diet pp, which may impaired rumen function and further induced a rumen acidosis, causing in turn a reduced DMI (Hayirli and Grummer 2004). However, group CLA-20 did not show such a depressed feed intake shortly after calving. Perhaps, the previous supposed negative influence of supplemented CLA on rumen microbes and thus rumen fermentation may had the effect that group CLA-20 could consume more DMI without impairing rumen function.

Moreover, RT was comparable in all groups around calving. AlZahal et al. (2008) recorded a significantly increased RT of lactating cows due to feeding high amounts of grain and observed also a negative relationship between RpH and RT (R^2=0.77). Therefore, the significantly increased RT of group CON-60 compared with CON-20 before calving may be caused by higher rumen fermentation due to the high NFC proportion in diet and also enhanced consumption of DM. After calving, concentrate proportion in diets were adjusted to 50 % in all groups while fat supplementation continued. Nevertheless, RT of group CON-60 was highest pp, indicating an increase in rumen fermentation compared to the remaining groups due to better adaption of group CON-60 to concentrate level pp. Grummer (1995) assumed that cows fed high amounts of concentrate a.p. may adapt rumen microbes to lactation diets, support development of ruminal papillae and thus enhance absorbency of rumen epithelium. However, despite the same DMI, NFC and NDF intake pp, cows of group CLA-60 did not show such increased RT, which may strengthen the established hypothesis that CLA supplementation may have had adverse effects on rumen microbes and thus inhibit rumen fermentation.

Effects of parturition itself on RpH and RT

At the time of calving, RpH and RT of the presented cow were characterized by short-term fluctuations (Fig. 7), whereby RT was repeatedly subjected to abrupt declines, which could be mediated by water intakes (Cooper-Prado et al. 2011). The sharp decrease in RpH and steep increase in RT in the last 6 h ap confirmed the negative relationship of both parameters (AlZahal et al. 2008; Lohölter et al. 2013a) and indicated the beginning of parturition. Comparable developments of RpH and RT could be observed in 75% of the animals at time of calving. However, to our knowledge, no previous studies examined in detail the development of RpH and RT just a few hours before and after parturition. Hence, the observed variations in both parameters may be caused by a recumbent position of the cow and a feed intake of almost zero. Due to less activity of the cow, rumen movement may be lower leading to a reduced mixing of rumen contents. Furthermore, the ruminating activity and hence salivation could be depressed, whereas heat production may be enhanced by efforts during calving process, resulting in reduced RpH and also increased RT. Nevertheless, shortly after parturition both parameters returned back to their initial level, which may have resulted from water intake.

Conclusion

Compared with low concentrate proportions in diet ap, high concentrate proportions tended to increase DMI and reduce RpH. Groups CON-60 and CLA-60 spent more than 4 h per day below RpH 5.6, indicating the presence of SARA during late pregnancy. Before calving, cows spent less than 3 h below RpH 5.6. SARA could be detected in each group pp through increasing DM and thus NFC intakes. However, it seems that the lipid-encapsulated CLA preparation influences RpH and RT. Hence, further studies are necessary to examine the underlying effects of dietary supplemented CLA on rumen metabolism. Based on the present results, rumen probes could be a useful management tool for animal health surveillance, whereby it should be noted that a proper function of the used probes cannot be guaranteed. However, they are capable of monitoring effects of different feeding strategies, for example early detection of SARA due to a diet high in energy density. Furthermore, devices for continuous RpH and RT measurement may also help to predict parturition. Mean RpH increased slightly ap, whereas few hours before parturition, a sharp decrease of RpH could be observed, accompanied with increased RT. However, rumen parameters fluctuate during day depending, for example, on feeding time and diet composition and thus must be regularly monitored to know herd level and to exclude misinterpretations.

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References

AlZahal, O.; Kebreab, E.; France, J.; Froetschel, M.; McBride, B.W., 2008: Ruminal temperature may aid in the detection of subacute ruminal acidosis. *Journal of Dairy Science* **91**, 202-207.

AlZahal, O.; Steele, M.A.; Valdes, E.V.; McBride, B.W., 2009: The use of a telemetric system to continuously monitor ruminal temperature and to predict ruminal pH in cattle. *Journal of Dairy Science* **92**, 5697-5701.

Baumgard, L.H.; Corl, B.A.; Dwyer, D.A.; Saebo, A.; Bauman, D.E., 2000: Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *American Journal of Physiology, Regulatory, Integrative and comparative Physiology* **278**, R179-R184.

Cooper-Prado, M.J.; Long, N.M.; Wright, E.C.; Goad, C.L.; Wettemann, R.P., 2011: Relationship of ruminal temperature with parturition and estrus of beef cows. *Journal of Animal Science* **89**, 1020-1027.

Degen, C.; Lochner, A.; Keller, S.; Kuhnt, K.; Dänicke, S.; Jahreis, G., 2011: Influence of in vitro supplementation with lipids from conventional and Alpine milk on fatty acid distribution and cell growth of HT-29 cells. *Lipids in Health and Disease* **10**, 131.

Folch, J.; Lees, M.; Stanley, G.H.S., 1957: A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *Journal of Biological Chemistry* **226**, 497-509.

Friggens, N.C.; Emmans, G.C.; Kyriazakis, I.; Oldham, J.D.; Lewis, M., 1998: Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. *Journal of Dairy Science* **81**, 2228-2239.

Gasteiner, J.; Boswerger, B.; Guggenberger, T., 2012: Practical use of a novel ruminal sensor on dairy farms. *Praktische Tierarzt* **93**, 730-739.

Gasteiner, J.; Fallast, M.; Rosenkranz, S.; Haeusler, J.; Schneider, K.; Guggenberger, T., 2009: Measuring rumen pH and temperature by an indwelling and wireless data transmitting unit and application under different feeding conditions. *Wiener Tierärztliche Monatsschrift* **96**, 188-194.

GfE, 1991: (Society of Nutrition Physiolgy). Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern (Guidelines for determining the digestibility of crude ruminants). *Journal of Animal Physiology and Animal Nutrition* **65**, 229-234.

GfE, 2001: *(Society of Nutrition Physiolgy). 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.

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

Gozho, G.N.; Plaizier, J.C.; Krause, D.O.; Kennedy, A.D.; Wittenberg, K.M., 2005: Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *Journal of Dairy Science* **88**, 1399-1403.

Grummer, R.R., 1995: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science* **73**, 2820-2833.

Hayirli, A.; Grummer, R.R., 2004: Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. *Canadian Journal of Animal Science* **84**, 337-347.

Kafil, H.; Amjad ul, I.; Gupta, S.K., 2011: Management of sub-acute ruminal acidosis in dairy cattle for improved production: a review. *Journal of Advanced Veterinary Research* **1**, 80-93.

Kaur, R.; Garcia, S.C.; Horadagoda, A.; Fulkerson, W.J., 2010: Evaluation of rumen probe for continuous monitoring of rumen pH, temperature and pressure. *Animal Production Science* **50**, 98-104.

Kay, J.K.; Roche, J.R.; Moore, C.E.; Baumgard, L.H., 2006: Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture. *Journal of Dairy Research* **73**, 367-377.

Kennelly, J.J.; Robinson, B.; Khorasani, G.R., 1999: Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and composition in early-lactation Holstein cows. *Journal of Dairy Science* **82**, 2486-2496.

Kleen, J.L.; Hooijer, G.A.; Rehage, J.; Noordhuizen, J., 2003: Subacute ruminal acidosis (SARA): a review. *Journal of Veterinary Medicine Series a-Physiology Pathology Clinical Medicine* **50**, 406-414.

Lohölter, M.; Meyer, U.; Rauls, C.; Rehage, J.; Dänicke, S., 2013a: Effects of niacin supplementation and dietary concentrate proportion on body temperature, ruminal pH and milk performance of primiparous dairy cows. *Archives of Animal Nutrition* **67**, 202-218.

Lohölter, M.; Rehage, R.; Meyer, U.; Lebzien, P.; Rehage, J.; Dänicke, S., 2013b: Evaluation of a device for continuous measurement of rumen pH and temperature considering localization of measurement and dietary concentrate proportion. *Landbauforschung* **63**, 61-68.

Maekawa, M.; Beauchemin, K.A.; Christensen, D.A., 2002: Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *Journal of Dairy Science* **85**, 1165-1175.

Mishra, M.; Martz, F.A.; Stanley, R.W.; Johnson, H.D.; Campbell, J.R.; Hilderbrand, E., 1970: Effect of diet and ambient temperature-humudity on ruminal pH, oxidation reduction potential, ammonia and lactic acid in lactating cows. *Journal of Animal Science* **30**, 1023-1028.

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

Pappritz, J.; Lebzien, P.; Meyer, U.; Jahreis, G.; Kramer, R.; Flachowsky, G.; Dänicke, S., 2011: Duodenal availability of conjugated linoleic acids after supplementation to dairy cow diets. *European Journal of Lipid Science and Technology* **113**, 1443-1455.

Petzold, M.; Meyer, U.; Kersten, S.; Spilke, J.; Kramer, R.; Jahreis, G.; Dänicke, S., 2013: Effects of conjugated linoleic acids and dietary concentrate proportion on performance, milk composition, milk yield and metabolic parameters of periparturient dairy cows. *Archives of Animal Nutrition* **67**, 185-201.

Sato, S.; Mizuguchi, H.; Ito, K.; Ikuta, K.; Kimura, A.; Okada, K., 2012: Technical note: Development and testing of a radio transmission pH measurement system for continuous monitoring of ruminal pH in cows. *Preventive Veterinary Medicine* **103**, 274-279.

Tanaka, K., 2005: Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. *Animal Science Journal* **76**, 291-303.

VDLUFA, 2007: *Handbuch der Landwirtshcaftlichen Veruschs- und Untersuchungsmethodik. (VDLUFA-Methodenbuch). Band III. Die chemische Untersuchung von Futtermitteln.* VDLUFA-Verlag. Darmstadt. Germany.

PAPER IV

Feeding conjugated linoleic acids and various concentrate proportions to late pregnant cows and its consequence on blood metabolites of calves

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Abstract

The study aimed to investigate the influence of maternal conjugated linoleic acid (CLA) supplementation and various concentrate proportions in diets during late pregnancy on blood metabolites of unsuckled compared to suckled calves to examine possible CLA effects on calf metabolism. Pregnant German Holstein cows had *ad libitum* access to silage-based rations three weeks prior to calving. Cows received 100 g/d control fat (CON) or CLA supplement, either in a low (20 %; CON-20, CLA-20) or high (60%; CON-60, CLA-60) concentrate diet. In total 5 to 6 calves were used out of potentially available calves per group to carry out the study. Blood samples were obtained from unsuckled calves immediately after parturition and from the same calves after staying 16 to 24 h with their dam. Calves had *ad libitum* access to colostrum in this time. A*ntepartum* dry matter intake of dams of group CLA-60 was highest, whereas birth weight of calves remained unaffected. Nearly all blood metabolites were increased in suckled calves compared to unsuckled calves due to colostrum intake, whereas concentrations of non-esterified fatty acids decreased slightly and serum albumin showed similar concentrations after colostrum intake. However, no significant maternal diet effect and no effect by intake of CLA enriched colostrum could be observed. Results indicate that CLA supplementation and various concentrate levels in dairy cow diets during the final weeks of pregnancy did not affect the metabolic status of the offspring.

Keywords: conjugated linoleic acids, maternal nutrition, calves, blood, metabolism

1. Introduction

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of linoleic acid characterized by conjugated double bonds. It has been reported that CLA exerts several physiological effects, like anticarcinogenic, antiatherogenic and immunomodulatory effects or growth and lean body mass promotion (reviewed by Tanaka (2005)). Especially, *trans*-10,*cis*-12 CLA isomer is frequently added to dairy cow diets because of its milk fat reducing properties (Baumgard et al., 2000). However, the mode of action of maternal CLA supplementation on their offspring has been scarcely investigated. The bovine fetus grows about 75 % during the last two month of pregnancy, whereby fetal development and growth rate depend on maternal nutrition (Funston et al., 2010). Previous studies demonstrated that CLA fed to pregnant humans or rats were transferred from maternal to fetal blood and hence may affect metabolic functions of fetuses (Ringseis et al., 2004; Müller et al., 2007). Additionally, Dänicke et al. (2012) observed an altered fatty acid profile of erythrocyte lipids

in calves after feeding CLA to early pregnant cows and suggested long-term effects of CLA on cows and their offspring. Moreover, preceding studies showed that *trans*-10,*cis*-12 CLA was consistently transferred into milk fat and that its proportion in milk fat increased dose-dependently during treatment period (Moore et al., 2004; Pappritz et al., 2011). Hence, colostrum from cows fed CLA could also have important implications on calves.

An experiment with dairy cows during transition period was used to study possible CLA impacts on metabolism of newborn calves. The experiment was previously described by Petzold et al. (2013) and for present investigation a part of the calves of the four experimental groups was used. The objective of this trial was to examine the effects of maternal CLA supplementation in a low or high concentrate diet during late pregnancy on blood parameters of unsuckled compared to suckled calves to elucidate whether an intrauterine exposure to CLA or the intake of CLA enriched colostrum affects calf metabolism. Compared to an adapted feeding, the impact of a high concentrate diet during late pregnancy on calf metabolism should be investigated due to the fact that maternal nutrition plays an important role in pre- and postnatal calf development, nutrition and metabolism (Funston et al., 2010). It was supposed that the level of energy intake of late pregnant cows could affect calf metabolism.

2. Material and Methods

2.1. Experimental design, animals, feeding

The study was performed at the Experimental Station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI) in Braunschweig, Germany, according to European Community regulations concerning the protection of experimental animals. The present trial was part of a more comprehensive feeding study (Petzold et al., 2013). 64 pregnant German Holstein cows were assigned to one of four dietary treatments. 21 days prior to calving, group CON-20 (n=16) and CLA-20 (n=16) received 100 g/d control fat (CON) or CLA supplement in a low (20%) concentrate diet, whereas group CON-60 (n=16) and CLA-60 (n=16) were fed 100 g/d CON or CLA supplement in a high (60%) concentrate diet.

Cows had *ad libitum* access to partial mixed rations (PMR) consisting of 20% or 60% concentrate and 80% or 40% roughage (60% corn silage and 40% grass silage on dry matter [DM] -basis), respectively. PMR were offered in self-feeding stations (type RIC, manufacturer Insentec, B.V., Marknesse, The Netherlands). The fat supplements were included into 2 kg concentrate supplied via computerized concentrate feeding station (manufacturer Insentec, B.V., Marknesse, The Netherlands). The composition of concentrates

and PMR are presented in Table 1. A commercial rumen-protected CLA preparation (Lutrell® Pure, BASF SE, Ludwigshafen, Germany), containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA, and a rumen-protected control fat preparation (Silafat®, BASF SE, Ludwigshafen, Germany), containing stearic acid instead of conjugated linoleic acids, were used as CLA and CON fat supplements, respectively. *Postpartum* (p.p.), cows were fed a PMR for *ad libitum* consumption based on 50% concentrate and 50% roughage (60% corn silage and 40% grass silage on DM-basis) while fat supplementation continued. All diets were formulated to meet the nutritional requirements of cows stated by the Society of Nutrition Physiology (GfE, 2001). Cows had *ad libitum* access to water.

In total only 5 to 6 calves were used out of 16 potentially available calves per group due to sampling only those calves where parturition was under personal control of staff and further through equal distribution of gender and pluri- and primiparous dams. Calves spent approximately 16 to 24 h p.p. with their mothers. In this time, calves had *ad libitum* access to colostrum of their corresponding dams. Calves were observed to consume colostrum within the first hour of life.

2.2. Sample collection and analyses

Each cow was equipped with an ear transponder to record daily individual feed and water intake. Representative PMR samples were taken daily, samples of concentrates were collected once, samples of corn and grass silage were taken twice a week and pooled monthly. The chemical composition of the feed was analyzed according to methods of VDLUFA (Naumann and Bassler, 1997). *Postpartum*, cows were milked twice daily. Live weight was recorded once a.p. and automatically daily p.p..

Blood samples were obtained from each of the confined unsuckled calves from *Vena jugularis externa* immediately after parturition. The second blood samples were drawn from calves after staying approximately 16 to 24 h with their mother. In this time, calves had ad libitum access to colostrum. Hence, amounts of colostrum intake between these blood samples could not be recorded. Blood was centrifuged at 2000 x g and 15 °C for 15 minutes after incubating 30 minutes by 30 °C. Concentrations of albumin, aspartate amino-tranferase (ASAT), β-hydroxybutyrate (BHB), γ-glutamyl-transferase (GGT), glutamate dehydrogenase (GLDH), glucose, non-esterified fatty acids (NEFA), total bilirubin, total cholesterol and total protein in blood serum of calves were determined photometrically by an automatic clinical chemistry analyser (Eurolyser, Qinlab Diagnostic GbR, Martinsried, Germany). Birth weight (BW) of calves was determined after separating from their dams.

Table 1
Components and chemical composition of concentrates and partial mixed rations (PMR); Means.

Variable	Concentrate				Partial mixed ration		
	CON[a]	CLA[b]	PMR-20/60	PMR-50	PMR-20[c]	PMR-60[d]	PMR-50[e]
Components [%]							
Wheat	41.0	41.0	41.0	41.0			
Dried sugar beet pulp	25.5	25.5	30.5	30.3			
Rapeseed meal	20.0	20.0	20.0	20.0			
Soybean meal	6.5	6.5	6.5	6.5			
Vitamin/mineral premix[f]	2.0	2.0	2.0	-			
Vitamin/mineral premix[g]	-	-	-	2.0			
Calcium carbonate	-	-	-	0.2			
CLA supplement	-	5.0	-	-			
Control fat supplement	5.0	-	-	-			
Analysed chemical profile							
Dry matter [g/kg]	886	886	876	879	335	450	447
Nutrient [g/kg DM]							
Crude ash	58	65	64	67	67	65	66
Crude protein	188	187	195	192	109	136	138
Ether extract	80	64	25	29	35	32	35
Crude fibre	94	94	97	103	207	174	172
Acid detergent fibre	134	126	130	136	231	202	200
Neutral detergent fibre	274	259	265	275	436	392	399
Energy[h] [MJ /kg DM]							
ME	13.9	13.8	13.5	13.6	10.5	12.1	11.7
NE_L	8.8	8.7	8.6	8.7	6.3	7.5	7.3
CLA[i] [g/kg DM]							
C18:2 *t*10,*c*12	0.0	4.6	0.0	0.0	0.0	0.0	0.0
C18:2 *c*9,*t*11	0.0	4.4	0.0	0.0	0.0	0.0	0.0

[a] CON, control fat.
[b] CLA, Conjugated linoleic acid.
[c] *Antepartum* PMR containing 20% concentrate on DM basis.
[d] *Antepartum* PMR containing 60% concentrate on DM-basis.
[e] *Postpartum* PMR containing 50% concentrate on DM basis.
[f] For dry cows. Ingredients per kg mineral feed: 60 g Ca, 105 g Na, 80 g P, 50 g Mg, 7000 mg Zn, 4800 mg Mn, 1250 mg Cu, 100 mg I, 40 mg Se, 30 mg Co, 800,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
[g] For lactating dairy cows. Ingredients per kg mineral feed: 140 g Ca, 120 g Na, 70 g P, 40 g Mg, 6000 mg Zn, 5400 mg Mn, 1000 mg Cu, 100 mg I, 40 mg Se, 25 mg Co, 1,000,000 IU vitamin A, 100,000 IU vitamin D_3, 1500 mg vitamin E.
[h] Calculation based on nutrient digestibilities measured with wethers (GfE 1991).
[i] Calculation based on analysed concentrates and silage.

2.3. Statistical analyses

The software package SAS (Version 9.2, SAS Institute, Cary, NC, USA) was used for all statistical evaluation. Blood serum data were processed using the PROC MIXED procedure containing dietary treatment of dams during late pregnancy (split up into concentrate feeding, CLA supplementation and the concentrate x CLA interaction), time of sampling (before and after colostrum intake) and their interactions as fixed factors. Because of repeated measurements during experiment, individual animal effects were considered by using

REPEATED procedure. BW were analysed by using analysis of variance (ANOVA) according to a two factorial design, considering sex of calves, respective diet of their dam (CON-20, CON-60, CLA-20, CLA-60) and their interactions as fixed factors. DMI, PMR and concentrate intake of cows was processed using a random-regressions model with fixed regression coefficients for the groups and random regression coefficients to take into consideration repeated measures per animal, as described in detail by Petzold et al. (2013). All results are shown as least square means (LSMeans) and standard errors (SE). Using Tukey's multiple range test differences with $p < 0.05$ were considered to be significant and a tendency was declared when $p < 0.1$.

3. Results

Based on analyses and intakes of concentrates and silages (Table 1 and 2), dams of group CLA-20 consumed 8.3 g/d *trans*-10,*cis*-12 and 7.9 g/d *cis*-9,*trans*-11 CLA and group CLA-60 6.9 g/d *trans*-10,*cis*-12 and 6.6 g/d *cis*-9,*trans*-11 CLA, respectively. DMI and PMR intake of cows were significantly ($p<0.001$) influenced by dietary treatments during late pregnancy (Day 21 to 1*antepartum*), whereby the intake of the CON- and CLA-concentrate remained unaffected (Table 2). Cows of group CLA-60 consumed significantly more total DM and PMR compared to cows of group CON-20 and CLA-20 in this time. BW of sampled calves remained unaffected by maternal nutrition and amounted to 45.0 ±1.9 kg, 45.0 ±1.8 kg, 43.4 ±2.3 kg and 44.6 ± 2.3 kg for group CON-20, CON-60, CLA-20 and CLA-60 respectively. BW of male calves (46.9 ±1.1 kg) were higher (p=0.039) compared to female calves (42.1 ±1.7 kg). With the exception of serum albumin and serum NEFA, measured blood metabolites were significantly influenced by colostrum intake, whereas the other fixed factors remained without influence (Table 3). All recorded blood metabolites increased due to colostrum intake (ASAT from 24 to 69 IU/l, BHB from 0.13 to 0.20 mmol/l, GGT from 11 to 2167 IU/l, GLDH from 5.5 to 8.5 IU/l, glucose from 62 to 97 mg/dl, total bilirubin from 0.49 to 0.98 mg/dl, total cholesterol from 19 to 28 mg/dl and total protein from 45 to 54 g/l), whereby NEFA decreased slightly from 0.59 to 0.46 mmol/l and serum albumin showed similar concentrations. Besides serum albumin, NEFA and total cholesterol before and after colostrum intake as well as glucose and total protein before and GGT after colostrum intake, all blood metabolites were within the normal reference range according to Kraft and Dürr (2005) and Dirksen et al. (2006).

Table 2
Dry matter (DM), partial mixed ration (PMR) and concentrate intake of cows fed different amounts of concentrate supplemented with conjugated linoleic acid (CLA) or control fat (CON) during late pregnancy; LSMeans±SE.

	Control fat (100 g/d)				CLA (100 g/d)				
Parameter	**CON-20**[1]		**CON-60**[2]		**CLA-20**[1]		**CLA-60**[2]		*p-value*
	(n=5)		*(n=6)*		*(n=5)*		*(n=5)*		
DMI [kg/d]	13.2[a]	±0.8	15.8[ab]	±0.9	12.3[a]	±0.9	18.3[b]	±0.8	<0.001
PMR intake[3] [kg/d]	11.6[a]	±0.9	14.0[ab]	±1.0	10.5[a]	±1.0	16.7[b]	±0.9	<0.001
Roughage[3] [kg/d]	10.5[c]	±0.6	6.0[a]	±0.7	9.5[bc]	±0.7	7.0[ab]	±0.6	<0.001
Concentrate[3] [kg/d]	1.1[a]	±0.4	8.1[b]	±0.4	1.0[a]	±0.4	9.7[c]	±0.4	<0.001
CON/CLA-concentrate intake[3,4] [kg/d]	1.7	±0.1	1.8	±0.1	1.8	±0.1	1.5	±0.1	0.167

DMI, dry matter intake.
[1] Group CON-20 and CLA-20 received a low (20%) concentrate diet *antepartum*.
[2] Group CON-60 and CLA-60 received a high (60%) concentrate diet *antepartum*.
[3] On DM-basis.
[4] Supplied via computerized concentrate feeding station. CLA-concentrate containing 4.6 g/kg *trans*-10,*cis*-12 CLA and 4.4 g *cis*-9,*trans*-11 CLA on DM-basis. CON-concentrate containing stearic acid instead of conjugated linoleic acids.
[a,b,c] Different superscripts indicate significant differences between the groups ($p < 0.05$).

Table 3

Effects of feeding different amounts of concentrate supplemented with conjugated linoleic acids (CLA) or control fat (CON) to late pregnant cows on serum blood parameters of their calves before and after colostrum intake; LSMeans±SE.

	Before colostrum intake				**After colostrum intake**								
	Control fat (100 g/d)		CLA (100 g/d)		Control fat (100 g/d)		CLA (100 g/d)		*p-value*				
Item	**CON-20**[a]	**CON-60**[b]	**CLA-20**[a]	**CLA-60**[b]	**CON-20**[a]	**CON-60**[b]	**CLA-20**[a]	**CLA-60**[b]	Concentrate	CLA	Concentrate x CLA	Time	Diet[e] x Time
	(n=5)	*(n=6)*	*(n=5)*	*(n=5)*	*(n=5)*	*(n=6)*	*(n=5)*	*(n=5)*					
Prot[c]	45.1 ±1.6	45.8 ±1.4	43.2 ±1.6	45.9 ±1.6	53.7 ±6.4	50.5 ±5.9	54.1 ±6.4	56.4 ±6.4	0.858	0.751	0.600	0.009	0.862
Alb[c]	21.2 ±0.9	20.5 ±0.9	20.7 ±0.9	22.1 ±0.9	21.6 ±0.9	20.6 ±0.9	19.5 ±0.9	21.4 ±0.9	0.670	0.938	0.141	0.364	0.492
ASAT[c]	21.3 ±4.8	20.7 ±4.4	25.9 ±4.8	30.0 ±4.8	61.0 ±14	72.6 ±13	78.4 ±14	64.7 ±14	0.960	0.391	0.448	<0.001	0.825
GGT[c]	13.0 ±2.6	7.68 ±2.4	8.11 ±3.4	13.8 ±2.6	1084 ±924	1394 ±844	2589 ±924	3598 ±924	0.634	0.195	0.797	0.008	0.526
GLDH[c]	4.89 ±1.9	6.39 ±1.8	4.02 ±2.1	6.53 ±2.6	7.21 ±1.6	8.39 ±1.5	8.72 ±1.6	9.63 ±1.8	0.343	0.750	0.906	0.016	0.784
Chol[c]	18.2 ±2.0	20.3 ±1.8	19.1 ±2.0	19.3 ±2.0	27.9 ±5.1	26.8 ±4.6	30.0 ±5.1	29.0 ±5.1	0.994	0.727	0.882	0.001	0.912
Gluc[c]	67.3 ±11	51.8 ±10	64.6 ±11	65.3 ±11	94.4 ±21	96.5 ±19	101.2 ±21	96.3 ±21	0.744	0.750	0.866	0.002	0.912
NEFA[d]	0.75 ±0.14	0.66 ±0.13	0.58 ±0.14	0.37 ±0.14	0.51 ±0.11	0.48 ±0.10	0.50 ±0.11	0.36 ±0.11	0.213	0.119	0.546	0.154	0.809
BHB[d]	0.12 ±0.02	0.14 ±0.02	0.11 ±0.03	0.13 ±0.03	0.20 ±0.04	0.25 ±0.08	0.20 ±0.04	0.14 ±0.04	0.886	0.288	0.357	0.031	0.643
Bili[c]	0.54 ±0.13	0.51 ±0.13	0.49 ±0.13	0.40 ±0.13	1.08 ±0.17	0.97 ±0.16	1.11 ±0.17	0.74 ±0.17	0.204	0.445	0.489	<0.001	0.780

[a] Dams of calves in group CON-20 and CLA-20 received a low (20%) concentrate diet *antepartum*.

[b] Dams of calves in group CON-60 and CLA-60 received a high (60%) concentrate diet *antepartum*.

[c] Reference values according to Kraft & Dürr (2005):

Prot= total protein [g/l]: 50-70 g/l; Alb= albumin [g/l]: 30-40 g/l; ASAT= aspartate amino-transferase [IU/l]: < 80 IU/l;
GGT= γ-glutamyl-transferase [IU/l]: < 50 IU/l; GLDH= glutamate dehydrogenase [IU/l]: < 30 IU/l; Chol= total cholesterol [mg/dl]: >75 mg/dl;
Gluc= glucose [mg/dl]: 80-125 mg/dl; Bili= total bilirubin [mg/l]: < 3 mg/l.

[d] Reference value according to Dirksen et al. (2006):

BHB= β-hydroxybutyrate [mmol/l]: <1.20 mmol/l; NEFA= non-esterified fatty acids [mmol/l]: < 0.35 mmol/l.

[e] Diet = Concentrate feeding x CLA supplementation.

4. Discussion

The present study aimed to examine the effects of maternal CLA supplementation and various concentrate proportions in diets during late pregnancy on blood metabolites of unsuckled compared to suckled calves to investigate possible impacts of dietary interventions of dams on calf metabolism. Therefore, CLA supplementation was initiated 21 days before anticipated calving in either a low or high concentrate diet. DM and PMR intake of cows of group CLA-60 was highest during late pregnancy, which may be a result of a pronounced concentrate effect on total DMI *antepartum* (Friggens et al., 1998). Nevertheless, the level of energy intake of dams during late pregnancy did not influenced blood metabolites of newborn calves and no differences in BW of calves could be observed. Unlike these findings, Funston et al. (2010) supposed that diets high in energy fed to pregnant cows have beneficial effects on calf metabolism and development due to glucose as an essential substrate for fetal development and growth are available in sufficient quantities. Furthermore, it was of interest whether an intrauterine exposure to CLA may exert effects on pre- and postnatal calf metabolism. However, no maternal diet effect between the groups could be found, even if Ringseis et al. (2004) and Müller et al. (2007) demonstrated that CLA fed to pregnant humans or rats were transferred from maternal to fetal blood. However, no comparable study could be found, investigating a CLA transfer through the placenta in cattle. Moreover, colostrum intake could also have important effects on newborn calves due to a consistent and dose dependent transfer of *trans*-10,*cis*-12 CLA into milk fat during CLA supplementation period (Moore et al., 2004; Pappritz et al., 2011). Based on the literature, it can be assumed that calves of CLA-groups received colostrum enriched with *trans*-10,*cis*-12 CLA. However, no differences in blood parameters within the groups of suckled calves could be observed, indicating that the intake of CLA enriched colostrum did not have effects on calves. Maybe the time of colostrum intake was too short and thus the ingested amount of CLA enriched colostrum was too low causing an effect. However, it should be noted that the amount of colostrum intake of calves could not be recorded. Consequently, there may be a huge variation in ingested colostrum among the calves, which in turn could have an influence on their metabolic state. Colostrum intake is necessary for passive immunity and provides calf with important nutrients (Blum and Hammon, 2000; Blum, 2006). Nearly all blood metabolites of calves changed markedly due to colostrum intake. Total protein serum concentrations increased due to colostrum intake and may be resulted from immunoglobulin absorption; especially of immunoglobulin G (Blum and Hammon, 2000; Kühne et al., 2000). In contrast, serum albumin concentrations were below the reference range (Kraft and Dürr, 2005) in unsuckled and suckled calves. However,

albumin blood level should rise in the first week of life depending on colostrum intake, as a consequence of enhanced hepatic synthesis (Blum and Hammon, 2000; Kühne et al., 2000). Moreover, serum levels of liver enzymes GGT and GLDH and enzyme ASAT were increased in suckled compared to unsuckled calves. Colostrum milked first was observed to have elevated levels of these enzymes compared to milk of a later milking time point. Additionally, milk replacer did not induce increased enzyme blood levels and let suggest that these enzymes were absorbed from colostrum (Blum and Hammon, 2000). Total cholesterol concentration in serum increased due to colostrum intake and might partly be dependent on ingested amount of fat (Blum and Hammon, 2000), but postabsorptive factors could also have regulated blood cholesterol level (Kühne et al., 2000). Contrary to suckled, unsuckled calves were characterized by low blood glucose levels, as observed also by Kühne et al. (2000) and Hadorn et al. (1997), indicating that colostrum supply may influence blood glucose concentration of neonatal calves (Blum and Hammon, 2000). Hadorn et al. (1997) discussed, that colostrum intake could stimulate lactase activity in small intestine, supporting lactose digestion and further glucose and galactose absorption. Moreover, high quantities of substrates for gluconeogenesis may be available due to colostrum intake (Hadorn et al., 1997). For all unsuckled calves increased NEFA serum concentrations were observed, followed by a slight decrease after colostrum intake. Elevated NEFA values indicate an energy deficit and subsequent lipid mobilization (Dirksen et al., 2006). Hence, newborn calves may have had a slight energy deficit before colostrum intake, whereby reduced NEFA values of suckled calves may be a result of a less extent of body fat mobilisation due to the availability of lactate (Hadorn et al., 1997).

In conclusion, colostrum intake changed markedly blood metabolites of newborn calves. However, no maternal diet effect and no effect by intake of CLA enriched colostrum could be observed, indicating that CLA supplementation and various concentrate levels in dairy cow diets during late pregnancy did not have an impact on the metabolic status of the offspring. Marginal changes between the groups within unsuckled and suckled calves may be partly caused by differences in time of sampling and amounts of ingested colostrum. Nevertheless, a higher number of replications per group are needed and additionally the amount of ingested colostrum of newborn calves should be recorded to get a more detailed impression of the influence of maternal nutrition on calf metabolism.

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References

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

Blum, J.W., 2006. Nutritional physiology of neonatal calves. J Anim Physiol Anim Nutr 90, 1-11.

Blum, J.W., Hammon, H., 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livest Prod Sci 66, 151-159.

Dänicke, S., Kowalczyk, J., Renner, L., Pappritz, J., Meyer, U., Kramer, R., Weber, E.M., Döll, S., Rehage, J., Jahreis, G., 2012. Effects of conjugated linoleic acids fed to dairy cows during early gestation on hematological, immunological, and metabolic characteristics of cows and their calves. J Dairy Sci 95, 3938-3953.

Dirksen, G., Gründer, H.D., Stöber, M.H., 2006. Innere Medizin und Chirugie des Rindes [Internal medicine and surgery of boovine animals]. Parey in MVS Medizinverlage Stuttgart GmbH & Co. KG Stuttgart, Germany.

Friggens, N.C., Emmans, G.C., Kyriazakis, I., Oldham, J.D., Lewis, M., 1998. Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. J Dairy Sci 81, 2228-2239.

Funston, R.N., Larson, D.M., Vonnahme, K.A., 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J Anim Sci 88, E205-E215.

GfE, 2001. (Society of Nutrition Physiolgy). 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.

Hadorn, U., Hammon, H., Bruckmaier, R.M., Blum, J.W., 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J Nutr 127, 2011-2023.

Kraft, W., Dürr, U.M., 2005. Klinische Labordiagnostik in der Tiermedizin [Clinical laboratory diagnostics in veterinary medicine]. Schattauer Verlag, Stuttgart, Germany.

Kühne, S., Hammon, H.M., Bruckmaier, R.M., Morel, C., Zbinden, Y., Blum, J.W., 2000. Growth performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J Anim Sci 78, 609-620.

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

Müller, A., Keller, U., Seliger, G., Barthel, C., Steinhart, H., Eder, K., 2007. Concentrations of conjugated linoleic acids in neonatal blood in relationship to those in maternal blood. Prostaglandins Leukot Essent Fatty Acids 76, 213-219.

Naumann, C., Bassler, R., 1997. Die chemische Untersuchung von Futtermitteln [The chemical evaluation of animal feed]. VDLUFA-Verlag, Darmstadt, Germany.

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

Petzold, M., Meyer, U., Kersten, S., Spilke, J., Kramer, R., Jahreis, G., Dänicke, S., 2013. Effects of conjugated linoleic acids and dietary concentrate proportion on performance, milk composition, milk yield and metabolic parameters of periparturient dairy cows. Arch Anim Nutr 67, 185-201.

Ringseis, R., Saal, D., Müller, A., Steinhart, H., Eder, K., 2004. Dietary conjugated linoleic acids lower the triacylglycerol concentration in the milk of lactating rats and impair the growth and increase the mortality of their suckling pups. J Nutr 134, 3327-3334.

Tanaka, K., 2005. Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. Anim Sci J 76, 291-303.

GENERAL DISCUSSION

Early lactating cows are typically not able to consume enough energy to meet their energetic requirements for maintenance and milk synthesis. As a consequence, dairy cows enter into a negative energy balance at the onset of lactation, which is associated with enormous metabolic stress, health and fertility problems (Butler et al. 1981; Bell 1995; Grummer 1995; Goff & Horst 1997; Beam & Butler 1999; Drackley 1999). Due to the fact that milk fat presents with 50 % the largest proportion of total milk energy and is consequently the energetically most expensive milk component to synthesize (Tyrrell & Reid 1965), a reduction of milk fat synthesis was considered to be an option to minimize the energy deficit and thus the metabolic imbalances of early lactating cows. As a result CLA supplements are frequently added in dairy cow diets to reduce the fat and energy content of milk of dairy cows. Especially the *trans*-10,*cis*-12 CLA isomer is known to have milk fat reducing properties (Baumgard et al. 2000).

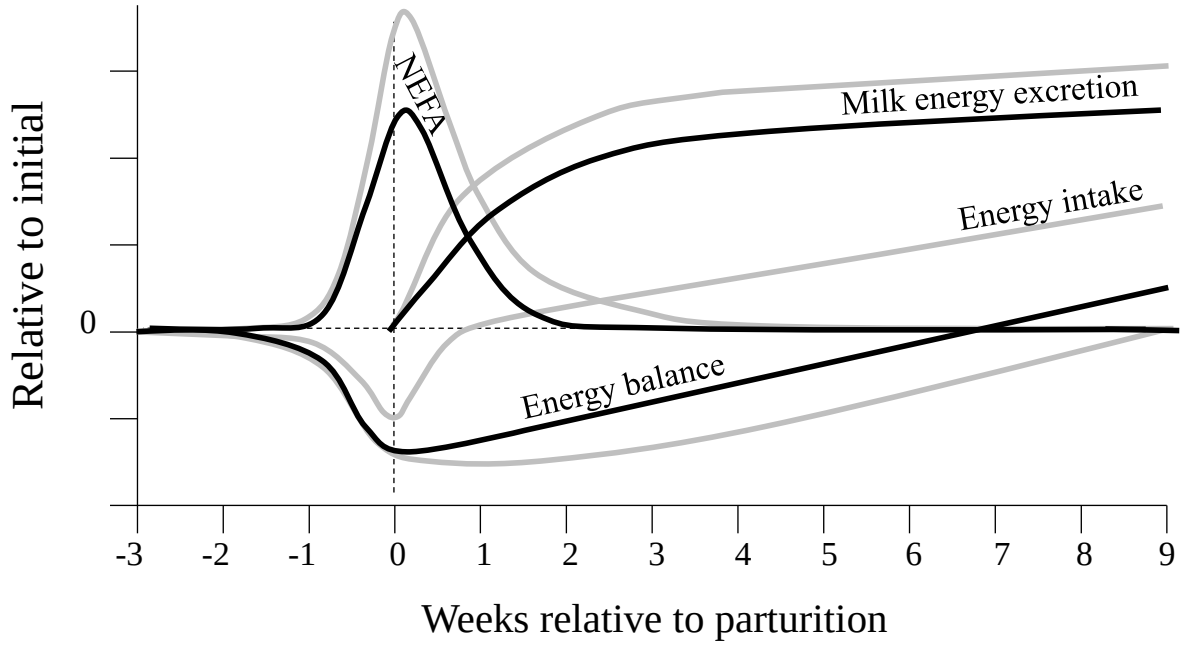


Figure 4. Physiological changes of the periparturient dairy cow (▬) and supposed physiological alterations with CLA supplementation (▬). NEFA = non-esterified fatty acids.

As presented in Figure 4, it is supposed that cows need less energy for milk production due to a CLA induced decline in milk fat content and yield and thus milk energy excretion. Hence, cows require less feed energy if the milk yield remains unchanged, which in turn may counteract the limited energy intake during early lactation. Consequently, CLA supplements have the potential to minimize negative energy balances of cows at the onset of lactation with

a supposed earlier change into a positive energy balance. Additionally, saving energy during times of energy deficits may also reduce the risk of metabolic disorders, immunosuppression or liver stress of early lactating cows due to a lesser extent of body fat mobilization, reflected in a reduced increase of blood NEFA values. Nevertheless, previous studies examining effects of dietary supplemented CLA on energy balance and lipid metabolism of dairy cows during early lactation showed inconsistent results and post-treatment effects of CLA are rarely investigated. In particular the relationship between dietary concentrate feed proportion and supplemented CLA in diets immediately before calving has received no attention in past literature, as most investigations were carried out exclusively during early lactation. However, this may be a crucial point since influencing the metabolic situation of cows immediately before parturition is a possibility to examine and to better understand the lipid metabolism modifying properties of added CLA and thus its influence on energy balance and metabolism. It is hypothesised that a high-energy feeding during late pregnancy may stimulate p.p. lipolysis and thereby results in more pronounced CLA effects in dairy cows.

Consequently, the first aim of this thesis was to examine the influence of supplemented CLA on performance, milk yield, milk composition, estimated energy balance and serum NEFA and BHB concentrations of dairy cows fed low (20%, group CLA-20) and high (60%, group CLA-60) concentrate feed proportions in the diet a.p. to gain detailed information about CLA effects on energy and lipid metabolism **(Paper I)**. Compared to an adapted feeding, the high-concentrate diet was fed to cows three weeks prior to calving to induce an increased lipolysis and thus a ketogenic metabolic situation p.p.. After calving, the concentrate feed proportion was adjusted to 50% and lasted for 60 days. Cows received 100 g CLA supplement (containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA) per day over the entire experimental period, whereby a group-specific termination of CLA supplementation on day 32 p.p. was conducted to determine possible post-supplementation effects **(Paper I and II)**. Results were compared with control groups (groups CON-20, CON-60), receiving a control fat (CON) supplement where CLA isomers were substituted by stearic acid. In the same trial, blood metabolites and the proliferation of PBMC of transition cows were examined to assess the impact of CLA on the bovine metabolism and immune function (**Paper II**). Periparturient dairy cows are immunosuppressed (Mallard et al. 1998) and it is known that their immune function is sensitive to fatty acids (Lacetera et al. 2004), like CLA. However, information about the impact of CLA on the bovine immune system during transition period, which is influenced by strong fluctuations of NEFA, is rare.

Considering that also less knowledge about the mode of action of CLA on rumen metabolism exist, a part of cows of the four experimental groups were equipped with rumen probes to investigate CLA effects on rumen parameters of cows fed low and high concentrate feed proportions in the diet a.p. **(Paper III)**. Rumen probes are designed for continuous RpH and RT measurement and thus enabled to study the impacts of dietary concentrate feed proportion and CLA supplementation of *ad libitum* fed and free-ranging cows around parturition on RpH and RT.

Moreover, approximately 75% of the bovine fetus weight increased during the last two month of pregnancy; consequently maternal nutrition plays a key role in fetal development and growth rate (Funston et al. 2010) and it is also known that *trans*-10,*cis*-12 CLA is consistently transferred into milk fat during supplementation period (Moore et al. 2004; Pappritz et al. 2011b). Thus, maternal CLA supplementation might exert marked effects on calf development, nutrition and metabolism. Therefore, this thesis further aimed to test the hypothesis that an intrauterine exposure to CLA during late pregnancy and the intake of CLA enriched colostrum affects calf metabolism **(Paper IV)**.

1 General aspects

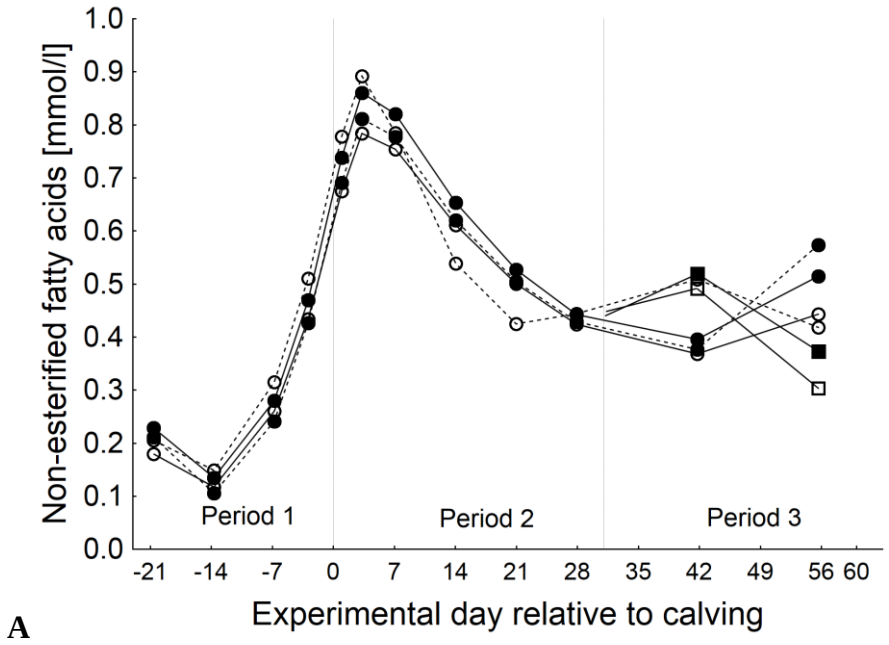
To study the effects of supplemented CLA on performance and various physiological parameters of periparturient dairy cows and their calves, it was intended that each cow of group CLA-20 and CLA-60 receive 10 g *trans*-10,*cis*-12 CLA and *cis*-9,*trans*-11 CLA per day, respectively, which is a common dosage in practical feeding. Nevertheless, according to analysed concentrations in the CLA-concentrate, cows received approximately 20% less CLA than expected (8 g/d of the respective CLA-isomer). Also, Pappritz et al. (2011a) analysed lower CLA concentrations than expected and assumed that the process of pelleting may reduce the amount of fatty acids. The *cis*-9,*trans*-11 CLA isomer was equally present due to the manufacturing process of the CLA preparation. However, *cis*-9,*trans*-11 CLA isomer neither affect milk fat synthesis nor lipid metabolism of lactating cows (Baumgard et al. 2000; Baumgard et al. 2002a).

Moreover, it was hypothesised that CLA effects are more pronounced in energetically overfed cows during late pregnancy. Influencing the metabolic situation of cows immediately before calving could be helpful to lead to a better understanding of how CLA modifies the lipid metabolism of dairy cows immediately after calving and during early lactation. Therefore, compared to a feeding adjusted to the requirements, a high-concentrate diet was fed to dairy cows before calving. As reported in Background Chapter 2, it is known that feeding high

amounts of concentrates prepartum increase the energy intake of dairy cows and thus may counteract the generally reduced feed consumption in this time (Grummer 1995; Drackley 1999). However, adverse effects are quite often including overconditioning (Grummer 1995), the risk of developing rumen acidosis or displaced abomasum and thereby lead to a reduction in feed intake (Hayirli & Grummer 2004). Especially, overconditioning during the dry period is negatively related to feed intake a.p., which predisposes dairy cows to a higher susceptibility of metabolic diseases like ketosis or fatty liver after calving. Compared to thinner cows, obese cows are observed to have a sluggish appetite a.p. and a greater decline in feed intake p.p., leading to a more severe negative energy balance and an increased lipolysis of adipose tissue during early lactation, which is reflected in higher blood NEFA and BHB concentrations and in a greater loss of body weight in this time (Grummer 1995; Smith et al. 1997; Rukkwamsuk et al. 1998; Bobe et al. 2004; Hayirli & Grummer 2004). Thus, the high-concentrate diet a.p. should induce the mentioned ketogenic metabolic situation and increased lipolysis in cows p.p. to investigate and better understand how CLA works.

However, differently as expected, no decline in feed intake of cows fed a high concentrate proportion in the diet a.p. could be observed until calving. Depending on DMI and energy content of the feedstuff, groups CON-60 and CLA-60 fed on high concentrate a.p. consumed significantly more net energy than groups CON-20 and CLA-20 a.p., leading to a marked energy surplus before calving **(Paper I)**. McNamara et al. (2003) also observed that cows fed a high energy density before calving had increased net energy intakes and thus positive energy balances a.p. but reported further that those cows experienced the greatest degree of negative energy balance after calving. However, cows of group CON-60 and CLA-60 did not consume less DM nor had a greater negative energy balance in comparison with their respective counterparts during early lactation **(Paper I**). Moreover, live weight (LW) and blood NEFA and BHB values (Figure 5) did not differ between the respective groups during the entire experimental period, even if NEFA serum concentrations increased shortly after calving in all groups, indicating an energy deficit and subsequent lipid mobilisation (Dirksen et al. 2006) and serum BHB concentrations let suggest that cows of group CLA-60 and CON-60 may be subjected to subclinical ketosis in early lactation (Dirksen et al. 2006). It seems that the approach to induce a ketogenic metabolic situation of cows p.p. by feeding a high concentrate proportion in the diet during late pregnancy failed. Consequently, the possibility to investigate the impact of CLA under such a metabolic state was not given **(Paper I and II)**. Perhaps, as realized in other studies (Loor et al. 2007; Kuhla et al. 2009), to restrict or deprive

feed intake at the onset of lactation might have been a more successful method to induce a ketogenic metabolic state p.p..



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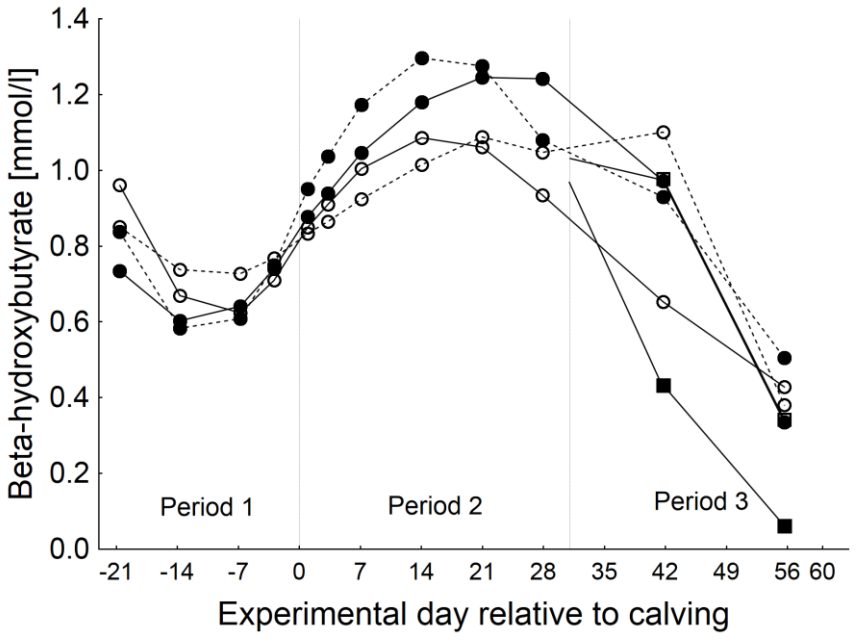

B

Figure 5. (**A**) Non-esterified fatty acid and (**B**) beta-hydroxybutyrate concentrations in serum of periparturient cows in the course of the experiment.
Antepartum (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Postpartum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]).

2 Dry matter intake and rumen metabolism

In the present study, cows of group CLA-60 consumed significantly more DM (22-24 %) compared to cows of group CON-20 and CLA-20 during late pregnancy and even if the concentrate proportion was adjusted to 50 % in all groups after parturition, DMI of group CLA-60 was also highest during the first 2 weeks p.p. compared to group CLA-20 **(Paper I)**. Data regarding CLA effects on DMI are largely identical. Most of the previous studies observed that DMI remained unaffected (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Kay et al. 2006; Odens et al. 2007; Sigl et al. 2010) and only a few studies recorded a reduced DMI by CLA supplementation (Moallem et al. 2010; Pappritz et al. 2011b), whereby no study could be found where CLA increased DMI. Consequently, observed differences in DMI may be caused by a pronounced concentrate effect on total DM (Friggens et al. 1998). It is well known that DMI increases with an increase of digestibility in the diet (NRC 1988), which can also be confirmed in the present study (Figure 6A). A positive relationship (r=0.968) between daily non-fibre carbohydrate (NFC) intake, which represents the intake of soluble and thus easily digestible carbohydrates like starch, sugar or pectin, and DMI was observed. On the other side, the rumen fill is the limiting factor for DMI when feeding high amounts of roughage to ruminants (NRC 1988). Moreover, it could also be possible that cows of group CLA-60 may have had a better rumen adaption to concentrate level p.p.. Grummer (1995) assumed that feeding high amounts of concentrates a.p. may adapt rumen microbes to lactation ration, support development of rumen papillae and thus increase the absorbency of rumen epithelium. However, DMI of group CON-60 were not increased during the first 2 weeks p.p.. For inexplicable reasons, findings indicate that CLA supplementation in a high concentrate diet a.p. lead to an increase in DMI immediately after calving **(Paper I)**, which may present a possibility to minimize energy deficits and metabolic disorders of early lactating cows. This topic is discussed later in detail in Chapter 4 and 5.

Moreover, cows fed on high concentrate were characterized by reduced RpH compared to cows fed on low concentrate during late pregnancy, whereby RT remained unaffected **(Paper III)**. As presented in Figure 6B, the intake of NFC and thus easily digestible carbohydrates are negatively related to RpH (-0.293). Feeding high amounts of NFC reduces RpH, because of its rapid bacterial fermentation and thus rising production of short chain fatty acids, especially of propionate, and later lactate (Kafil et al. 2011). In contrast, an increased intake of crude fibre (CF) lead to an enhancement in chewing time of cows due to reducing the

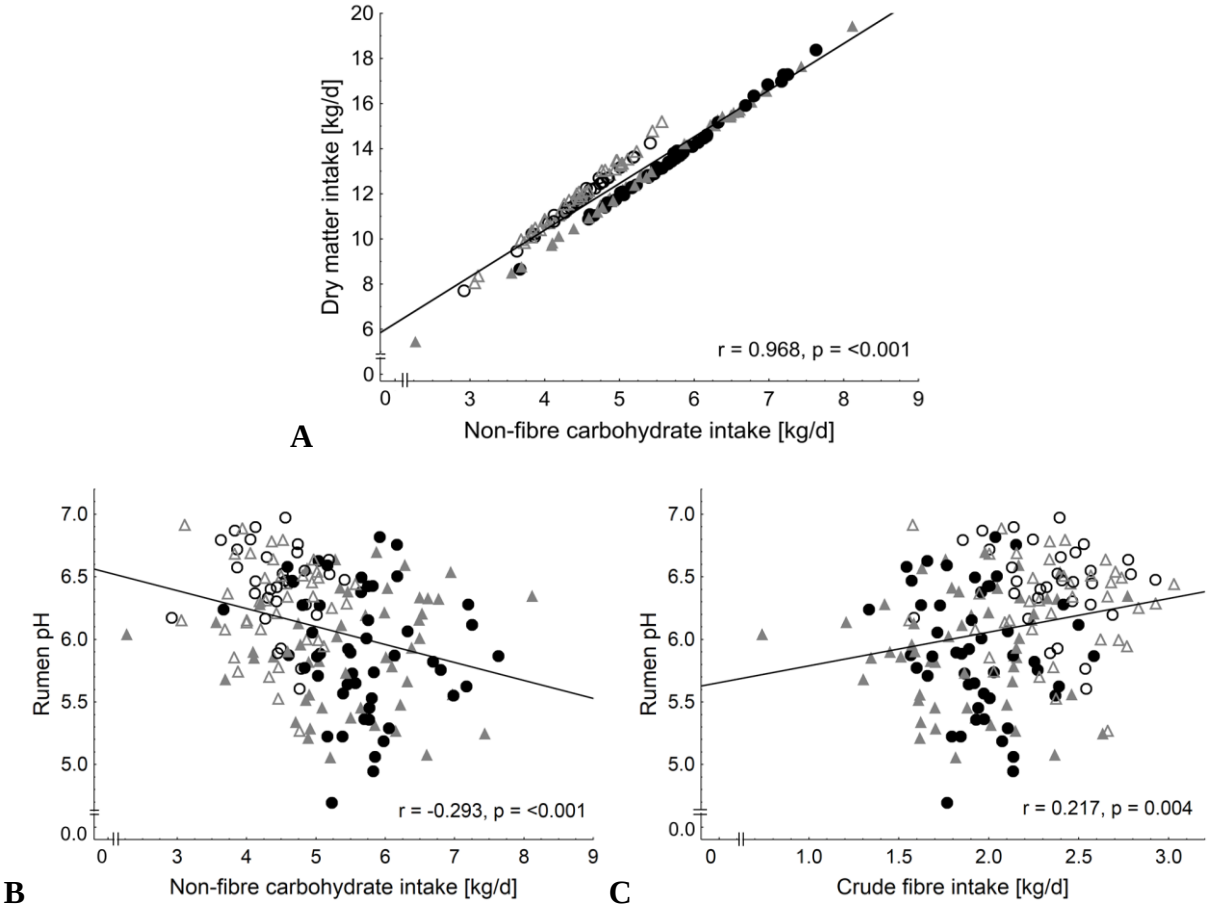

Figure 6. Relationship between (**A**) non-fibre carbohydrate intake and dry matter intake, (**B**) non-fibre carbohydrate and rumen pH and (**C**) crude fibre intake and rumen pH during late pregnancy (day -21 to -8 *antepartum*).
Group CON-20 (△, n= 4) and CLA-20 (○, n=4) received a control fat (CON) or a conjugated linoleic acid (CLA) supplement in a low (20%) and group CON-60 (▲, n=4) and CLA-60 (●, n=5) in a high (60%) concentrate diet. Data based on **Paper III**, values presented as daily means.

particle size of the feed, which results in a greater amount of salvia per unit DMI (Maekawa et al. 2002) and thus increases RpH (Figure 6C, r=0.217). However, it seems that CLA supplementation might have influenced rumen metabolism, even if only minor changes on RpH and RT were observed. Despite the same DM, NFC and CF intake among the respective counterparts, a reduced time below RpH 5.8 and a lower RT were observed in group CLA-20 during late pregnancy and in group CLA-60 after calving, respectively **(Paper III)**. Pappritz et al. (2011a) examined the duodenal availability of a lipid-encapsulated CLA preparation, which was also used in the present study. The authors recorded, based on intakes of 8 g *trans*-10,*cis*-12 CLA isomer per day, that only 5 % of CLA were available in the duodenum. Perhaps, an insufficient CLA rumen-protection may have negatively affected rumen microbes and thus may have inhibited rumen fermentation of group CLA-20 and CLA-60, leading to an increase in RpH and consequently RT may be reduced because of the negative relationship

between RpH and RT (R^2=0.77) (AlZahal et al. 2008). In addition, dietary CLA supplementation has been associated with a modified rumen fermentation pattern as indicated by an altered profile of short chain fatty acids, increased starch degradation and a drop in rumen microbial protein synthesis in dairy cows (Pappritz et al. 2011a). However, Pappritz et al. (2011a) suggest that CLA effects on rumen fermentation were negligible through recording also an unaffected rumen concentration of total volatile fatty acids and NH_3, an unchanged acetic acid-to-propionic acid ratio and an uninfluenced RpH (Pappritz et al. 2011a). Likewise, Huang et al. (2009) observed only marginal CLA effects on rumen metabolism. Besides an increased lipid digestibility, CLA supplementation did not influenced DM or crude protein digestibility in sheep. Nevertheless, a minor CLA effect on rumen metabolism cannot be ruled out, which however cannot completely described on the basis of RpH and RT. Unfortunately, RpH and RT represent parameters, which are easily to manipulate. Hence, cows of group CLA-20 and CLA-60 may only have had an enhanced water intake or an increased salvia production due to higher ruminating activity, leading to a marginal rise in RpH and lower RT.

3 Lipid metabolism in mammary gland

The milk fat reducing properties of CLA, especially of the *trans*-10,*cis*-12 CLA isomer, are well examined in lactating dairy cows. Nevertheless, some studies observed besides a reduced milk fat content an increased milk yield (Kay et al. 2006; Medeiros et al. 2010; Hammon et al. 2011; von Soosten et al. 2011) and milk protein (Medeiros et al. 2010) or milk lactose content (Kay et al. 2006; Hammon et al. 2011), indicating that spared energy was redistributed and used for milk protein and lactose synthesis, resulting in higher milk yield. In the present study, CLA supplementation did not influence milk yield, milk protein and milk lactose content, which is in accordance with previous studies (Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Sigl et al. 2010; Pappritz et al. 2011b). However, contrary to expectation and despite to the fact that CLA supplementation was initiated 21 days before parturition, only a slight reduction of milk fat yield was recorded for group CLA-20 during early lactation. Group CLA-20 synthesized approximately 120-190 g and 150-290 g less milk fat per day than the other groups during the first 31 and 32-60 days in milk (DIM), respectively, being only significant in the third week p.p. compared to group CON-60 (**Paper I**). It is known that CLA supplementation led to an immediately decrease in milk fat synthesis during established lactation (Loor & Herbein 1998; Chouinard et al. 1999a; Chouinard et al. 1999b; Baumgard et al. 2000; Giesy et al. 2002; Perfield et al. 2002; de Veth et al. 2005; Sippel et al. 2009), whereby CLA supplementation during transition period (Bernal-Santos et

al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005) or during early lactation (Liermann 2008; Pappritz et al. 2011b; von Soosten et al. 2011) indicated that CLA is less effective at reducing milk fat synthesis until several weeks after calving.

Reasons are widely unknown, whereas differences in the uptake of *trans*-10,*cis*-12 CLA by mammary gland can be excluded, because of its consistent transfer into milk fat during supplementation period (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005; Kay et al. 2006). Accordingly, significantly increased proportions of *trans*-10,*cis*-12 CLA isomer in the fatty acid profile in milk fat on day 21 p.p. of CLA-groups compared to CON-groups were observed in the present study (Table 2).

Table 2. Fatty acid profile in milk fat on day 21 p.p.; LSMeans.

	Control fat (100 g/d)		CLA (100 g/d)			
Fatty acid [% of total FAME]	**CON-20**[1] *(n=16)*	**CON-60**[2] *(n=15)*	**CLA-20**[1] *(n=16)*	**CLA-60**[2] *(n=16)*	SEM	*p-value*
$C_{4:0}$	4.07	3.89	3.97	4.03	0.16	0.855
$C_{6:0}$	2.88	2.70	2.80	2.82	0.13	0.796
$C_{8:0}$	1.46	1.39	1.47	1.46	0.07	0.836
$C_{10:0}$	3.11	2.97	3.27	3.14	0.22	0.833
$C_{12:0}$	3.13	3.00	3.31	3.16	0.25	0.855
$C_{14:0}$	9.91	9.69	10.31	10.44	0.50	0.695
$C_{14:1}$	0.73	0.65	0.64	0.68	0.04	0.434
$C_{15:0}$	1.35	1.44	1.59	1.44	0.11	0.508
$C_{16:0}$	28.99	27.65	28.04	28.84	0.68	0.462
$C_{16:1}$	2.09	2.00	1.74	1.69	0.14	0.134
$C_{17:0}$	1.30	1.41	1.35	1.34	0.04	0.204
$C_{18:0}$	10.10	10.14	10.28	10.45	0.48	0.954
$C_{18:1}$ *trans*	2.27	2.62	3.35	2.82	0.29	0.082
$C_{18:1}$ *cis*-9	23.54	25.02	22.28	22.25	1.43	0.493
$C_{18:2}$ *trans*-9,*trans*-12	0.00	0.00	0.00	0.00		
$C_{18:2}$ *cis*-9,*cis*-12	1.59	1.72	1.75	1.79	0.07	0.212
CLA						
$C_{18:2}$ *cis*-9,*trans*-11	0.303	0.357	0.345	0.373	0.018	0.057
$C_{18:2}$ *trans*-10,*cis*-12	0.001[a]	0.003[a]	0.024[b]	0.021[b]	0.002	<0.001
Other CLA	0.067[a]	0.075[ab]	0.087[bc]	0.093[c]	0.004	<0.001
n-3	0.336	0.375	0.380	0.384	0.014	0.051
$C_{20:0}$	0.105	0.108	0.110	0.111	0.005	0.827
Other	2.67	2.83	2.91	2.69	0.011	0.395
Summation						
< C16	27.23	26.31	28.04	27.74	1.26	0.787
C16	31.08	29.65	29.78	30.52	0.67	0.399
> C16	41.69	44.04	42.18	41.74	1.60	0.708

CLA = Conjugated linoleic acid; CON = Control fat.

[1]Group CON-20 and CLA-20 received a low (20%) concentrate diet antepartum.

[2]Group CON-60 and CLA-60 were fed a high (60%) concentrate diet before calving. After calving the concentrate proportion was adjusted to 50% in each group, while fat supplementation continued.

[abc]Different superscripts indicate significantly differences between the groups (p<0.05).

An explanation for a lack of CLA response in milk fat may be the differing sources of milk fatty acids for milk fat synthesis during early lactation. Milk fatty acids vary in their carbon chain length and are primarily classified according to their origin: fatty acids synthesized *de novo* in the mammary gland ($<C_{16}$), fatty acids from the uptake of preformed fatty acids originating from feed or body fat mobilization ($>C_{16}$) and fatty acids from both sources (C_{16}) (McGuire & Bauman 2002). It is known that *trans*-10,*cis*-12 CLA reduces milk fat content through an inhibited expression of genes that encode for enzymes involved in milk fat synthesis (Baumgard et al. 2002b). Previous studies observed that CLA reduced the secretion of all fatty acids. However, the reduction was greatest for the most short and medium chain fatty acids, whereas the percentage of long chain fatty acids was increased (Loor & Herbein 1998; Bauman & Griinari 2001; Loor & Herbein 2001; Perfield et al. 2002; Moore et al. 2004; Kay et al. 2006), indicating that CLA reduces the milk fat synthesis due to an inhibition of *de novo* fatty acid synthesis in mammary gland (Harvatine & Bauman 2011). During established lactation nearly 50 % of the fatty acids in milk triglycerides originate from mammary *de novo* synthesis from acetate and BHB and approximately 50 % are derived preformed from plasma lipoprotein triglycerides (Bell 1995). However, at the onset of lactation cows are typically in a negative energy balance, associated with increased levels of blood NEFA due to an increased adipose tissue mobilization (Drackley 1999). This in turn increases the uptake of NEFA by the mammary gland and their utilization to synthesized milk fat triglycerides. NEFA can account up to 40 % of milk fatty acids at day 4 p.p. (Bell 1995). Perhaps, the adequate mammary epithelial CLA uptake is prevented by the completive binding and cellular incorporation of NEFA (Moore et al. 2004) and thus CLA cannot effectively inhibit *de novo* milk fatty acid synthesis, which could explain the unaffected percentages of $<C_{16}$, C_{16} and $>C_{16}$ fatty acids in milk fat on day 21 p.p. (Table 2) and also the slight reduction of milk fat content of group CLA-20 in contrast to group CLA-60. Compared to obese cows, thinner cows are known to have a reduced lipolysis-reaction p.p. and thus lower blood NEFA concentrations, which may result in a decline in milk fat (Holter et al. 1990). However, as mentioned above, LW and blood NEFA and BHB values did not differ between the groups. Nevertheless, it could also be possible that genes relevant for milk fat synthesis are generally insensitive to manipulate during early lactation (Moore et al. 2004).

However, contrary to expectations and to other studies with comparable CLA doses (Bernal-Santos et al. 2003; Moore et al. 2004; Castaneda-Gutierrez et al. 2005; von Soosten et al. 2011), dietary supplemented CLA did not reduce the milk fat content after several weeks p.p. **(Paper I)**. Perhaps, an insufficient CLA rumen protection against microbial degradation and

thus relatively low amounts of CLA available at duodenum may have led to a failure of CLA effect on milk fat (Pappritz et al. 2011a). Moreover, the milk yield level of cows used in the present study was relatively low compared to high-yielding cows, which could also cause a lack of response in milk fat. However, even though not significantly different, group CLA-20 produced less milk per day (Period 2: 0.7-2.2 kg; Period 3: 1.0-2.7 kg/d) than the other groups. Consequently, less milk protein (Period 2: 30-60 g/d; Period 3: 60-90 g/d) and lactose (Period 2: 60-140 g/d; Period 3: 80-190 g/d) were synthesized **(Paper I)**. Perhaps, the slightly reduced milk fat content of group CLA-20 during the entire experimental period is mainly a result of a decreased milk yield. Nevertheless, a CLA effect on milk fat of group CLA-20 cannot be excluded. In Period 3, half of the animals of the CLA-groups changed to CON supplementation and group CLA-20-CON, receiving CON instead of CLA supplement, showed a significantly increased milk fat yield compared to group CLA-20 approximately one week upon terminated CLA supplementation. This observation may also be strengthened the assumption that CLA was stored in adipose tissue during treatment period and was frequently mobilized after completion of supplementation. However, the milk fat reducing effect was only apparent for a short time, which is in accordance with other studies (Baumgard et al. 2000; Castaneda-Gutierrez et al. 2005; Pappritz et al. 2011b).

However, even though not significantly different over the entire experimental period, it seems that the milk fat reduction in group CLA-20 may have led to an energy saving in contrast to other groups, providing cows of group CLA-20 additional energy during early lactation. This topic is discussed in the following chapter.

4 Energy balance and lipid mobilization

Dairy cows are often subjected to an enormous energy deficit at the onset of lactation, which were compensated by the use of their body fat reserves (Grummer 1995; Drackley 1999). The milk fat reducing properties of *trans*-10,*cis*-12 CLA suggests an energy-saving effect in dairy cows. Saving energy may improves the energy deficit of early lactating cows and further may lead to an earlier change into a positive energy balance, resulting in a lesser extent of body fat mobilization or even in an increased adipose tissue lipogenesis. Consequently, one of the main focuses of the present study was to examine whether dietary supplemented CLA has the potential to minimize a negative energy balance and the extent of adipose tissue mobilization of cows at the onset of lactation **(Paper I)**.

Contrary to other studies and as mentioned in the previous chapters, only a slightly reduced (non-significant) milk fat production during early lactation and a significantly increased DMI in the first two weeks after calving could be observed in group CLA-20 and CLA-60,

respectively **(Paper I)**, which may provide cows additional energy at the onset of lactation. However, each group changed from a positive energy balance a.p. to a negative energy balance p.p.. No improvement of energy balance was recorded for group CLA-20 p.p., even if cows reached a positive energy balance approximately one week earlier than others. Whereas group CLA-60 had indeed a significantly less negative estimated energy balance within the first two weeks of lactation **(Paper I),** indicating an improved energy utilisation due to CLA supplementation. Differences in estimated energy balances can be directly attributed to the significantly increased DMI and net energy intake of group CLA-60, as there were no differences in LW, milk yield, milk composition and milk energy output within the groups. Consequently, a reduced lipid mobilization was expected for group CLA-60, indicating by lower blood NEFA values (Dirksen et al. 2006). The development of increased serum NEFA concentrations indicate that the body fat mobilisation was highest within the first week of lactation in each group (Figure 5A), which coincides with the nadir of negative estimated energy balance. However, overall blood NEFA and BHB levels provide no evidence for a reduction in lipid mobilization **(Paper I)**. Unchanged NEFA values were unexpected but agree with other CLA studies during transition period or early lactation (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Pappritz et al. 2011b; von Soosten et al. 2011). An explanation for this lack of CLA effect on NEFA blood values is widely unknown and a sustained body fat mobilization due to a redistribution of spared energy to higher milk yield can be excluded since milk yields remained unchanged in the present study. Moore et al. (2004) assumed that the signal to mobilize adipose tissue at the onset of lactation is neither dependent on the energy balance nor on the fatty acid requirements of the mammary gland, but instead reflects more the oxidation needs of extra-mammary tissues in a homeorhetic effort to spare glucose, which is required for mammary lactose synthesis. Moreover, in previous studies LW, body condition score (BCS) or body fat thickness (BFT) were generally used to assess the body fat mobilization in lactating dairy cows, whereby the latter two ones mainly reflect alterations in subcutaneous adipose depot. However, no differences in LW, BCS or BFT could be observed in studies with early lactating cows and a comparable CLA dose like supplemented in the present study (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Medeiros et al. 2010; Pappritz et al. 2011b), which may also indicate that CLA does not have an influence on body fat mobilization and thus NEFA blood values. However, Odens et al. (2007) observed a reduced BW and BCS loss of cows during early lactation through CLA supplementation (33.6 g/d), which was accompanied by lower NEFA blood concentrations. It seems that lower doses

of CLA may only affect lipid metabolism to a minor extent, which cannot be detected by LW, BCS, BFT and NEFA sensitively enough. Indeed, recent studies investigating CLA effects on body fat composition confirm this subscription of an energy-saving effect and its consequences on adipose tissue. Von Soosten et al. (2011) observed a decelerated mobilization of the retroperitoneal adipose depot in primiparous cows during the first 42 DIM after a CLA supplementation of 6 g/d, which may confirm the expected energy-saving effect through CLA supplement. Moreover, CLA induced a numeric reduction in body fat mass mobilization (von Soosten et al. 2012) and decreases adipocyte size (Akter et al. 2011) in these cows compared to control, whereas blood NEFA remained also unaffected. The authors concluded that CLA may have effects on lipolysis or lipogenesis or on both in different adipose tissues of dairy cows, causing the observed effects (Akter et al. 2011; von Soosten et al. 2012). It could also be observed that on mid-lactating dairy cows a CLA-induced milk fat depression was accompanied with an upregulated expression of lipid synthesis-related enzymes and key regulators, including LPL, FAS, stearoyl-CoA desaturase, FABP4, SREBP-1, PPARγ and thyroid hormone responsive spot 14, in the adipose tissue (Harvatine et al. 2009). Likewise, Saremi et al. (2011) recorded an increased mRNA abundance of PPARγ in the visceral adipose tissue of CLA-treated cows used in the above mentioned studies (Akter et al. 2011; von Soosten et al. 2011; von Soosten et al. 2012). These findings may provide the evidence of short-term alterations in energy partitioning in dairy cows through CLA treatment. Even if a 4-day abomasal infusion of 7.5 g/d CLA resulted in a reduced voluntary feed intake, CLA-induced milk fat reduction led to an energy-saving effect, whereby spared energy is potentially repartitioned towards adipose tissue (Harvatine et al. 2009).

Overall, CLA supplementation showed a clear energy-saving effect during early lactation, leading to an improvement in estimated energy balance, even if caused by an increased DMI and not through a reduced milk fat reduction, as observed in other studies. No influence on lipid mobilization could be shown by investigated parameters, whereas based on literature a CLA effect on adipose tissue and thus on lipid mobilization could be seen. Further studies are necessary.

5 Metabolism and liver function

At the onset of lactation, dairy cows are unable to meet their energy requirements for maintenance and milk production due to a limited DMI potential (Bell 1995; Grummer 1995; Goff & Horst 1997). However, this status of negative energy balance and hence increased lipid mobilization are associated with an increased incidence of infections and metabolic

diseases like ketosis or fatty liver (Grummer 1995; Goff & Horst 1997; Drackley 1999) accompanied with a subsequent loss of performance and fertility problems (Butler et al. 1981; Beam & Butler 1999). However, as shown in previous chapters, CLA supplementation led to an improvement in estimated energy balance, even if induced by an increased DMI and not through a reduced milk fat reduction. Consequently, CLA supplementation may have the potential to prevent health problems of early lactating cows. Indeed, several physiological properties have been attributed to CLA, including for example anticarcinogenic and antidiabetic effects or prevention of cholesterol-induced atherosclerosis in dairy cows (Belury 2002; Tanaka 2005), whereby studies on mice indicate that CLA led to lower tissue and higher liver weights with an accompanying development of liver steatosis (Tsuboyama-Kasaoka et al. 2000). The liver plays a key role in coordinating metabolic processes like gluconeogenesis, triglyceride synthesis and secretion, fatty acid oxidation and ketogenesis to ensure an adequate nutrient supply of the dairy cow (Drackley et al. 2001). However, during early lactation the liver lipid metabolism is additionally burdened by an excessive lipolysis and consequently greater hepatic uptake of NEFA. If infiltration becomes more severe, an accumulation of triglycerides within hepatocytes occur, impairing liver function (Grummer 1993; Goff & Horst 1997; Drackley 1999). Hence, possible adverse effects of CLA on hepatic lipid metabolism may result in additional liver stress during this time of energy deficit. Consequently, the question arises if CLA, dietary supplemented in a dose able to reduce milk energy output, have an impact on metabolism and liver function of periparturient dairy cows **(Paper II)**.

As mentioned above, present findings revealed that CLA supplements may be able to reduce metabolic disorders. However, blood NEFA and BHB values remained unaffected (Figure 5), indicating that CLA supplements did not alter body lipid mobilization and thus metabolism of early lactating cows. Moreover, blood glucose concentration remained unchanged by CLA supplementation, which is in accordance with other long-term studies during transition period and during early or established lactation (Perfield et al. 2002; Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Kay et al. 2006; Sigl et al. 2010; von Soosten et al. 2011) and short-term experiments (Baumgard et al. 2000; Baumgard et al. 2002a), revealing that CLA does not have an influence on glucose level. Overall, CLA supplementation did not affect investigated blood metabolites **(Paper II)**, which confirm observations from previous studies (Baumgard et al. 2002a; Bernal-Santos et al. 2003; Liermann 2008; Sigl et al. 2010). Results demonstrate that dietary supplemented CLA does not have an impact on metabolism of periparturient dairy cows.

Concentrations of investigated blood metabolites were mainly affected by variations in feed intake. DMI was reduced around calving and coincides with higher blood NEFA values **(Paper I)**. Around and especially immediately after calving, increased blood NEFA concentrations were paralleled by low triglyceride, cholesterol and IGF-1 and high bilirubin values **(Paper II)**. As mentioned above, the liver function may be reduced due to lower DMI and consequently sustained lipid mobilization. However, group CLA-20 was affected more severe than group CLA-60, which may result from the observed differences in DMI **(Paper I)**. Cell and liver damage indicating enzymes like γ-glutamyl-transferase (GGT) and glutamate dehydrogenase (GLDH) remained unaffected by treatments during the entire experimental period and were within their reference range according to Kraft & Dürr (2005) (Figure 7). Only aspartate amino-transferase (ASAT) showed higher activities after calving (Figure 7). However, increased ASAT activities are not necessary pathological due to the fact that liver cells need to adapt to a greater turnover rate of lipids during early lactation (Bostedt 1974). Hence, there was no indication that liver cells were damaged at this time. Compared to the other groups, cows of group CLA-20 may only have had a less optimal energy and nutrient supply and thus greater liver stress around calving via reduced DMI. Consequently, it can be assumed that CLA supplements did not adversely affect liver function.

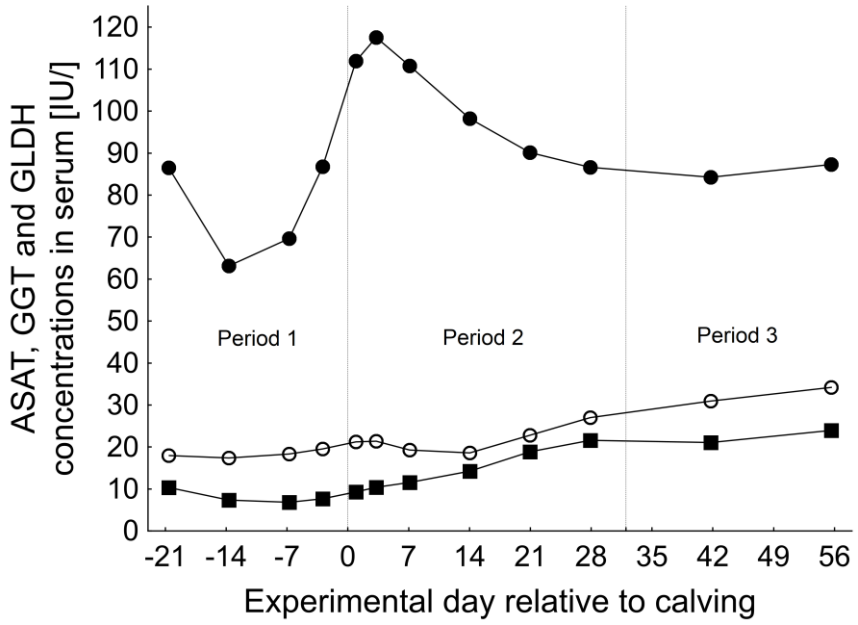

Figure 7. Aspartate amino-transferase (ASAT), γ-glutamyl-transferase (GGT) and glutamate dehydrogenase (GLDH) in serum of periparturient dairy cows in the course of the experiment.
Reference range according to Kraft & Dürr (2005): ASAT: <80 IU/l; GGT: <50 IU/l; GLDH: <30 IU/l (Means; n=63) (– • – ASAT; – o – GGT; – ■ –GLDH).

This agrees with previous studies, observing that liver weight (von Soosten et al. 2011) and hepatic total lipid and cholesterol concentration (Bernal-Santos et al. 2003; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; Schlegel et al. 2012) were not affected by CLA supplementation in early lactating cows. Furthermore, Schlegel et al. (2012) investigated CLA effects on the hepatic expression of genes involved in lipid metabolism and observed that none of the genes (genes encoding fatty acid transport; genes involved in mitrochondrial and peroxisomal β-oxidation; genes of carnitine metabolism; genes of ketogenesis; genes involved in fatty acid and triacylglycerol synthesis, genes involved in cholesterol metabolism; genes involved in lipioprotein metabolism) were differently altered due to CLA supplementation. Consequently, the authors concluded that CLA do not have an impact on hepatic lipid metabolism. However, findings are contrary to observations in mice. Differences in hepatic lipid metabolism may be caused by variations in fat metabolism of mice and ruminants (Pearce 1983) or by differences in supplementation level (Bauman et al. 2008). The development of fatty liver in mice was shown in a study of Tsuboyama-Kasaoka et al. (2000) using diets containing 1% *trans*-10,*cis*-12 CLA, which strongly differ from that dose used in dairy cows ranging from 0.02 % - 0.08% *trans*-10,*cis*-12 CLA of total DMI (Bernal-Santos et al. 2003; Selberg et al. 2004; Castaneda-Gutierrez et al. 2005; von Soosten et al. 2011; Schlegel et al. 2012).

6 Immune function

Data regarding CLA effects on bovine immune function were rarely investigated. Renner et al. (2012a) examined the fatty acid profile and proliferation of PBMC after a long-term supplementation (day 1 to 189 p.p.) of either 4 g or 8 g *trans*-10,*cis*-12 CLA per day to dairy cows and observed that supplemented CLA influenced the fatty acid profile in bovine PBMC. The proportion of *trans*-10,*cis*-12 CLA of total fatty acids in bovine PBMC was observed to be increased, whereas the proportion of *trans*-9 $C_{18:1}$ and *cis*-12 $C_{24:1}$ was reduced. However, observed alterations in the fatty acid profile did not have an impact on the function of PBMC since no differences in the mitogen-induced activation of PBMC were observed (Renner et al. 2012a). Results indicate that the function of bovine PBMC was not influenced by CLA supplementation, which agrees with findings from other studies (Hussen et al. 2011; Renner et al. 2012b) and the present observation **(Paper II)**. No differences in mitogen-stimulated proliferation of PBMC could be observed between the groups during experimental periods (Figure 8)**.**

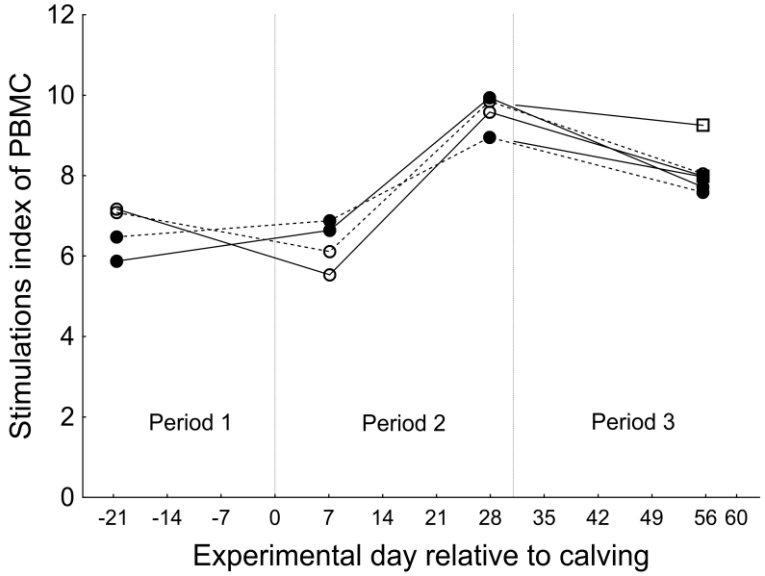

Figure 8. Stimulations index of peripheral blood mononuclear cells (PBMC) of periparturient cows in the course of the experiment.
Antepartum (Period 1), group CON-20 and CLA-20 received control fat (——) or CLA supplement (- - -) in a low (○) and group CON-60 and CLA-60 in a high (●) concentrate diet. *Postpartum* (Period 2 and 3), concentrate proportion was adjusted while fat supplementation continued. In Period 3, half of the animals of CLA-groups changes to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]).

However, the mitogen-stimulated proliferation of PBMC was reduced around calving and rose with progressing lactation in each group. This is in accordance with other studies (Dänicke et al. 2012; Renner et al. 2012a; Renner et al. 2012b), observing also that the simulations index (SI) changed in the course of lactation. Dairy cows are known to be immune-compromised around parturition (Mallard et al. 1998), reflected in an impaired lymphocyte function (Franklin et al. 1991; Goff 2006) and also in a reduced response of PBMC to mitogen-stimulation (Nonnecke et al. 2003; Loiselle et al. 2009). Consequently, findings indicate a depressed immune function of dairy cows around parturition. Lacetera et al. (2004) observed that NEFA reduce the mitogen-stimulated proliferation of PBMC in a dose-dependent manner. Hence, higher NEFA concentrations, as occurring through increased body fat mobilization, are discussed to contribute to immune-modulation in dairy cows around calving. In the present study, NEFA serum concentrations increased shortly a.p. and were highest within the first week of lactation in each group (Figure 5A), which coincides with reduced SI of PBMC (Figure 8). Also, Renner et al. (2012b) observed that the ability to stimulate PBMC was lowest (at day 1 p.p.), when NEFA blood concentration were increased and reached their peak (von Soosten et al. 2011). With the progress of lactation, the SI of PBMC increased while blood NEFA concentration decreased, as also reported by Renner et al. (2012b). On average, a relationship between blood NEFA concentrations and SI of PBMC

is recognizable. However, based on the present data, no correlation between both parameters could be found (Figure 9). Furthermore, the SI decreased slightly from day 28 p.p.. An reduction of mitogen-stimulated proliferation of PBMC could also be observed by Renner et al. (2012a) during early lactation, whereby the SI reached the minimum at day 49 p.p.. An explanation cannot be given, but at this time point cows changed from a negative to a positive estimated energy balance (Renner et al. 2012a). Further studies are necessary to investigate the underlying mechanism of this decline in more detail.

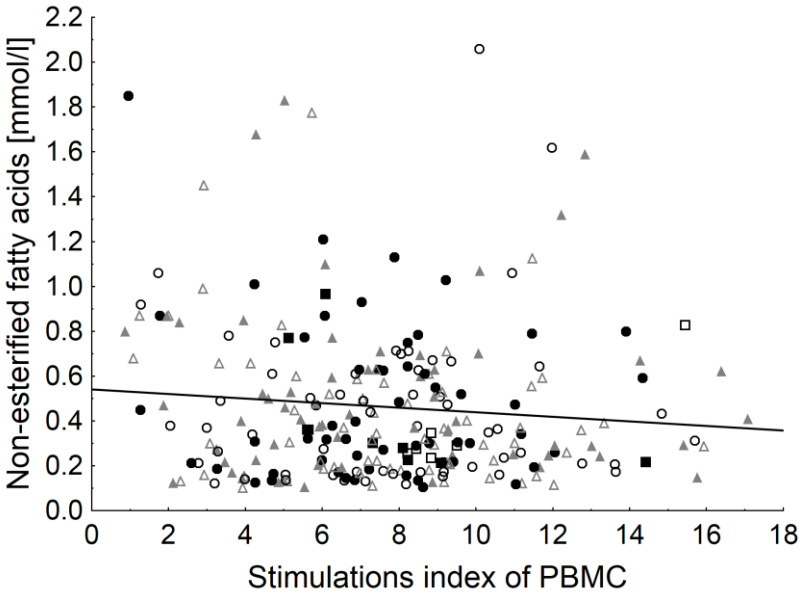

Figure 9. Relationship between non-esterified fatty acids in serum and stimulations index of peripheral blood mononuclear cells (PBMC) during the course of the experiment (day 21 a.p., 7, 28 and 56 p.p.); r = -0.097; p = 0.125.
Antepartum, group CON-20 (△, n= 4) and CLA-20 (○, n=4) received a control fat (CON) or a conjugated linoleic acid (CLA) supplement in a low (20%) and group CON-60 (▲, n=4) and CLA-60 (●, n=5) in a high (60%) concentrate diet. After calving the concentrate proportion was adjusted to 50% while fat supplementation continued. At day 32 p.p., half of the animals of CLA-groups changed to control fat supplementation (CLA-20-CON [□] and CLA-60-CON [■]). Data based on **Paper I and II**, values presented as daily means.

Moreover, it was expected that CLA, supplemented in a dose able to induce a milk fat reduction, reduces lipid mobilization during early lactation, resulting in reduced NEFA concentrations around calving and thus may improve capability to stimulate the immune system. However, CLA supplementation neither influenced milk energy output nor blood NEFA values nor the mitogen-stimulated proliferation of PBMC in the present study **(Paper I)**, indicating that CLA did not have the potential to ameliorate the effects of a compromised immune function. This assumption can be confirmed by previous studies, observing unaffected NEFA levels and stimulation indices even if CLA supplementation led to a milk

fat reduction (Pappritz et al. 2011b; von Soosten et al. 2011; Renner et al. 2012a; Renner et al. 2012b)

7 Calf metabolism and development

The conceptus growth is sensitive to direct or indirect effects of maternal nutrition. Especially during the last two month of pregnancy, when up to three-quarter of weight increase of the bovine fetus occurs, maternal dietary intake plays an essential role in proper fetal development and growth rate (Funston et al. 2010). Consequently, it is of interest whether an intrauterine exposure to CLA may also exert effects on pre- and postnatal calf development or metabolism. Based on the literature and as reported in the Background Chapter 3.5 only little information is available about possible effects of maternal CLA supplementation on calves. Moreover, colostrum intake could also have important effects on newborn calves because of a consistent and dose-dependent transfer of *trans*-10,*cis*-12 CLA into milk fat during CLA supplementation period (Moore et al. 2004; Pappritz et al. 2011b). Hence, the present study also aimed to investigate the effects of maternal supplementation of CLA and various concentrate feed proportions during late pregnancy on blood metabolites of unsuckled and suckled calves to examine potential CLA impacts on calf metabolism **(Paper IV)**.

Based on the above-mentioned results from the literature (Moore et al. 2004; Pappritz et al. 2011b) and due to the fact that the proportion of *trans*-10,*cis*-12 CLA in milk fat was significantly increased at day 21 p.p. in the present study **(Paper I)**, it can be assumed that calves received colostrum enriched with *trans*-10,*cis*-12 CLA. However, a CLA effect on calves due to an intake of CLA enriched colostrum could not be observed within the period of investigation. Blood parameters did not differ within suckled calves. Perhaps, the time of colostrum intake was too short and thus the amount of ingested colostrum was too low causing an effect **(Paper IV)**. Nevertheless, it should be noted that the amount of ingested colostrum could not be recorded. Consequently, there may be a huge variation in colostrum intake between the calves, which in turn could have influenced their metabolic situation. Colostrum intake provides calf with essential nutrients and is necessary for passive immunity (Blum & Hammon 2000; Blum 2006). Consequently, measured blood metabolites of newborn calves changed markedly due to colostrum intake, whereby no maternal diet effect could be observed **(Paper IV)**. Findings may indicate that maternal CLA supplementation did not have an influence on metabolism of calves. Results are in accordance with a study of Dänicke et al. (2012), observing also no maternal diet effect on blood metabolites of unsuckled and suckled calves after feeding CLA to early pregnant cows.

In addition the development of calves was observed until an age of 50 days after birth. After calves were separated from their dams, approximately after 16 to 24 h p.p., calves were kept in small stables (87 x 175 cm) on straw for 7 d and received 6 l of pooled colostrum per day in 2 equal proportions in the morning and afternoon. For another 42 d, calves were kept in group boxes with straw bedding and were fed a commercial milk replacer (skim milk powder 38%, sweet whey powder 21%, whey powder 20%, and premix and vegetable oil 21%) using automatic self-feeders. Another blood sample was drawn from calves at day 50 of age and body weights were recorded for a total of 50 days. Birth weight of male calves was significantly higher compared to female calves, whereas birth weights remained uninfluenced by maternal nutrition during late pregnancy (Table 3). Body weights of calves at day 50 of age as well as body weight gain during the milk replacer feeding period did not differ among the groups (Table 3). Present observation also coincides with results reported by Dänicke et al. (2012).

Table 3. Effects of feeding different amounts of concentrate supplemented with conjugated linoleic acids (CLA) or control fat (CON) to late pregnant cows on performance of calves until day 50 of age; LSMeans.

	Control fat (100 g/d)		CLA (100 g/d)			*p-value*			
Parameter	**CON-20**[1]	**CON-60**[2]	**CLA-20**[1]	**CLA-60**[2]	PSEM	Diet	Sex	Diet x Sex	Birth weight
	(n=5)	*(n=6)*	*(n=5)*	*(n=5)*					
Birth weight [kg]	45.0	45.1	43.4	44.6	2.1	0.937	0.039	0.368	
BW at day 50 [kg]	80.7	77.3	73.5	77.1	3.7	0.648	0.185	0.672	
BW gain day 8-50 [kg/d]	0.696	0.614	0.573	0.660	0.07	0.682	0.848	0.966	0.594

BW = body weight; PSEM = Pooled standard error of the mean.
[1]Dams of calves in group CON-20 and CLA-20 received a low concentrate diet *antepartum*.
[2]Dams of calves in group CON-60 and CLA-60 received a high concentrate diet *antepartum*.

Moreover, it could be observed that blood metabolites of calves changed depending on measured day (Table 4), which is a normal physiological process. Blood metabolites of newborn calves vary in the course of their development depending on quality and quantity of ingested colostrum or milk replacer (Blum & Hammon 2000; Blum 2006). However, no maternal diet effect could be found (Table 4).

Based on the present results it can be concluded that maternal CLA supplementation during late pregnancy did not have an influence on calf development and metabolism. However, it should be noted that only minor CLA effects on dams of calves could be shown **(Paper I, II and III)**, which may be caused by an unexpectedly low rumen-protection of the CLA supplement (Pappritz et al. 2011a). Perhaps, the intrauterine exposure of CLA was too low causing an effect on calf metabolism or development.

Table 4. Effects of feeding different amounts of concentrate supplemented with conjugated linoleic acids (CLA) or control fat (CON) to late pregnant cows on serum blood parameters of their calves on day 1 and 50 after calving; LSMeans±SE.

	Day 1				**Day 50**						
	Control fat (100 g/d)		CLA (100 g/d)		Control fat (100 g/d)		CLA (100 g/d)		*p-value*		
Parameter	**CON-20**[1]	**CON-60**[2]	**CLA-20**[1]	**CLA-60**[2]	**CON-20**[1]	**CON-60**[2]	**CLA-20**[1]	**CLA-60**[2]	Diet	Day	Diet x Day
	(n=5)	*(n=6)*	*(n=5)*	*(n=5)*	*(n=5)*	*(n=6)*	*(n=5)*	*(n=5)*			
Prot [g/l][3]	53.7 ±6.4	50.5 ±5.9	54.1 ±6.4	56.4 ±6.4	57.3 ±3.2	59.2 ±3.0	55.3 ±3.2	64.3 ±3.2	0.626	0.157	0.862
Alb [g/l][3]	21.6 ±0.9	20.6 ±0.9	19.5 ±0.9	21.4 ±0.9	30.4 ±1.6	32.4 ±1.5	30.4 ±1.6	31.3 ±1.6	0.525	<0.001	0.724
ASAT [IU/l][3]	61.0 ±14.4	72.6 ±13.2	78.4 ±14.4	64.7 ±14.4	64.1 ±6.2	70.1 ±5.6	58.1 ±6.2	59.3 ±6.2	0.792	0.413	0.735
GGT [IU/l][3]	1084.1 ±897	1394.5 ±818	2589.1 ±897	3598.4 ±897	23.3 ±2.8	21.2 ±2.6	14.8 ±2.8	19.8 ±2.8	0.450	0.001	0.447
GLDH [IU/l][3]	7.2 ±1.6	8.4 ±1.5	8.7 ±1.6	9.7 ±1.8	20.6 ±6.2	28.2 ±5.7	6.8 ±6.2	15.0 ±6.2	0.141	0.011	0.119
Chol [mg/dl][3]	27.9 ±5.1	26.8 ±4.6	30.0 ±5.1	29.0 ±5.1	87.7 ±9.5	105.2 ±8.7	89.5 ±9.5	93.1 ±9.5	0.737	<0.001	0.479
Gluc [mg/dl][3]	94.4 ±20.9	96.5 ±19.1	101.2 ±20.9	96.3 ±20.9	89.5 ±7.4	101.6 ±6.8	89.2 ±7.4	101.7 ±7.4	0.961	0.885	0.926
NEFA [mmol/l][4]	0.51 ±0.11	0.48 ±0.10	0.50 ±0.11	0.36 ±0.11	0.19 ±0.03	0.22 ±0.03	0.26 ±0.03	0.27 ±0.03	0.886	0.001	0.569
BHB [mmol/l][4]	0.20 ±0.05	0.26 ±0.08	0.21 ±0.04	0.15 ±0.04	0.26 ±0.10	0.34 ±0.10	0.54 ±0.12	0.52 ±0.10	0.344	0.006	0.199
Bili [mg/l][3]	1.1 ±0.17	1.0 ±0.16	1.1 ±0.17	0.7 ±0.17	1.3 ±0.05	1.3 ±0.05	1.3 ±0.05	1.3 ±0.05	0.516	0.006	0.446

[1]Dams of calves in group CON-20 and CLA-20 received a low concentrate diet *antepartum*.

[2]Dams of calves in group CON-60 and CLA-60 received a high concentrate diet *antepartum*.

[3]Reference values according to Kraft & Dürr (2005):

Prot= total protein: 50-70 g/l; Alb= albumin: 30-40 g/l; ASAT= aspartate amino-transferase: < 80 IU/l; GGT= γ-glutamyl-transferase: < 50 IU/l; GLDH= glutamate dehydrogenase: < 30 IU/l; Chol= total cholesterol: >75 mg/dl; Gluc= glucose: 80-125 mg/dl; Bili= total bilirubin: < 3 mg/l.

[4]Reference value according to Dirksen et al. (2006):

BHB= β-hydroxybutyrate: <1.20 mmol/l; NEFA= non-esterified fatty acids: < 0.35 mmol/l.

CONCLUSIONS

Contrary to other studies, supplementation of 8 g/d *trans*-10,*cis*-12 CLA failed to show a consistent reduction in milk fat yield, milk fat content and milk energy concentration, even if initiated 21 days before anticipated calving. Only a slight and non-significant reduction in milk fat yield was observed in cows, receiving a CLA supplement in a low concentrate diet a.p., whereby milk yield, milk protein and milk lactose were not influenced. No reduction in milk energy output and no improvement in estimated energy balance were found, which is also reflected in unaltered blood NEFA and BHB values. Based on these results, it is not recommend starting CLA supplementation before parturition.

CLA supplementation in a high concentrate diet a.p. led to an increase of DM and NE_L intake around calving and thus caused an improvement of negative estimated energy balance during the first weeks of lactation. Milk yield, milk composition, LW and blood NEFA and BHB values remained unaffected, indicating an unaltered metabolic situation of cows. Findings cannot be completely clarified on the basis of available data. Further research is necessary to elucidate the underlying metabolic mechanism and the energy partitioning in CLA fed cows in more detail.

The CLA effect on milk fat yield was reversible as treated group, which were fed a low concentrate diet a.p., returned back to levels similar as control within one week after terminated CLA supplementation. In accordance with previous studies, no post-supplementation effects on investigated parameters were observed.

Supplementation of CLA increased the proportion of *trans*-10,*cis*-12 CLA in the milk fatty acid profile on day 21 p.p., whereas the proportion of *cis*-9,*trans*-11 CLA remained uninfluenced. Unlike to other studies, CLA treatment did not influence the proportions of short, medium and long chain fatty acids in milk fat.

CLA supplementation did not affect blood metabolites and stimulation ability of PBMC, indicating that metabolic situation, liver and lipid metabolism and immune function of periparturient cows are likely to be not influenced by CLA supplementation.

Continuous RpH and RT measurement suggest that CLA supplementation may have a minor influence on gross rumen fermentation. Accordingly, previous studies observed that rumen metabolism was little but not adversely affected by CLA treatment. However, further experiments should be conducted to clarify potential CLA effects on rumen microbes and thus rumen fermentation.

Neither maternal CLA supplementation during late pregnancy nor the intake of supposed CLA enriched colostrum had an impact on metabolism and development of calves, since blood metabolites, body weights and body weight gains remained unaffected.

SUMMARY

Dairy cows are physiologically in a negative energy balance during early lactation as their feed intake potential is limited and thus the amount of consumed energy does not meet the energy requirements for maintenance and for milk production. Hence, early lactating dairy cows are dependent on the use of their body fat reserves to provide additional energy, which in turn may lead to enormous metabolic disorders, health and fertility problems. Conjugated linoleic acids (CLA), especially the *trans*-10,*cis*-12 CLA isomer, are known to have milk fat reducing properties. Consequently, CLA supplements are frequently added in dairy cow diets to lower the energy requirements for milk production through a reduction in milk fat synthesis. Hence, the use of CLA supplements is an option to minimize the gap of energy intake and energy requirement and thus metabolic disturbances of early lactating cows via a lesser extent of body fat mobilization. However, available data regarding CLA effects on energy and lipid metabolism of early lactating cows are inconsistent and the impact of a CLA addition before calving and after terminated supplementation has been rarely investigated. Moreover, there is a lack of information about the impact of CLA on the bovine immune function and on rumen metabolism, whereby also less is known about physiological effects of maternal CLA supplementation during late pregnancy on calves. As most studies were carried out exclusively during early lactation, the relationship between dietary concentrate feed proportion and CLA supplementation during late pregnancy has not yet been examined in detail. However, this could be a crucial point since influencing the metabolic situation of cows immediately before calving is an opportunity to investigate and better understand the lipid metabolism modifying properties of supplemented CLA and hence its impact on energy metabolism. It was hypothesised that a high-energy feeding during late pregnancy may stimulate *postpartum* (p.p.) lipolysis and thereby results in more pronounced CLA effects in dairy cows. Therefore, the present study aimed to investigate the influence of supplemented CLA on energy and lipid metabolism, on immune function and on rumen metabolism of periparturient dairy cows fed different concentrate feed proportions during late pregnancy and on calf development and metabolism.

For this purpose, 64 pregnant German Holstein cows had *ad libitum* access to partial mixed rations, based on concentrate and roughage (60% corn silage and 40% grass silage on dry matter [DM] -basis), three weeks prior to calving until day 60 p.p.. Before calving, cows received a control fat (CON) or a CLA supplement either in a low-concentrate (20%, group CON-20 and CLA-20) or high-concentrate diet (60%, group CON-60 and CLA-60).

Compared to a adapted feeding, the high-concentrate diet was fed to induce a ketogenic metabolic situation of cows p.p.. A commercial rumen-protected CLA preparation, containing 10% *trans*-10,*cis*-12 CLA and 10% *cis*-9,*trans*-11 CLA, and a rumen-protected CON preparation, containing stearic acid instead of conjugated linoleic acids, were used as CLA and CON supplements, respectively. After parturition, the concentrate proportion was adjusted to 50% while fat supplementation was continued. After day 32 p.p., half of the animals of CLA-groups changed to CON supplementation (CLA-20-CON and CLA-60-CON), while in groups CLA-20 and CLA-60 the CLA supplementation continued. During the trial, performance data were regularly recorded. Blood chemistry samples were taken over the entire experimental period, whereby additional blood samples for isolation of peripheral blood mononuclear cells (PBMC) were collected on day -21, 7, 28 and 56 relative to calving.

Based on the calculated CLA concentrations in the CLA-concentrate, cows received approximately 8 g *trans*-10,*cis*-12 CLA per day. However, a ketogenic metabolic situation of cows p.p. was not achieved and respective impacts of CLA could not be examined, as blood non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) did not differ between the respective groups. The evaluation of performance data revealed that CLA supplementation in a high concentrate diet *antepartum* (a.p.) lead to increase dry matter intake (DMI) before and shortly after calving. Consequently, the negative estimated energy balance was alleviated in group CLA-60, whereas milk yield and composition, live weight and blood NEFA and BHB values remained unaffected, indicating an unaltered metabolic situation. Moreover, even if initiated three weeks before parturition, a reduction in milk fat synthesis was not achieved by CLA supplementation and milk yield, milk protein and milk lactose remained also unaffected. Only a slight (non-significant) milk fat reduction was recorded for group CLA-20 during early lactation, synthesizing approximately 8.6-10.6% and 8.7-12.6 % less g milk fat per day than the other groups during the first 31 and 32 to 60 days in milk. No improvement in milk energy output, estimated energy balance and blood NEFA and BHB concentration were found. Nevertheless, CLA supplementation increased the proportion of *trans*-10,*cis*-12 CLA in milk fat from an average of 0.002% to 0.02% of total fatty acid methyl esters. With exception of a reversible CLA effect on milk fat in group CLA-20, no post-treatment effects occurred. Furthermore, CLA supplementation did neither affect immune function nor metabolism of periparturient cows, since the stimulation-ability of PBMC and investigated blood metabolites remained unaffected. However, some time-dependent alterations were observed, which may partly attributed to metabolic challenges around calving through a limited DMI potential.

For evaluation of CLA effects on rumen metabolism, a part of the animals of the four experimental groups were equipped with rumen probes, which enabled to study the impact of dietary concentrate feed proportion and CLA supplementation on rumen pH (RpH) and rumen temperature (RT) around parturition. High proportions of dietary concentrate decreased RpH, whereas RT remained unaffected. Groups fed on high concentrate spent more than four hours per day below RpH 5.6, indicating a rising risk of sub-acute rumen acidosis. Moreover, CLA might have influenced rumen metabolism, since minor changes on RpH and RT were observed. Despite the same DM, non-fibre carbohydrate (NFC) and crude fibre (CF) intakes between the respective counterparts, a reduced time below RpH 5.8 and a lower RT were observed in group CLA-20 during late pregnancy and in group CLA-60 after calving, respectively.

In total 5 to 6 calves were used out of 16 potential available calves per group for investigation whether an intrauterine exposure to CLA may also exert effects on pre- and postnatal calf development and metabolism. Blood samples were obtained from unsuckled calves immediately after parturition and from the same calves after staying 16 to 24 h with their respective dam. In this time, calves had *ad libitum* access to colostrum. Afterwards, calves were separated from dams and spent 7 days in small stables, receiving 6 l pooled colostrum per day. For another 42 days, calves were kept in group boxes and were fed a commercial milk replacer. The third blood sample was taken from calves at day 50 of age. Body weights were observed for a total of 50 days. Blood metabolites changed in the course of calf development, whereby no maternal diet effect and no effect by the intake of CLA enriched colostrum could be observed. Body weights and the body weight gain during the milk replacer period remained unaffected.

It can be concluded under the conditions of the present study that CLA supplementation are ineffective in reducing the milk energy output during early lactation, even if CLA supplementation was initiated three weeks before calving. However, CLA supplementation in a high concentrate diet a.p. could partially alleviate a negative energy balance during early lactation through an increased DMI. But, supplemented CLA seems to have no influence on the metabolic situation, liver and lipid metabolism and immune function of periparturient cows. Further investigations on this topic are necessary with regard to the underlying metabolic processes and the energy partitioning in CLA fed cows during early lactation. A minor CLA effect on rumen metabolism cannot be ruled out and neither maternal CLA supplementation during late pregnancy nor the intake of CLA enriched colostrum had an influence on calf metabolism and development.

ZUSAMMENFASSUNG

Milchkühe sind physiologisch bedingt während der Frühlaktation in einer negativen Energiebilanz. Ihr Futteraufnahmepotential ist begrenzt und entspricht somit nicht dem Energiebedarf für Erhaltung und Milchproduktion. Daher nutzen frühlaktierende Kühe ihre Körperfettreserven, um zusätzlich Energie zu gewinnen, was wiederum zu enormen Stoffwechselstörungen, Gesundheits- und Fruchtbarkeitproblemen führen kann. Konjugierte Linolsäuren (CLA), insbesondere das *trans*-10,*cis*-12 CLA Isomer, haben Milchfett reduzierende Eigenschaften. Folglich werden CLA-Supplemente häufig in Milchkuhrationen hinzugefügt, um den Energiebedarf für die Milchproduktion durch eine Reduzierung der Milchfettsynthese zu senken. Somit stellen CLA-Supplemente eine Möglichkeit dar, das Energiedefizit und daher auch Stoffwechselstörungen von frühlaktierenden Kühen zu minimieren. Allerdings sind bisherige Daten bezüglich den Auswirkungen von CLA auf den Energie- und Fettstoffwechsel von frühlaktierenden Kühen widersprüchlich und der Einfluss einer CLA-Zugabe vor der Abkalbung und nach beendeter Supplementierung ist nur selten untersucht worden. Des Weiteren ist wenig über den Einfluss von CLA auf das bovine Immunsystem und den Pansenstoffwechsel als auch über die physiologischen Auswirkungen einer mütterlichen CLA-Supplementierung während der Spätträchtigkeit auf die Kälber bekannt. Da die meisten Studien ausschließlich während der Frühlaktation durchgeführt worden, ist die Beziehung zwischen dem Konzentratanteil in der Ration *antepartum* (a.p.) und einer CLA-Supplementierung nicht näher untersucht. Jedoch könnte dies von Bedeutung sein, da die Beeinflussung der Stoffwechselsituation von Kühen unmittelbar vor der Abkalbung eine Möglichkeit darstellt, die Fettstoffwechsel-modifizierenden Eigenschaften von CLA und damit deren Wirkung auf den Energiestoffwechsel besser zu untersuchen und verstehen zu können. Es wird angenommen, dass eine energiereiche Fütterung während der Spätträchtigkeit eine postpartale Lipolyse stimulieren und somit zu deutlich ausgeprägteren CLA Effekten führen kann. Deshalb hatte die vorliegende Studie zum Ziel den Einfluss von CLA auf den Energie- und Fettstoffwechsel, auf das Immunsystem und auf den Pansenstoffwechsel von peripartalen Kühen, welche unterschiedliche Konzentratanteile in der Ration während der Spätträchtigkeit erhielten, sowie auf die Entwicklung und den Stoffwechsel von Kälbern zu untersuchen.

Hierzu hatten 64 trächtige Milchkühe, drei Wochen vor bis 60 Tage nach der Abkalbung, uneingeschränkten Zugang zu partiellen Mischrationen, welche auf Konzentrat und Grundfutter (60% Maissilage und 40% Grassilage auf Trockenmassebasis) basierten. Vor der

Abkalbung erhielten die Kühe eine Kontrollfett- (CON) oder eine CLA-Ergänzung entweder in einer niedrigen Konzentratration (20%, Gruppe CON-20 und CLA-20) oder einer hohen Konzentratration (60%, Gruppe CON-60 und CLA-60). Im Vergleich zu einer angepassten Fütterung sollte der hohe Konzentratanteil a.p. eine präpartale Prädisposition für eine ketogene Stoffwechsellage *postpartum* (p.p.) induzieren. Ein handelsübliches, pansengeschütztes CLA-Präparat wurde eingesetzt, welches 10% *trans*-10,*cis*-12 CLA und 10 % *cis*-9,*trans*-11 CLA enthielt. Das pansenstabile CON-Präparat beinhaltete anstatt CLA Stearinsäure. Nach der Abkalbung wurde der Konzentratanteil auf 50% angepasst, wobei die Fettergänzung gleich blieb. Nach Tag 32 p.p. wechselten die Hälfte der Tiere der CLA-Gruppen zur CON Supplementierung (CLA-20-CON und CLA-60-CON), wobei in den Gruppen CLA-20 und CLA-60 die CLA-Ergänzung fortgeführt wurde. Während des Versuches wurden Leistungsdaten erhoben und Blutproben für Analysen gewonnen, wobei an den Tagen -21, 7, 28 und 56 relativ zur Abkalbung zusätzlich Blutproben für die Isolation von peripheren mononuklearen Blutzellen (PBMC) gezogen worden. Die Kühe erhielten circa 8 g *trans*-10,*cis*-12 CLA pro Tag. Eine postpartale ketogene Stoffwechsellage wurde nicht erzielt und somit konnten entsprechende CLA Wirkungen nicht untersucht werden. Eine CLA-Zulage in einer hoch konzentrierten Ration a.p. führte zu einer Steigerung der Trockenmasseaufnahme (DMI) um die Abkalbung. Folglich war eine Verbesserung der negativen Energiebilanz zu verzeichnen, wobei die Milchmenge, Milchzusammensetzung, Lebendmasse und NEFA und BHB Blutwerte unbeeinflusst blieben, was auf eine unveränderte Stoffwechselsituation hindeutet. Obwohl CLA-Supplementierung schon drei Wochen a.p. begann, konnte keine Milchfettreduzierung erzielt werden. Nur eine leichte Milchfettreduktion wurde in Gruppe CLA-20 während der Frühlaktation verzeichnet, wobei keine Verbesserung in der Milchenergieausscheidung, kalkulierten Energiebilanz und NEFA und BHB Blutwerten festgestellt werden konnte. Die CLA-Zulage bewirkte einen Anstieg des *trans*-10,*cis*-12 CLA Anteils in der Milch von 0.002% auf 0.02% der Fettsäuremethylester. Außer einem umkehrbaren CLA Effekt auf das Milchfett von Gruppe CLA-20 traten nach Absetzen des CLA-Supplements keine Unterschiede bei den untersuchten Parametern auf. Die CLA-Zulage hat weder das Immunsystem noch den Stoffwechsel von peripartalen Kühen beeinflusst, da die Stimulierbarkeit von PBMC als auch die untersuchten Blutparameter unverändert blieben. Allerdings waren einige zeitabhängige Veränderungen zu verzeichnen, welche teilweise den metabolischen Herausforderungen um die Abkalbung aufgrund einer reduzierten Futteraufnahme zugeschrieben werden können. Zur Beurteilung der CLA Effekte auf den Pansenstoffwechsel wurde ein Teil der Tiere der vier Gruppen mit Pansensonden

ausgestattet, welche die Untersuchung des Einflusses von Kraftfutteranteil in der Ration und CLA-Zulage auf den Pansen-pH (RpH) und die Pansentemperatur (RT) ermöglichten. Hohe Konzentratanteile in der Ration reduzierten den RpH, wobei die RT unverändert blieb. Der RpH war bei der Verfütterung von Rationen mit einem hohen Konzentratanteil täglich für über vier Stunden unter einem RpH von 5.6, was auf ein erhöhtes Risiko einer subakuten Pansenazidose hinweist. Des Weiteren wurden geringfügige Veränderung bei RpH und RT durch die CLA-Zulage beobachtet. Im Vergleich zu den entsprechenden CON-Gruppen und trotz gleicher DMI, Nichtfaser-Kohlenhydrat- und Rohfaseraufnahme, war die Zeit unter einem RpH von 5.8 in Gruppe CLA-20 während der Spättrachtigkeit reduziert und die RT bei Gruppe CLA-60 nach der Abkalbung geringer. Für die Untersuchung, ob eine intrauterine Exposition von CLA Auswirkungen auf die pre-und postnatale Entwicklung und den Stoffwechsel von Kälbern hat, wurden 5 bis 6 Kälber aus insgesamt 16 möglichen Kälbern je Gruppe verwendet. Blutproben wurden aus ungesäugten Kälbern unmittelbar nach der Geburt gezogen. Eine Zweite wurde von denselben Kälbern nach einem 16- bis 24-stündigem Aufenthalt mit ihrer entsprechenden Mutter gewonnen. In dieser Zeit hatten die Kälber uneingeschränkten Zugang zu Kolostrum. Danach wurden diese von den Muttertieren getrennt und es folgte nach 7 Tagen eine 42-tägige Milchaustauscherperiode. Eine weitere Blutprobenentnahme erfolgte bei den Kälbern im Alter von 50 Tagen. Die Köpergewichte wurden über die gesamten 50 Tage erfasst. Die Blutparameter änderten sich im Laufe der Entwicklung der Kälber, wobei kein mütterlicher Fütterungseffekt oder ein Effekt durch die Aufnahme von CLA angereichertem Kolostrum zu sehen war. Die Körpergewichte und die tägliche Zunahme während der Milchaustauscherperiode waren unbeeinflusst.

Zusammengefasst kann aus den vorliegenden Untersuchungen geschlossen werden, dass eine CLA-Zulage, trotz einer beginnenden Supplementierung vor dem Abkalben, unwirksam in der Reduzierung der Milchenergieausscheidung während der Frühlaktation ist. Allerdings führt eine CLA-Zulage in einer hohen Konzentratration a.p. durch eine erhöhte DMI teilweise zu einer Verbesserung der negativen Energiebilanz in der Frühlaktation. Jedoch scheint CLA keinen Einfluss auf Stoffwechsellage, den Leber- und Fettstoffwechsel als auch die Immunfunktion von peripartalen Kühen zu haben. Weitere Untersuchungen sind notwendig, um die zugrundeliegenden metabolischen Prozesse und die Energieverteilung in CLA gefütterten Kühen zu verdeutlichen. Ein geringfügiger CLA-Einfluss auf den Pansenstoffwechsel kann nicht ausgeschlossen werden und scheinbar hat weder die mütterliche CLA-Zulage, noch die Aufnahme von CLA-angereicherter Milch einen Einfluss auf die Entwicklung und den Stoffwechsel von Kälbern.

REFERENCES (cited in Introduction, Background and General Discussion)

ADR, 2013. (Arbeitsgemeinschaft Deutscher Rinderzüchter e.V.). Jahresbericht. Available: http://www.adr-web.de/list_adr_publikationen.html.

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

AlZahal O, Kebreab E, France J, Froetschel M, McBride BW. 2008. Ruminal temperature may aid in the detection of subacute ruminal acidosis. Journal of Dairy Science. 91:202-207.

Ballard FJ, Hanson RW, Kronfeld DS. 1968. Factors controlling concentration of mitochondrial oxaloacetate in liver during spontaneous bovine ketosis. Biochemical and Biophysical Research Communications. 30:100-104.

Bauman DE, Baumgard LH, Corl BA, Griinari JM. 1999. Biosynthesis of conjugated linoleic acid in ruminats. Proceedings of the American Society of Animal Science. Available: http://www.animal-science.org/content/77/E-Suppl/1.32.full.pdf.

Bauman DE, Currie WB. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. Journal of Dairy Science. 63:1514-1529.

Bauman DE, Griinari JM. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science. 70:15-29.

Bauman DE, Griinari JM. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition. 23:203-227.

Bauman DE, Perfield JW, 2nd, Harvatine KJ, Baumgard LH. 2008. Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. Journal of Nutrition. 138:403-409.

Baumgard LH, Corl BA, Dwyer DA, Bauman DE. 2002a. Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. Journal of Animal Science. 80:1285-1293.

Baumgard LH, Corl BA, Dwyer DA, Saebo A, Bauman DE. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. American Journal of Physiology, Regulatory, Integrative and comparative Physiology. 278:R179-R184.

Baumgard LH, Matitashvili E, Corl BA, Dwyer DA, Bauman DE. 2002b. trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. Journal of Dairy Science. 85:2155-2163.

Beam SW, Butler WR. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. Journal of Reproduction and Fertility. 54:411-424.

Bell AW. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science. 73:2804-2819.

Belury MA. 2002. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. Annual Review of Nutrition. 22:505-531.

Bernal-Santos G, Perfield JW, 2nd, Barbano DM, Bauman DE, Overton TR. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. Journal of Dairy Science. 86:3218-3228.

Blum JW. 2006. Nutritional physiology of neonatal calves. Journal of Animal Physiology and Animal Nutrition. 90:1-11.

Blum JW, Hammon H. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 66:151-159.

Bobe G, Young JW, Beitz DC. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. Journal of Dairy Science. 87:3105-3124.

Bostedt H. 1974. Enzyme activity in the blood serum of cows during the period before and after parturition. Berliner und Muenchener Tieraerztliche Wochenschrift. 87:365-371.

Butler WR, Everett RW, Coppock CE. 1981. The relationships between energy-balance, milk production and ovulation in postpartum holstein cows. Journal of Animal Science. 53:742-748.

Calder PC. 2008. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukotrienes and Essential Fatty Acids. 79:101-108.

Castaneda-Gutierrez E, Overton TR, Butler WR, Bauman DE. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. Journal of Dairy Science. 88:1078-1089.

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

Chouinard PY, Corneau L, Saebo A, Bauman DE. 1999b. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. Journal of Dairy Science. 82:2737-2745.

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

Dänicke S, Kowalczyk J, Renner L, Pappritz J, Meyer U, Kramer R, Weber EM, Döll S, Rehage J, Jahreis G. 2012. Effects of conjugated linoleic acids fed to dairy cows during early gestation on hematological, immunological, and metabolic characteristics of cows and their calves. Journal of Dairy Science. 95:3938-3953.

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

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

Delany JP, Blohm F, Truett AA, Scimeca JA, West DB. 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 276:R1172-R1179.

Dirksen G, Gründer HD, Stöber MH, 2006. Innere Medizin und Chirugie des Rindes [Internal medicine and surgery of boovine animals]. Parey in MVS Medizinverlage Stuttgart GmbH & Co. KG Stuttgart, Germany.

Drackley JK. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: the final frontier? Journal of Dairy Science. 82:2259-2273.

Drackley JK, Overton TR, Douglas GN. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. Journal of Dairy Science. 84:E100-E112.

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

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

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

Franklin ST, Young JW, Nonnecke BJ. 1991. Effects of Ketones, Acetate, Butyrate, and Glucose on Bovine Lymphocyte Proliferation. Journal of Dairy Science. 74:2507-2514.

Friggens NC, Emmans GC, Kyriazakis I, Oldham JD, Lewis M. 1998. Feed intake relative to stage of lactation for dairy cows consuming total mixed diets with a high or low ratio of concentrate to forage. Journal of Dairy Science. 81:2228-2239.

Funston RN, Larson DM, Vonnahme KA. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. Journal of Animal Science. 88:E205-E215.

GfE. 1991. (Society of Nutrition Physiolgy). Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern (Guidelines for determining the digestibility of crude ruminants). Journal of Animal Physiology and Animal Nutrition. 65:229-234.

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

Gillis MH, Duckett SK, Sackmanni JR, Keisler DH. 2003. Effect of rumen-protected conjugated linoleic acid (CLA) or linoleic acid on leptin and CLA content of bovine adipose depots. Journal of Animal Science. 81:12-12.

Goff JP. 2006. Major advances in our understanding of nutritional influences on bovine health. Journal of Dairy Science. 89:1292-1301.

Goff JP, Horst RL. 1997. Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science. 80:1260-1268.

Griinari JM, Bauman DE, 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. Volume 1. Pages 180-200. AOCS Press, Champaign, Illinois, USA.

Grummer RR. 1993. Etiology of Lipid-Related Metabolic Disorders in Periparturient Dairy-Cows. Journal of Dairy Science. 76:3882-3896.

Grummer RR. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Journal of Animal Science. 73:2820-2833.

Hammon HM, Hotger K, Gors S, Becker M, Weber C, Troscher A, Metges CC, 2011. Glucose metabolism in dairy cows supplemented with rumen-protected conjugated linoleic acid (CLA) - first results. Proceedings of the Society of Nutrition Physiology. 20:92. (Abstract).

Harfoot CG, Hazlewood GP, 1988. Lipid metabolism in the rumen. In: P. N. Hobson (Ed.). The rumen microbial ecosystem. Pages: 285-322. Elsevier Applied Science Publishers. London. UK.

Harvatine KJ, Bauman DE. 2011. Characterization of the acute lactational response to trans-10, cis-12 conjugated linoleic acid. Journal of Dairy Science. 94:6047-6056.

Harvatine KJ, Perfield JW, Bauman DE. 2009. Expression of Enzymes and Key Regulators of Lipid Synthesis Is Upregulated in Adipose Tissue during CLA-Induced Milk Fat Depression in Dairy Cows. Journal of Nutrition. 139:849-854.

Hayirli A, Grummer RR. 2004. Factors affecting dry matter intake prepartum in relationship to etiology of peripartum lipid-related metabolic disorders: A review. Canadian Journal of Animal Science. 84:337-347.

Holter JB, Slotnick MJ, Hayes HH, Bozak CK, Urban WE, Mcgilliard ML. 1990. Effect of Prepartum Dietary Energy on Condition Score, Postpartum Energy, Nitrogen Partitions, and Lactation Production Responses. Journal of Dairy Science. 73:3502-3511.

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

Hussen J, Dänicke S, Schuberth HJ. 2011. The effect of a long term dietary supplementation with conjugated linoleic acid (CLA) on the composition of bovine peripheral blood mononuclear cells (PBMC) and the concentration of IgG isotypes in blood and milk. Proceedings of the Society of Nutrition Physiology. 20:85-85.

Hutchinson I, de Veth MJ, Stanton C, Dewhurst RJ, Lonergan P, Evans AC, Butler ST. 2011. Effects of lipid-encapsulated conjugated linoleic acid supplementation on milk production, bioenergetic status and indicators of reproductive performance in lactating dairy cows. Journal of Dairy Research. 78:308-317.

Ingvartsen KL, Andersen JB. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. Journal of Dairy Science. 83:1573-1597.

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

Kadegowda AKG, Piperova LS, Erdman RA. 2008. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. Journal of Dairy Science. 91:749-759.

Kafil H, Amjad ul I, Gupta SK. 2011. Management of sub-acute ruminal acidosis in dairy cattle for improved production: a review. Journal of Advanced Veterinary Research. 1:80-93.

Kay JK, Roche JR, Moore CE, Baumgard LH. 2006. Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture. Journal of Dairy Research. 73:367-377.

Kelley DS, Simon VA, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL. 2001. Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells, but did not alter their function. Lipids. 36:669-674.

Kepler CR, Tove SB. 1967. Biohydrogenation of unsaturated fatty acids. 3. Purification and properties of linoleate delta12-cis,delta11-trans-isomerase from butyrivibrio fibrisolvens. Journal of Biological Chemistry. 242:5686-5692.

Khanal RC, Dhiman TR. 2004. Biosynthesis of conjugated linoleic acid (CLA): a review. Pakistan Journal of Nutrition. 3:72-81.

Kraft J, Lebzien P, Flachowsky G, Möckel P, Jahreis G. 2000. Duodenal infusion of conjugated linoleic acid mixture influences milk fat synthesis and milk CLA content in dairy cows. British Society of Animal Science. 25:143-147.

Kraft W, Dürr UM, 2005. Klinische Labordiagnostik in der Tiermedizin [Clinical laboratory diagnostics in veterinary medicine]. Schattauer Verlag, Stuttgart, Germany.

Kuhla B, Albrecht D, Kuhla S, Metges CC. 2009. Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid, and glycogen metabolism. Physiological Genomics. 37:88-98.

Lacetera N, Scalia D, Bernabucci U, Ronchi B, Pirazzi D, Nardone A. 2005. Lymphocyte functions in overconditioned cows around parturition. Journal of Dairy Science. 88:2010-2016.

Lacetera N, Scalia D, Franci O, Bernabucci U, Ronchi B, Nardone A. 2004. Short communication: Effects of nonesterified fatty acids on lymphocyte function in dairy heifers. Journal of Dairy Science. 87:1012-1014.

Lehninger AL. 1946. A quantitative study of the products of fatty acid oxidation in liver suspensions. Journal of Biological Chemistry. 164:291-306.

Leroy J, Vanholder T, Mateusen B, Christophe A, Opsomer G, de Kruif A, Genicot G, Van Soom A. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction. 130:485-495.

Liermann T, 2008. Einfluss einer Zulage von pansengeschützter konjugierter Linolsäure(CLA) in Kombination mit Propylenglykol oder pansengeschütztem Fett auf Leistungsmerkmale, Stoffwechselparameter und den Energiestatus frischlaktierender Milchkühe [Effects of feeding rumenprotected conjugated linoleic acids (CLA) alone or in combination with propylene glycol or rumenprotected fat on performance and metabolic parameters and energy status of early lactation dairy cows]. Dissertation, Technische Universität München, München, Germany, 188 pages.

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

Loiselle MC, Ster C, Talbot BG, Zhao X, Wagner GF, Boisclair YR, Lacasse P. 2009. Impact of postpartum milking frequency on the immune system and the blood metabolite concentration of dairy cows. Journal of Dairy Science. 92:1900-1912.

Loor JJ, Everts RE, Bionaz M, Dann HM, Morin DE, Oliveira R, Rodriguez-Zas SL, Drackley JK, Lewin HA. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiological Genomics. 32:105-116.

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

Loor JJ, Herbein JH. 2001. Alterations in blood plasma and milk fatty acid profiles of lactating Holstein cows in response to ruminal infusion of a conjugated linoleic acid mixture. Animal Research. 50:463-476.

Loor JJ, Lin XB, Herbein JH. 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. British Journal of Nutrition. 90:1039-1048.

Maekawa M, Beauchemin KA, Christensen DA. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. Journal of Dairy Science. 85:1165-1175.

Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Vankampen CL, Wagter L, Wilkie BN. 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. Journal of Dairy Science. 81:585-595.

McGuire MA, Bauman DE, 2002. Encyclopedia of dairy sciences. Milk biosynthesis and secretion. Elsevier Science, p. 1828–1834, London, GB.

McNamara S, Murphy JJ, Rath M, O'Mara FP. 2003. Effects of different transition diets on energy balance, blood metabolites and reproductive performance in dairy cows. Livestock Production Science. 84:195-206.

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

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

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

Müller A, Keller U, Seliger G, Barthel C, Steinhart H, Eder K. 2007. Concentrations of conjugated linoleic acids in neonatal blood in relationship to those in maternal blood. Prostaglandins Leukotrienes and Essential Fatty Acids. 76:213-219.

Nonnecke BJ, Kimura K, Goff JP, Kehrli ME. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. Journal of Dairy Science. 86:2359-2368.

NRC, 1988. (National Research Council). Subcommittee on Dairy Cattle Nutrition. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, D.C.

Odens LJ, Burgos R, Innocenti M, VanBaale MJ, Baumgard LH. 2007. Effects of varying doses of supplemental conjugated linoleic acid on production and energetic variables during the transition period. Journal of Dairy Science. 90:293-305.

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

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

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

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids. 32:853-858.

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

Pearce J. 1983. Fatty-Acid Synthesis in Liver and Adipose-Tissue. Proceedings of the Nutrition Society. 42:263-271.

Perfield JW, 2nd, Lock AL, Griinari JM, Saebo A, Delmonte P, Dwyer DA, Bauman DE. 2007. Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. Journal of Dairy Science. 90:2211-2218.

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

Peterson DG, Matitashvili EA, Bauman DE. 2003. The inhibitory effect of t10, c12 CLA on lipid synthesis in bovine mammary epithelial cells involves reduced proteolytic activation of the transcription factor SREBP-1. FASEB Journal. 17:Abstract No. 681.

Renner L, Pappritz J, Kramer R, Kersten S, Jahreis G, Dänicke S. 2012a. Fatty acid profile and proliferation of bovine blood mononuclear cells after conjugated linoleic acid supplementation. Lipids in Health and Disease. 11:1-7.

Renner L, von Soosten D, Sipka A, Döll S, Beineke A, Schuberth HJ, Dänicke S. 2012b. Effect of conjugated linoleic acid on proliferation and cytokine expression of bovine peripheral blood mononuclear cells and splenocytes ex vivo. Archives of Animal Nutrition. 66:73-85.

Ringseis R, Saal D, Müller A, Steinhart H, Eder K. 2004. Dietary conjugated linoleic acids lower the triacylglycerol concentration in the milk of lactating rats and impair the growth and increase the mortality of their suckling pups. Journal of Nutrition. 134:3327-3334.

Roberts CJ, Reid IM, Rowlands GJ, Patterson A. 1981. A fat mobilisation syndrome in dairy cows in early lactation. Veterinary Record. 108:7-9.

Rukkwamsuk T, Wensing T, Geelen MJH. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. Journal of Dairy Science. 81:2904-2911.

Saebo A, Saebo PC, Griinari JM, Shingfield KJ. 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.

Saremi B, Sauerwein H, von Soosten D, Dänicke S, Mielenz M. 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. Journal of Dairy Science. 94 (E-Suppl.):343. (Abstract).

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

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

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

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

Smith TR, Hippen AR, Beitz DC, Young JW. 1997. Metabolic characteristics of induced ketosis in normal and obese dairy cows. Journal of Dairy Science. 80:1569-1581.

Tanaka K. 2005. Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. Animal Science Journal. 76:291-303.

Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC, Yaqoob P. 2004. Effects of cis-9,trans-11 and trans-1 0,cis-12 conjugated linoleic acid on immune cell function in healthy humans. American Journal of Clinical Nutrition. 80:1626-1633.

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

Tyrrell HF, Reid JT. 1965. Prediction of Energy Value of Cows Milk. Journal of Dairy Science. 48:1215-1223.

Vangroenweghe F, Lamote I, Burvenich C. 2005. Physiology of the periparturient period and its relation to severity of clinical mastitis. Domestic Animal Endocrinology. 29:283-293.

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

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

Wang YW, Jones PJH. 2004. Conjugated linoleic acid and obesity control: efficacy and mechanisms. International Journal of Obesity. 28:941-955.

West DB, Blohm FY, Truettt AA, DeLany JP. 2000. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. Journal of Nutrition. 130:2471-2477.

West DB, Delany JP, Camet PM, Blohm F, Truett AA, Scimeca J. 1998. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 275:R667-R672.

Wieland O, Weiss L, Egerneufeldt I. 1964. Enzymatic regulation of liver acetyl-CoA metabolism in relation to ketogenesis. Advances in Enzyme Regulation. 2:85-99.

Wiltrout DW, Satter LD. 1972. Contribution of Propionate to Glucose Synthesis in the Lactating and Nonlactating Cow. Journal of Dairy Science. 55:307-317.

Yamasaki M, Ikeda A, Oji M, Tanaka Y, Hirao A, Kasai M, Iwata T, Tachibana H, Yamada K. 2003. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague Dawley rats fed various fat-level diets. Nutrition. 19:30-35.

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Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

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