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(Direktor: Prof. Dr. Klaus Eder)

„Untersuchungen zu Wirkungen von konjugierten Linolsäuren (CLA) auf Fleischqualitätsparameter und den hepatischen Lipidstoffwechsel beim Rind und zu Veränderungen im hepatischen Lipidstoffwechsel der Milchkuh beim Übergang von der Trächtigkeit in die Laktation“

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vorgelegt von

Diplom-Agraringenieurin

Gloria Schlegel

Gutachter: Prof. Dr. K. Eder

Prof. Dr. G. Erhardt

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Abkürzungsverzeichnis

a.p.	ante partum
ACACA	Acetyl-CoA-Carboxylase
ACLY	ATP-Citrat-Lyase
ALDH9A1	Gen, das für die 4-N-Trimethylaminobutyraldehyd-Dehydrogenase codiert
Apo	Apolipoprotein
ApoB100	Apolipoprotein B100
BB	γ-Butyrobetain
BBD	γ-Butyrobetain-Dioxygenase
BBOX1	Gen, das für die γ-Butyrobetain-Dioxygenase codiert
BHBA	*β-hydroxy butyric acid*
bHLH-Zip	basic Helix-Loop-Helix-Leucin-Zipper
bzw.	beziehungsweise
CACT	Carnitin-Acylcarnitin-Translokase
CE	Cholesterolester
CLA	*conjugated linoleic acid(s)*
CoA	Coenzym-A
CPT	Carnitin-Palmitoyl-Transferase
d	Tag
ER	Endoplasmatisches Retikulum
FABP	*fatty acid binding protein*
FASN	*fatty acid synthase*

FDPS	Farnesyl-Diphosphat-Synthase
FGF21	*fibroblast growth factor 21*
g	Gramm
GPAT	Glycerol-3-Phosphat-Acyltransferase
HMGCR	3-Hydroxymethylglutaryl-CoA-Reduktase
HMGCS	3-Hydroxymethylglutaryl-CoA-Synthase
HTMLA	3-Hydroxy-*N*-TML-Aldolase
Insig	*Insulin induced gene*
kg	Kilogramm
LDL	*low density lipoprotein(s)*
LDLR	LDL Rezeptor
LM	*Musculus longissimus dorsi*
LPL	Lipoproteinlipase
LXRα	Liver-X-Rezeptor α
mRNA	*messenger ribonucleic acid*
MTP	Mikrosomales Triglycerid-Transferprotein
MJ	Megajoule
NEFA	*non-esterified fatty acids*
NEL	Netto-Energie Laktation
OCTN	*novel organic cation transporter*
p.p.	post partum
PPAR	Peroxisomenproliferator-aktivierter Rezeptor
PPARα	Peroxisomenproliferator-aktivierter Rezeptor α

PPARA	Gen, das für den Peroxisomenproliferator-aktivierten Rezeptor α codiert
PPRE	*peroxisome proliferator respone element*
RXR	Retinoid-X-Rezeptor
Scap	Sterol-aktivierendes Protein
SCD	Stearoyl-CoA-Desaturase
SLC	*solute carrier family*
SLC22A5	*solute carrier family 22 member 5*; Gen, das für OCTN2 codiert
SRE	*sterol regulatory element*
SREBP	s*terol regulatory element binding protein*
SREBF1	Gen, das für das *sterol regulatory element binding protein-1* codiert
TAG	Triacylglycerid(e)
TMABA-DH	4-*N*-Trimethylaminobutyraldehyd-Dehydrogenase
TML	N^6-Trimethyllysin
TMLD	Trimethyllysin-Dioxygenase
TMLHE	Gen, das für die Trimethyllysin-Dioxygenase codiert
u.a.	unter anderem
v.a.	vor allem
VLDL	*very low density lipoprotein(s)*
VLDLR	VLDL Rezeptor
z. B.	zum Beispiel

1. Einleitung

Die sogenannte Transitphase der Milchkühe, die den Zeitraum von drei Wochen vor der Abkalbung bis drei Wochen nach der Abkalbung umfasst, stellt für den Organismus eine hohe Belastung dar. Die Umstellung von der Trächtigkeit auf die Laktation macht die Adaption des Stoffwechsels an die neuen Anforderungen, insbesondere den höheren Energiebedarf, notwendig. Diese Adaption geht mit einer Vielzahl von Veränderungen und Regulationsvorgängen im Stoffwechsel der Milchkuh einher. Der Rückgang der Futteraufnahme in der Transitphase, der oft schon in der Trächtigkeit beginnt, erschwert die adäquate energetische Versorgung der Milchkuh über die Nahrungsaufnahme. In der Folge kommt es zur Mobilisierung von Depotfett, einer im Energiemangelzustand im Stoffwechsel der Milchkuh physiologischerweise auftretenden Maßnahme zur Energiegewinnung. Nimmt jedoch die Körperfettmobilisierung ein überschießendes, krankhaftes Ausmaß an, belastet die massive Anflutung nicht-veresterter Fettsäuren (*non-esterified fatty acids,* NEFA) die Leber. Ist die Kapazität der Leber, die NEFA zu verstoffwechseln, erschöpft, werden Stoffwechselkrankheiten wie Leberverfettung und, oft sekundär und subklinisch, Ketose begünstigt (Drackley, 1999). Die Bedeutung dieser Krankheiten ist enorm, da zahlreiche hochleistende Milchkühe in den ersten Laktationswochen an mindestens einer dieser beiden Erkrankungen leiden. Abgesehen von der Beeinträchtigung des Wohlbefindens der Tiere verringert sich der wirtschaftliche Erlös des Milchviehhalters durch Behandlungen und verminderte Milchleistung. Verschiedene nutritive Ansätze wurden verfolgt, um die Energieversorgung der Milchkühe in dieser kritischen Phase zu verbessern. Der verstärkte Kraftfuttereinsatz sowie der Einsatz pansenstabiler Fette als Energieträger wurden ebenso intensiv untersucht wie die Zulage verschiedener Zusatzstoffe, die beispielsweise die energetische Ausnutzung des Futters verbessern oder als glukoplastische Substanzen die Glukoneogenese steigern sollen (Grummer, 1995; Nielsen und Ingvartsen, 2004; Andersen et al., 2008; Moallem et al., 2009).

1.1 Konjugierte Linolsäuren (CLA)

Konjugierte Linolsäuren (*conjugated linoleic acids*, CLA) sind Fettsäuren mit speziellen Wirkungen auf den Organismus. So konnte beobachtet werden, dass bei verschiedenen Spezies durch Verabreichung von CLA der Fettgehalt der Milch abgesenkt werden kann (Baumgard et al., 2000; Masters et al., 2002; Hayashi et al., 2007). Der Einsatz von CLA bei der Milchkuh erlangte schließlich großes Interesse im Hinblick auf die Möglichkeit durch die

verringerte Abgabe von Milchfett über die Milch den Energieoutput zu reduzieren und für eine bessere Energiebilanz der Tiere in der Frühlaktation zu sorgen (Liermann, 2008). Orale CLA-Zulagen müssen in pansengeschützter Form an Rinder verabreicht werden, denn andernfalls werden sie im Pansen (teil)hydriert und können keine Wirkung entfalten. CLA enthalten 18 Kohlenstoffatome und zwei konjugierte Doppelbindungen, die an den Positionen 6 bis 15 auftreten können (Mitchell und McLeod, 2008). Es gibt 28 mögliche Positions- und geometrische CLA-Isomere, von denen bislang mindestens 16 identifiziert worden sind (Banni, 2002; House et al., 2005). CLA kommen natürlicherweise in Milch, Käse und Fleisch von Wiederkäuern vor. Das vorherrschende *cis*-9,*trans*-11 Isomer bekam deshalb den Namen „rumenic acid“ (Chin et al., 1992). Im Fett von Wiederkäuern macht es 75 - 90 % der gesamten CLA aus (Bauman et al., 2003). Anaerobe Pansenbakterien (*Butyrivibrio fibrisolvens*) bilden *cis*-9,*trans*-11 CLA als kurzlebiges Zwischenprodukt bei der Biohydrogenierung von Linolsäure zu Stearinsäure, das dem weiteren Abbau zu *trans*-11 Vaccensäure unterliegt (Harfoot und Hazlewood, 1988). Da *cis*-9,*trans*-11 CLA im Pansen schnell weiter abgebaut wird, ist davon auszugehen, dass die CLA im Milch- und Körperfett der Wiederkäuer nicht ausschließlich aus der Biohydrogenierung im Pansen stammen können. Des Weiteren wird der CLA-Gehalt im Fettgewebe von Wiederkäuern auch bei α-Linolensäure-reicher Fütterung gesteigert, ohne dass CLA ein Zwischenprodukt in der Biohydrogenierung der α-Linolensäure darstellt (Kramer et al., 2004; Poulson et al., 2004). Der Grund dafür ist, dass dabei im Pansen jedoch *trans*-11 Vaccensäure entsteht, die im Gegensatz zur CLA angereichert und in größerem Umfang von der Milchkuh im Darm absorbiert wird (Kepler et al., 1966; Harfoot und Hazlewood, 1988; Kramer et al., 2004; Wallace et al., 2007). *Trans*-11 Vaccensäure stellt die Vorstufe für die Bildung von CLA in verschiedenen Geweben dar (Griinari et al., 2000; Kramer et al., 2004). Griinari et al. (2000) konnten schließlich erstmals bei laktierenden Milchkühen zeigen, dass die endogene Δ^9-Desaturase (Stearoyl-CoA-Desaturase, SCD) *trans*-11 Vaccensäure, die aus der Biohydrogenierung von Linol- und α-Linolensäure im Pansen stammt, in bedeutendem Umfang zu *cis*-9,*trans*-11 CLA umwandelt. Die durch unvollständige Biohydrogenierung langkettiger Fettsäuren entstehende *trans*-11 Vaccensäure dient folglich als Substrat für die endogene CLA-Synthese, deren Anteile am gesamten CLA-Gehalt im Milchfett auf 50 – 64 % bis zu 78 % (Griinari et al., 2000; Corl et al., 2001) und im Rindfleisch auf über 86 % geschätzt werden (Gillis et al., 2003). Bei Mäusen, Ratten und Menschen wurde die endogene CLA-Synthese aus mit der Nahrung aufgenommener *trans*-11 Vaccensäure ebenfalls beschrieben (Ip et al., 1999a; Adlof et al., 2000; Santora et al., 2000). Ins Rampenlicht der

wissenschaftlichen Forschung gelangten die CLA ursprünglich Anfang der Achtziger Jahre, nachdem die Arbeitsgruppe um Michael Pariza ein Gemisch verschiedener CLA-Isomere aus gebratenem Rinderhackfleisch isolierte und ihnen eine antikanzerogene Wirkung bei Mäusen mit Neoplasien der Haut nachweisen konnte (Ha et al., 1987). Seither wurden zahlreiche Wirkungen der CLA beschrieben und eine Reihe von Reviews fassen diese zusammen (u.a. Jahreis et al., 2000; Pariza et al., 2001; Belury, 2002; House et al., 2005). In den meisten Studien wurden Nager als Modelltiere verwendet. Es wurden antikanzerogene Effekte des *cis*-9,*trans*-11 Isomers bei Mäusen und Ratten beschrieben (Ip et al., 1994; Belury et al., 1996; Ip et al., 1999b). Weiterhin konnten antiinflammatorische bzw. immunmodulatorische Wirkungen sowie antiatherosklerotische Effekte bei Fütterung von CLA aufgezeigt werden (Cook et al., 1993; Lee et al., 1994; Nicolosi et al., 1997; Hayek et al., 1999). Das *trans*-10,*cis*-12 Isomer zeichnet bei Ratten verantwortlich für eine antidiabetische sowie antiadipöse Wirkung (Houseknecht et al., 1998; Ryder et al., 2001; Yamasaki et al., 2003). Antiadipöse Effekte im Sinne eines verringerten Körperfettanteils durch das *trans*-10,*cis*-12 Isomer konnten auch bei Mäusen gesehen werden. Einhergehend mit dem reduzierten Fettanteil wurde der Gehalt an Triacylglyceriden (TAG) im Fettgewebe verringert und der Magermasse- und Wasseranteil im Schlachtkörper erhöht (Park et al., 1997; Park et al., 1999). Allerdings gibt es auch Studien, die negative Auswirkungen von CLA-Gaben dokumentieren, beispielsweise die Entstehung von Hyperinsulinämie und Insulinresistenz (Tsuboyama-Kasaoka et al., 2000; Sisk et al., 2001). Bei Mäusen entwickelte sich als schwerwiegende negative Begleiterscheinung ausgelöst durch die Fütterung von *trans*-10,*cis*-12 CLA, nicht jedoch *cis*-9,*trans*-11 CLA, eine massive Zunahme des Lebergewichts infolge von Steatose einhergehend mit erhöhten hepatischen TAG- und Cholesterolgehalten (Belury und Kempa-Steczko, 1997; DeLany et al., 1999; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002; Degrace et al., 2003).

1.2 Wirkungen konjugierter Linolsäuren bei Nutztieren

Als Folge des großen Interesses an den Wirkungen der CLA wurden bis heute zahlreiche CLA-Studien an landwirtschaftlichen Nutztieren durchgeführt. Es wurden Auswirkungen von CLA-Gaben auf Leistungsparameter, Körperzusammensetzung, Fettsäuremuster von Geweben und Produkten, Fleisch- und Produktqualität, Stoffwechselparameter und Genexpressionsraten in verschiedenen Geweben dokumentiert. Die tierischen Leistungsdaten sind besonders in der Fleischproduktion ein entscheidender wirtschaftlicher Faktor. Verbesserte tägliche Zunahmen oder Futterverwertungen können für den Landwirt deutliche

finanzielle Vorteile bedeuten, da die Tiere schneller schlachtreif sind und daher weniger Stalltage benötigen oder das Endgewicht mit geringerem Futteraufwand und somit geringeren Futterkosten, die oft den größten Kostenfaktor darstellen, erreichen (Kirchgeßner, 2004). Die Auswirkungen von CLA-Fütterung auf Produktionsparameter sind teilweise widersprüchlich. Bei wachsenden Schweinen und laktierenden Sauen wurden die Futterverwertung verbessert und die täglichen Zunahmen gesteigert (Ostrowska et al., 1999; Eggert et al., 2001; Thiel-Cooper et al., 2001), während andere Autoren keine Effekte feststellten (Dugan et al., 1997; Bee, 2000; Wiegand et al., 2001; Tischendorf et al., 2002). Ähnlich ist das Bild bei wachsenden Rindern. Gillis et al. (2004b) erzielten durch CLA-Fütterung bessere mittlere tägliche Zunahmen und eine bessere Futterverwertung ohne Einfluss auf die Futteraufnahme bei weiblichen Fleischrindern, während bei Ochsen ein Rückgang der Futteraufnahme und der mittleren täglichen Zunahmen beobachtet wurde (Gassman et al., 2000; Flórez-Díaz et al., 2006). Die mittleren täglichen Zunahmen weiblicher Aufzuchtrinder sowie die Futteraufnahme laktierender Schafe wurden durch CLA-Fütterung nicht verändert (Block et al., 2003; Lock et al., 2006; Sinclair et al., 2010). Die Leistung von Masthähnchen wurde in der Regel durch CLA-Fütterung verschlechtert (Szymczyk et al., 2001; Badinga et al., 2003; Suksombat et al., 2007) oder nicht beeinflusst (Aletor et al., 2003; Du und Ahn, 2003). Schlachtkörper werden in Deutschland gemäß ihres Magerfleischanteils und des Verfettungsgrades beurteilt und entlohnt, für Rinderschlachtkörper gilt das EUROP-Klassifizierungssystem. Beide Kriterien bilden ausschlaggebende finanzielle Faktoren für den Fleischerzeuger. Auch bei der Kaufentscheidung der Konsumenten spielen sie eine nicht unwesentliche Rolle, da der Wandel des Ernährungsbewusstseins in den letzten Jahren dazu geführt hat, dass fettarmes, mageres Fleisch bevorzugt konsumiert wird. Sowohl die Fettauflage als auch der intramuskuläre Fettgehalt des Fleisches dürfen nicht zu ausgeprägt sein, um für den Käufer ein attraktives Angebot darzustellen (Warriss, 2000). Der Magerfleisch- bzw. Muskelmasseanteil am Schweineschlachtkörper wurde in vielen Studien bei geringerem Rückenspeckanteil erhöht (Dugan et al., 1997; Ostrowska et al., 1999; Thiel-Cooper et al., 2001; Wiegand et al., 2001; Tischendorf et al., 2002; Wiegand et al., 2002), was bei Rind und Schaf nicht zu beobachten war (Block et al., 2003; Wynn et al., 2006; Sinclair et al., 2010). Die Abnahme des Fettgehalts im Schlachtkörper oder in verschiedenen Teilstücken beim Hähnchen durch CLA-Fütterung wurde mehrfach beschrieben (Szymczyk et al., 2001; Du und Ahn, 2002; Suksombat et al., 2007). Des Weiteren ist die Fleischqualität häufig ein ausschlaggebendes Merkmal für Kaufentscheidungen. Den ersten Eindruck prägt die Fleischfarbe, von der jeder Konsument eine subjektive Wunschvorstellung hat (Barbut, 2001;

Walsh und Kerry, 2002). Schließlich beeinflussen sensorische Eigenschaften wie die Zartheit und Schmackhaftigkeit das Verbraucherverhalten. Die Zartheit ist nach der Fleischfarbe das zweitwichtigste Verbraucherkriterium für eine Kaufentscheidung und wird u.a. durch die Fleischreifung, die Kühlgeschwindigkeit und –temperatur und den pH-Wert im Fleisch beeinflusst (Feiner, 2006). Auf verschiedene physikalische Fleischqualitätsparameter zeigten sich beim Schwein in zahlreichen Studien Effekte durch die Zulage von CLA, z. B. positive Effekte auf die Marmorierung von Schweinfleisch, durch die Geschmack, Saftigkeit und Zartheit verbessert werden könnten (Wiegand et al., 2001; Dugan et al., 1999). Auch die Festigkeit des Schweinefleisches nahm zu, wofür der höhere Anteil an gesättigten Fettsäuren im Gewebe verantwortlich sein dürfte (Eggert et al., 2001; Thiel-Cooper et al., 2001; Wiegand et al., 2002). Einen gesteigerten intramuskulären Fettgehalt beobachteten u.a. Dugan et al. (2003), Joo et al. (2002) und Wiegand et al. (2002). Die Fleischfarbe, der Grill- und Lagerverlust sowie die sensorischen Eigenschaften wurden beim Schweinefleisch vorwiegend nicht (Eggert et al., 2001; Joo et al., 2002; Dugan et al., 2003), in seltenen Fällen negativ beeinflusst (D'Souza und Mullan, 2002). Untersuchungen an Rindfleisch von Tieren, die CLA erhalten hatten, ergaben zumeist keine signifikanten Einflüsse auf Qualitätsparameter wie Zartheit, Saftigkeit, sensorische Attribute oder Farbe (Gillis et al., 2004b; Poulson et al., 2004; Flórez-Díaz et al., 2006), in zwei Studien wurden jedoch negative Auswirkungen auf Saftigkeit, Zartheit und Marmorierung des Rindfleisches beschrieben (Gassman et al., 2000; Gillis et al., 2007). Die Qualität des Fleisches von Geflügel nimmt in der Regel durch die Fütterung einer CLA-Zulage ab. Das Geflügelfleisch wird härter, weniger saftig und dunkler, zum Teil auch mit verminderter sensorischer Qualität beschrieben. Mögliche Ursachen können die Veränderungen im Fettsäuremuster zugunsten des Gehaltes an gesättigten Fettsäuren oder auch der erhöhte Proteingehalt des Schlachtkörpers sein (Du und Ahn, 2002; Du et al., 2002; Du et al., 2003; Parrish et al., 2003). Außerdem wurde ein Anstieg der relativen Lebermasse bei mit CLA gefütterten Broilern (Badinga et al., 2003; Du und Ahn, 2003) und Legehennen (Schäfer et al., 2001) gezeigt, ohne dass jedoch der Leberfettgehalt zugenommen hat. Eine weitere Erscheinung, die in den letzten Jahren bedingt durch die Veränderungen im Ernährungsbewusstsein der Menschen aufgetreten ist, ist die Nachfrage nach sogenanntem „functional food". Darunter versteht man Nahrungsmittel, die eine positive Wirkung auf Stoffwechselvorgänge des Konsumenten ausüben und in der Folge Krankheiten lindern oder vorbeugen können (Jiménez-Colmenero et al., 2001). So werden z. B. Nahrungsmittel mit einem hohen Verhältnis mehrfach ungesättigter zu gesättigten Fettsäuren oder mit einem hohen Anteil an Omega-3 Fettsäuren bzw. hohem Verhältnis von Omega-3 zu

Omega-6 Fettsäuren als ernährungsphysiologisch günstig erachtet. Auch der Gehalt an *trans*-Fettsäuren sollte nicht zu hoch sein (Lawrie und Ledward, 2006). Das Fettsäuremuster des subkutanen Fettgewebes ist außerdem von Bedeutung für die Eigenschaften, z. B. die Konsistenz, der Fettauflage auf dem Fleisch (Feiner, 2006). Die Fütterung von CLA führt bei allen untersuchten Spezies zur Anreicherung von CLA in verschiedenen Geweben. Studien berichten von erhöhten Gehalten sowohl an *cis*-9,*trans*-11 als auch an *trans*-10,*cis*-12 CLA im subkutanen und anderen Fettgeweben, im Lendenfleisch und Rückenspeck sowie in der Leber und im Herzen von supplementierten Schweinen (Kramer et al., 1998; Bee, 2000; Thiel-Cooper et al., 2001; Wiegand et al., 2002). Bei weiblichen Fleischrindern und Ochsen führte die Fütterung pansengeschützter CLA zur Anreicherung von *cis*-9,*trans*-11 und *trans*-10,*cis*-12 CLA in Fett- und Muskelgeweben (Gassman et al., 2000; Gillis et al., 2004a; Poulson et al., 2004). Beim Geflügel konnte die verstärkte Inkorporation von CLA in die Lipide verschiedener Gewebe und Teilstücke bei CLA-Fütterung belegt werden (Simon et al., 2000; Szymczyk et al., 2001; Suksombat et al., 2007). Des Weiteren erhöhte sich durch die CLA-Zulage das Verhältnis von gesättigten zu einfach ungesättigten Fettsäuren in verschiedenen Geweben beim Schwein (Thiel-Cooper et al., 2001; Wiegand et al., 2002; Bee, 2000; Kramer et al., 1998), beim Geflügel (Simon et al., 2000; Szymczyk et al., 2001; Aletor et al., 2003; Badinga et al., 2003) sowie beim Rind (Gillis et al., 2004a; Flórez-Díaz et al., 2008). Flórez-Díaz et al. (2008) berichteten außerdem von einem geringeren Verhältnis von Omega-6 zu Omega-3 Fettsäuren.

1.3 Wirkungen von CLA auf Milchfettgehalt und Genexpressionsraten

Eine herausragende Entdeckung war die Möglichkeit durch die Verabreichung pansengeschützter CLA (Infusion in den Labmagen oder pansengeschützte orale Gabe) an Milchkühe eine Milchfettdepression auszulösen (Loor und Herbein, 1998; Chouinard et al., 1999). Verursacher der Milchfettdepression ist ausschließlich das *trans*-10,*cis*-12 Isomer, das *cis*-9,*trans*-11 Isomer hat keinen Einfluss auf den Milchfettgehalt (Baumgard et al., 2000). Es wurden Absenkungen des Milchfettgehalts von bis zu 50 % erzielt (u.a. Baumgard et al., 2001; Perfield et al., 2002; Bauman et al., 2003; de Veth et al., 2006; Sippel et al., 2009; Moallem et al., 2010). Die Milchfettreduktion geschieht dosisabhängig (Baumgard et al., 2001; Peterson et al., 2002), verringert sowohl den Gehalt an *de novo* synthetisierten als auch an aus dem Futter aufgenommenen Fettsäuren in der Milch (Baumgard et al., 2001; Perfield et al., 2002; de Veth et al., 2006) und ist reversibel (Chouinard et al., 1999; Moallem et al., 2010). Bei oraler Verabreichung pansengeschützter CLA mit einem Gehalt von 4 g bis zu

über 50 g *trans*-10,*cis*-12 CLA pro Kuh und Tag wurden neben der Milchfettdepression häufig Milchleistungssteigerungen sowie zunehmende Gehalte an *trans*-10,*cis*-12 CLA in der Milch ausgelöst (de Veth et al., 2006; Odens et al., 2007; Sippel et al., 2009; Moallem et al., 2010). Der Milchproteingehalt und die Futteraufnahme der Kühe blieben unverändert (Giesy et al., 2002; Perfield et al., 2002; Odens et al., 2007; Liermann, 2008; Sippel et al., 2009). In drei Studien wurde beobachtet, dass die CLA-Gabe den hepatischen TAG-Gehalt bei Milchkühen nicht beeinflusst (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005). Ebenso wurden der Δ^9-Desaturase-Index in der Milch sowie die Gehalte an NEFA, β-Hydroxybutyrat (*β-hydroxy butyric acid*, BHBA), Glukose und Insulin im Plasma der Milchkühe durch die CLA-Gabe nicht beeinflusst (Baumgard et al., 2002a; Bernal-Santos et al., 2003; Moore et al., 2004; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; de Veth et al., 2006; Liermann, 2008). Die Energiebilanz der Kühe nach der Abkalbung konnte durch die CLA-Fütterung verbessert bzw. schneller ausgeglichen werden (Odens et al., 2007; Liermann, 2008; Sippel et al., 2009). Eine geringere negative Energiebilanz zu Laktationsbeginn hat positive Auswirkungen auf die Gesundheit von Milchkühen und reduziert z. B. das Mastitisrisiko oder Fruchtbarkeitsstörungen (de Vries et al., 1999; de Vries und Veerkamp, 2000), so dass die Fütterung von CLA besonders in der Frühlaktation über die Energieeinsparung infolge der Milchfettdepression und eine verbesserte Energiebilanz der Tiere auch die Fruchtbarkeit positiv beeinflussen kann (Liermann, 2008). Der Einfluss einer CLA-Fütterung von Milchkühen auf Expressionsraten von Genen wurde bislang vorwiegend im Eutergewebe, weniger häufig im Fett- und Lebergewebe, untersucht. Im Eutergewebe von laktierenden Milchkühen, denen *trans*-10,*cis*-12 CLA verabreicht wurde, wurde mehrfach eine geringere mRNA-Expression verschiedener Gene des Lipidstoffwechsels nachgewiesen, die mit der reduzierten Milchfettsynthese in Einklang zu bringen sind. Gene, die für Enzyme der Aufnahme und des Transports von Fettsäuren, der *de novo* Synthese von Fettsäuren, der Desaturierung von Fettsäuren sowie der TAG-Synthese codieren, wurden bei Fütterung von *trans*-10,*cis*-12 CLA, nicht aber von *cis*-9,*trans*-11 CLA, im Eutergewebe bzw. in Euterepithelzellen herunterreguliert (Baumgard et al., 2001; Baumgard et al., 2002b; Peterson et al., 2004; Kay et al., 2007). Die Regulation dieser lipogenen Schlüsselenzyme im Euter erfolgt durch den Peroxisomenproliferator-aktivierten Rezeptor (PPAR) α oder das s*terol regulatory element binding protein* (SREBP) 1, so dass davon auszugehen ist, dass *trans*-10,*cis*-12 CLA auf mindestens einen dieser Transkriptionsfaktoren wirkt (Baumgard et al., 2002b; Kay et al., 2007). Im Zellversuch wurde die Aktivität des SREBP-1 vermindert, ohne dass dessen Genexpression beeinflusst wurde (Peterson et al., 2004), während bei *trans*-

10,*cis*-12 CLA-Infusion in den Labmagen auch die mRNA-Expression des Gens *SREBF1*, das für SREBP-1 codiert, im Eutergewebe reduziert wurde (Harvatine und Bauman, 2006). Im Fettgewebe wurde hingegen die mRNA-Expression lipogener Enzyme sowie des *SREBF1* durch die Infusion von *trans*-10,*cis*-12 CLA gesteigert (Harvatine et al., 2009). Die Expression von Genen des Lipidstoffwechsels in der Leber wurde bislang nur in geringem Umfang untersucht und schien bei laktierenden Milchkühen durch die Fütterung von *trans*-10,*cis*-12 CLA nicht beeinflusst zu werden (Selberg et al., 2005; Sigl et al., 2010). Auch die Expression einiger Gene, die für Enzyme der Glukoneogenese codieren, blieb unverändert bei CLA-Fütterung (Selberg et al., 2004). Die detaillierte Aufklärung der Wirkung pansengeschützter CLA auf den hepatischen Lipidstoffwechsel bei der Milchkuh hinsichtlich der Einflüsse auf Genexpressionsraten und möglicher negativer Nebeneffekte erfordert weitere Untersuchungen.

1.4 Bedeutung des Lipidstoffwechsels in der Transitphase der Milchkuh

Um die Transitphase der Milchkuh möglichst optimal gestalten zu können, ist es erforderlich, die Stoffwechselvorgänge und Anpassungsmechanismen, die in dieser physiologisch kritischen Phase ablaufen, zu verstehen. In den letzten 20 Jahren hat die Forschung auf diesem Gebiet stark zugenommen. Die Etablierung veterinärmedizinischer Eingriffe zur Probenentnahme am lebenden Tier sowie die weit entwickelte Anwendung molekularbiologischer Methoden wie der Genexpressionsmessung erlauben heutzutage die Untersuchung und den Vergleich phäno- wie genotypischer Veränderungen in verschiedenen Phasen des Laktationszyklus. Der Lipidstoffwechsel in der Leber leistet in der Transitphase einen entscheidenden Beitrag zur Anpassung des Stoffwechsels der Milchkuh an die Laktation, weshalb seine detaillierte Aufklärung einen Grundpfeiler für die Optimierung des Managements der Transitphase der hochleistenden Milchkuh darstellt (Drackley, 1999).

Die Carnitinsynthese der Milchkuh

Die vitaminähnliche Substanz L-Carnitin (L-3-Hydroxy-4-*N*,*N*,*N*-Trimethylaminobutyrat) spielt eine bedeutende Rolle im Intermediär- und Lipidstoffwechsel eukaryotischer Zellen. Die wichtigste Aufgabe des Carnitins (im Folgenden steht der Begriff Carnitin stets für das stoffwechselaktive L-Carnitin) im Fettsäurestoffwechsel ist die Beteiligung am Transport mittel- und langkettiger Fettsäuren (> C10) in das Innere der Mitochondrien, wo die β-Oxidation der Fettsäuren zur Energiegewinnung stattfindet. Zunächst erfolgen die Bildung von Acylcarnitin und die Freisetzung von Coenzym-A (CoA) durch die Carnitin-Palmitoyl-

Transferase (CPT) 1 an der äußeren Mitochondrienmembran. Die Carnitin-Acylcarnitin-Translokase (CACT) sorgt für den Übertritt des Acylcarnitins in den mitochondrialen Matrix-Raum, wo die CPT-2 Acylcarnitin spaltet und die Reveresterung des CoA mit der Fettsäure katalysiert. Carnitin kann durch die CACT das Mitochondrium verlassen und erneut den Transport von Fettsäuren in den Matrix-Raum vermitteln (McGarry und Brown, 1997; Vaz und Wanders, 2002). Der CoA-Ester der Fettsäure kann schließlich der mitochondrialen β-Oxidation zugeführt werden, die der Energiegewinnung in Form von ATP dient. Die Oxidation von Fettsäuren in der Leber ist besonders in Energiemangel- oder Fastenzuständen von Bedeutung. Durch die Mobilisierung von Körperfett werden NEFA freigesetzt, die in der Leber anfluten und verstoffwechselt werden müssen. Es liegt folglich nahe, dass die Verfügbarkeit von Carnitin in der Leber von Milchkühen in der Transitphase von Interesse ist. Carnitin kann über die Nahrung aufgenommen oder endogen synthetisiert werden. Im Milchviehfutter ist typischerweise wenig Carnitin enthalten, so dass die endogene Synthese den größeren Beitrag zur Versorgung leistet. Ausgangsstoffe für die Carnitinsynthese sind die Aminosäuren Lysin und Methionin. Im ersten Schritt erfolgt die Bildung von N^6-Trimethyllysin (TML), das im weiteren Verlauf durch die Trimethyllysin-Dioxygenase (TMLD) hydroxyliert wird, gefolgt von der aldolytischen Spaltung durch die 3-Hydroxy-*N*-TML-Aldolase (HTMLA) und der Dehydrogenierung durch die 4-*N*-Trimethylaminobutyraldehyd-Dehydrogenase (TMABA-DH), die schließlich in der Entstehung von γ-Butyrobetain (BB) resultiert (Vaz und Wanders, 2002; Strijbis et al., 2010). Während die Synthese des BB in vielen Geweben, die es dann an die Blutbahn abgeben, stattfinden kann, läuft der letzte Schritt der Carnitinsynthese, die Hydroxylierung von BB zu Carnitin durch die γ-Butyrobetain-Dioxygenase (BBD) bei der Milchkuh ausschließlich in der Leber und der Niere ab (Vaz und Wanders, 2002). Für die Carnitinaufnahme in die Gewebe sind spezifische Transporter notwendig, die *novel organic cation transporters* (OCTNs), die der *solute carrier family* (SLC) 22A zugeordnet werden. Die Regulation der Gene der Carnitinsynthese und des Carnitintransports erfolgt durch den PPARα, einen nukleären Kernrezeptor mit essentieller Stellung im Energie- und Lipidstoffwechsel (van Vlies et al., 2007; Koch et al., 2008; Ringseis und Eder, 2009; Wen et al., 2010; Wen et al., 2011; Wen et al., 2012). Dieser Transkriptionsfaktor beeinflusst insbesondere die Adaption des Stoffwechsels an Energiemangelsituationen und kann durch verschiedene Liganden aktiviert werden, zu denen die aus der Mobilisierung von Körperfett stammenden NEFA gehören (Desvergne und Wahli, 1999; Kersten et al., 1999; Leone et al., 1999). Bei Milchkühen wurde bereits der Anstieg der Carnitinkonzentration in der Leber (Grum et al., 1996; Carlson et al.,

2007a) sowie die Hochregulation des PPARα und verschiedener seiner Zielgene (Loor et al., 2005) in der Frühlaktation beschrieben - ob sich die Expression der Gene des Carnitinstoffwechsels verändert, ist jedoch bislang nicht bekannt. Carlson et al. (2006; 2007a; 2007b) untersuchten in mehreren Studien die Effekte einer Supplementierung mit Carnitin bei Milchkühen. Die Infusion von Carnitin bei laktierenden Kühen während einer Futterrestriktion wirkt stimulierend auf die Fettsäureoxidation in der Leber und senkt die TAG-Akkumulation in der Leber (Carlson et al., 2006). Die orale Zulage von Carnitin zur Ration von Milchkühen in der Transitperiode führte zu verringerten Lipid- und TAG-Gehalten, einem höheren Glykogengehalt und zur verstärkten Oxidation von Palmitat in der Leber (Carlson et al., 2007a). Ein limitierender Einfluss der Carnitinverfügbarkeit in der Leber auf die Kapazität der Fettsäureoxidation und folglich die Begünstigung der Entstehung der Leberverfettung und der Ketose bei der Transitkuh kann aus diesen Ergebnissen abgeleitet werden (Carlson et al., 2006; 2007a), was die Bedeutung und die Notwendigkeit der weiteren Aufklärung des Carnitinstoffwechsels in der Transitphase der Milchkuh unterstreicht.

Bedeutung des FGF21 im Energiemangelzustand

In jüngsten Studien wurde der Fibroblasten-Wachstumsfaktor 21 (*fibroblast growth factor 21*, FGF21) als wichtiger Transkriptionsfaktor, der an der Regulation der Anpassungsreaktionen des Stoffwechsels an Energiemangelzustände beteiligt ist, identifiziert (Kharitonenkov et al., 2005; Badman et al., 2007; Inagaki et al., 2007; Lundåsen et al., 2007). Der FGF21 gehört zu einer Unterfamilie der Fibroblasten-Wachstumsfaktoren, deren Mitglieder wie Hormone in die Zirkulation abgegeben werden und endokrin wirken. Er wurde bislang vorwiegend bei Mäusen und Menschen erforscht, wobei die genauen Wirkmechanismen dieses Proteins noch ungeklärt sind (Kharitonenkov, 2009; Kliewer und Mangelsdorf, 2010). Die Expression des *FGF21* erfolgt in der Leber, dem Pankreas, dem weißen Fettgewebe sowie dem Skelettmuskel (Nishimura et al., 2000; Izumiya et al., 2008; Kliewer und Mangelsdorf, 2010). Sie ist bei Mäusen im Fastenzustand sowie bei der Fütterung einer ketogenen Diät stark erhöht (Badman et al., 2007). Im Fasten spielt die Leber eine essentielle Rolle für die Aufrechterhaltung der Energieversorgung des Organismus. Zunächst werden die Glykogenreserven der Leber als Energiequelle genutzt, nach deren Erschöpfung im weiteren Verlauf des Energiemangels die Energiegewinnung durch die Glukoneogenese und die Ketogenese im Vordergrund steht. Ketonkörper sind insbesondere nötig, um die Energieversorgung des Gehirns im Fastenzustand sicherzustellen (Badman et al., 2007; Potthoff et al., 2009). Die erhöhte Expression des *FGF21* im Energiemangel führt zur Steigerung der Ketonkörperbildung in der

Leber sowie der Lipolyse im weißen Fettgewebe, bei der NEFA freigesetzt werden, die als Vorstufe für die Ketonkörpersynthese dienen (Badman et al., 2007; Inagaki et al., 2007). Des Weiteren stimuliert FGF21 im Fasten die Fettsäureoxidation, den Citratzyklus sowie die Glukoneogenese in der Leber von Mäusen (Potthoff et al., 2009). Bei FGF21-Knockout-Mäusen funktionieren diese Mechanismen nicht mehr bzw. signifikant schlechter, so dass die essentielle Beteiligung des FGF21 an der Regulation des Lipid- und Kohlenhydratstoffwechsels in der Leber und dem Fettgewebe bei Mäusen im Fastenzustand zweifelsfrei nachgewiesen ist (Badman et al., 2007; Potthoff et al., 2009; Domouzoglou und Maratos-Flier, 2011). Die Regulation der Expression des *FGF21* erfolgt über verschiedene Wege. Der Transkriptionsfaktor PPARα, der eine wichtige Rolle in der Regulation der Lipidhomöostase spielt, reguliert die Expression des *FGF21* in der Leber (Badman et al., 2007; Inagaki et al., 2007; Lundåsen et al., 2007). Während sie bei hungernden PPARα-Knockout-Mäusen vollständig ausblieb, erfolgte bei PPARα-Knockout-Mäusen, die eine ketogene Diät erhielten, dennoch eine geringe Expression des *FGF21* in der Leber. Somit liegen zumindest bei Auftreten einer Ketose auch PPARα-unabhängige Regulationsmechanismen vor (Inagaki et al., 2007; Domouzoglou und Maratos-Flier, 2011). Im Skelettmuskel und im Fettgewebe wird die Expression des *FGF21* über den PI3-Kinase/Akt1-Stoffwechselweg bzw. über den Transkriptionsfaktor PPARγ reguliert (Izumiya et al., 2008; Muise et al., 2008; Wang et al., 2008). Aufgrund der Bedeutung des FGF21 für den hepatischen Lipid- und Kohlenhydratstoffwechsel im Energiemangelzustand bei der Maus kann man seine Beteiligung am hepatischen Stoffwechsel in der Frühlaktation der Milchkuh ebenfalls annehmen. Erhöhte Gehalte an FGF21 im Plasma laktierender Milchkühe in der Frühlaktation sowie ein Anstieg der *FGF21*-mRNA-Expression in der Leber kurz nach der Abkalbung wurden beobachtet (Carriquiry et al., 2009; Schoenberg et al., 2011). Dass FGF21 eine Rolle in der Regulation des Lipidstoffwechsels im Rahmen der Anpassungsmechanismen und des Energiedefizits der Milchkühe zu Laktationsbeginn spielt, scheint folglich belegt, die genaue Aufklärung erfordert jedoch weiterführende Studien.

2. Zielstellung

2.1 Untersuchungen zur Wirkung von pansengeschützter CLA auf Leistungsdaten und Fleischqualität beim weiblichen Jungrind

In vorangegangenen Studien konnte gezeigt werden, dass der Einsatz von CLA bei verschiedenen Labortieren sowie bei Nutztieren das Fettsäuremuster verschiedener Gewebe, die Körperzusammensetzung und die Fleischqualitätsbeurteilung beeinflusst (u.a. Park et al., 1999; Thiel-Cooper et al., 2001; Du und Ahn, 2002; Gillis et al., 2004a). Die Veränderung der Körperzusammensetzung zugunsten von Muskelmasse bei verringertem Körperfettgehalt ist beim Nutztier von Vorteil, da Schlachttiere nach Magerfleischanteil bezahlt werden (Ostrowska et al., 1999; Tischendorf et al., 2002). Bei dem landwirtschaftlichen Nutztier Schwein konnte auch ein positiver Einfluss auf verschiedene Leistungsparameter beschrieben werden (Eggert et al., 2001; Thiel-Cooper et al., 2001). Verbesserte Leistungsparameter stellen immer eine wirtschaftlichere Tierproduktion dar. Die Verabreichung von CLA an Mastrinder in der Endmast führte zu verbesserter bzw. unveränderter Leistung ohne Auswirkungen auf die Fleischqualität (Gillis et al., 2004b; Flórez-Díaz et al., 2006). In der Phase der Anfangsmast erreichen die Tiere jedoch deutlich höhere tägliche Zunahmen als in der Endmast. Eine Supplementierung in diesem Mastabschnitt könnte folglich interessant sein und zu anderen Ergebnisse führen als bereits vorliegende Studien. In *Studie 1* wurden deshalb 36 Fleckviehfärsen im Alter von etwa fünf Monaten auf eine Kontrollgruppe ohne CLA-Supplementierung und zwei Behandlungsgruppen, die pansengeschützte CLA in unterschiedlichen Dosen erhielten, verteilt. Die Tiere wurden nach 16 Wochen Fütterung mit einem Lebendgewicht von etwa 325 kg geschlachtet. Es wurden tierindividuelle Leistungsdaten aufgezeichnet sowie die Schlachtkörperbeurteilung im Schlachthof durchgeführt. Weiterhin wurden Organe und Fettgewebe verwogen, Proben des *Musculus longissimus dorsi* (LM) und des subkutanen Fettgewebes entnommen und für Untersuchungen hinsichtlich Fleischqualität und Fettsäurezusammensetzung aufbereitet. Weitere Einzelheiten zur Versuchsdurchführung, Material und Methodik sowie die ausführliche Darstellung und Diskussion der Ergebnisse dieser Studie sind ersichtlich in:

Studie 1:

Schlegel G, Ringseis R, Shibani M, Most E, Schuster M, Schwarz FJ, Eder K (2012) Influence of a rumen-protected conjugated linoleic acid mixture on carcass traits and meat quality in young simmental heifers. *Journal of Animal Science* 90: 1532-1540. Reproduced with permission of the American Society of Animal Science.

2.2 Untersuchungen zur Wirkung von pansengeschützter CLA auf den hepatischen Lipidstoffwechsel bei der Milchkuh

Der Einsatz von CLA zeigte besonders bei Nagern und Labortieren zahlreiche positive Auswirkungen auf Stoffwechsel und Gesundheit. So konnten der CLA-Fütterung zum Beispiel antikanzerogene, antiatherogene und antidiabetogene Effekte nachgewiesen werden (Ha et al., 1987; Lee et al., 1994; Yamasaki et al., 2003). Allerdings wurden ebenso negative Nebenwirkungen beim CLA-Einsatz bei Labortieren beschrieben. Insbesondere bei Mäusen beeinträchtigt die Fütterung des *trans*-10,*cis*-12 CLA-Isomers den hepatischen Lipidstoffwechsel. Es kommt zur massiven Akkumulation von TAG in der Leber – schwere Leberverfettungen entstehen (Belury und Kempa-Steczko, 1997; Clément et al., 2002). CLA werden in der praktischen Nutztierfütterung bereits eingesetzt, insbesondere beim Rind. Die Fütterung von pansengeschützten CLA an laktierende Milchkühe resultiert in einer effektiven Reduzierung des Fettgehaltes der Milch, eine sogenannte Milchfettdepression kann ausgelöst werden (Loor und Herbein, 1998). Die Absenkung des Milchfettgehaltes beträgt in einer dosisabhängigen Weise bis zu 50 % (Baumgard et al., 2001). Da Milchfett der energiereichste Nährstoff in der Milch ist, ist die Reduzierung des Milchfettgehaltes eine potente Möglichkeit, den Energieoutput der laktierenden Milchkuh zu verringern (Bauman und Davis, 1974; Drackley, 1999). Dies hat besondere Bedeutung in der Frühlaktation, in der Milchkühe häufig in eine negative Energiebilanz geraten (Drackley, 1999). Allerdings gibt es kaum Studien, die untersuchen, ob möglicherweise bei der Milchkuh wie bei Mäusen negative Auswirkungen der CLA-Supplementierung auf den hepatischen Lipidstoffwechsel zu beobachten sind. Deshalb wurde mit *Studie 2* ein Versuch mit 40 Milchkühen durchgeführt, die auf eine Kontroll- und eine Behandlungsgruppe gleichmäßig aufgeteilt wurden. Davon erhielten die 20 Milchkühe in der Behandlungsgruppe eine pansengeschützte Mischung konjugierter Linolsäuren, die zur Aufnahme von 3,8 g des *trans*-10,*cis*-12 CLA-Isomers je Tier und Tag führte. Diese Menge repräsentiert eine wirksame Dosis, um eine Milchfettdepression bei den Tieren auszulösen, die somit auch praxisnahe Verhältnisse widerspiegelt. Den Tieren wurden zu vier ausgewählten Zeitpunkten – drei Wochen vor der Abkalbung sowie in der ersten, fünften und 14. Laktationswoche – Leberbiopsieproben sowie Blutproben entnommen. Die Leberbiopsieproben der fünften Laktationswoche wurden mithilfe der Microarray-Technik hinsichtlich des Transkriptprofils der Leber in der Hochlaktation untersucht und dieses im Hinblick auf Veränderungen im Lipidstoffwechsel ausgewertet. Die ausführliche Versuchsbeschreibung, Material und Methodik sowie die

Darstellung und Diskussion der Ergebnisse der *Studie 2* können in der folgenden Veröffentlichung nachgelesen werden:

Studie 2:

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. Reproduced with permission of Elsevier.

2.3 Untersuchungen zum hepatischen Carnitinstoffwechsel von Milchkühen zu Laktationsbeginn

Vorangegangene Studien konnten beobachten, dass die Carnitinkonzentration in der Leber von Milchkühen zu Laktationsbeginn im Vergleich zur Trächtigkeit oder zur späteren Laktation erhöht ist (Grum et al., 1996; Carlson et al., 2007a). Carnitin ist ein bedeutender Metabolit mit weitreichenden Aufgaben im Intermediärstoffwechsel. Seine wichtigste Funktion liegt im Transport mittel- und langkettiger aktivierter Fettsäuren aus dem Cytosol in die Mitochondrien, wo die β-Oxidation der Fettsäuren stattfindet. Die endogene Carnitinsynthese ist neben der geringen nutritiven Aufnahme notwendig, um den Carnitinbedarf des Organismus zu decken (Vaz und Wanders, 2002). Beim Nager und beim Schwein konnte nachgewiesen werden, dass die Carnitinsynthese sowie die Aufnahme von Carnitin in die Gewebe direkt durch den PPARα reguliert werden, der eine große Rolle bei der Adaption des Stoffwechsels an Energiemangelsituationen spielt (van Vlies et al., 2007; Wen et al., 2010; Wen et al., 2011; Wen et al., 2012). Bei Aktivierung des PPARα im Fastenzustand kommt es durch eine Stimulierung der Carnitinsynthese und der Aufnahme von Carnitin aus dem Blut zu gesteigerten Carnitinkonzentrationen in verschiedenen Geweben (van Vlies et al., 2007; Ringseis et al., 2009). Bei der Milchkuh wurde der PPARα bislang kaum, die Regulation der Carnitinhomöostase noch gar nicht untersucht. Die vorliegende Studie sollte deshalb klären, ob die Gene der Carnitinsynthese und der Carnitinaufnahme in die Gewebe in der Frühlaktation bei hochleistenden Milchkühen infolge einer Aktivierung des PPARα hochreguliert werden. Weitere Details zur Versuchsdurchführung, Material und Methodik sowie die Ergebnisse und deren Diskussion sind ersichtlich in:

Studie 3:

Schlegel G, Keller J, Hirche F, Geißler S, Schwarz FJ, Ringseis R, Stangl GI, Eder K (2012) Expression of genes involved in hepatic carnitine synthesis and uptake in dairy cows in the transition period and at different stages of lactation. *BMC Veterinary Research* 8: 28.

2.4 Untersuchungen zur hepatischen Expression des FGF21 bei Milchkühen zu Laktationsbeginn

In der negativen Energiebilanz muss im Stoffwechsel von Milchkühen eine Vielzahl von Anpassungsreaktionen ablaufen, um die Versorgung des Organismus mit Energie sicherzustellen. Dazu gehören zunächst die Mobilisierung von Energie aus in der Leber gespeichertem Glykogen und schließlich die Mobilisierung von Fettsäuren aus den Fettgeweben, durch deren Oxidation Acetyl-CoA entsteht, das wiederum im Citratzyklus oder der Ketogenese genutzt werden kann (Drackley, 1999; Potthoff et al., 2009). Eines der an der Regulation der Stoffwechselvorgänge im Energiemangel beteiligten Hormone ist der FGF21, der in Leber, Pankreas, weißem Fettgewebe und Skelettmuskelgewebe exprimiert wird (Kliewer und Mangelsdorf, 2010). FGF21 ist in der Lage, Stoffwechselwege wie die hepatische Lipidoxidation, die Ketogenese oder die Glukoneogenese zu stimulieren. Außerdem wurde nachgewiesen, dass FGF21 durch den PPARα reguliert wird (Badman et al., 2007; Inagaki et al., 2007). Da in *Studie 3* belegt werden konnte, dass bei Milchkühen, die sich in der Frühlaktation in einer negativen Energiebilanz befinden, der PPARα aktiviert wird und eine Reihe seiner Zielgene hochreguliert werden, wurde die Hypothese aufgestellt, dass auch die Expression des *FGF21* in der Leber der Milchkühe in den ersten Laktationswochen im Vergleich zur Trächtigkeit erhöht sein muss. Um diese Fragestellung zu bearbeiten, wurden im Lebergewebe der Milchkühe aus *Studie 3* die relativen mRNA-Konzentrationen des *FGF21* sowie verschiedener Gene des Fettsäurestoffwechsels, der Glukoneogenese und der Ketogenese bestimmt. Um die Bedeutung des FGF21 für die Anpassungsvorgänge des hepatischen Lipidstoffwechsels zu Laktationsbeginn herauszustellen, wurde außerdem untersucht, ob Beziehungen zwischen der relativen mRNA-Konzentration des *FGF21* in der Leber und verschiedenen Parametern des Lipidstoffwechsels bestanden. Die detaillierte Beschreibung des Versuchs, Material und Methodik sowie die Diskussion der Ergebnisse sind dargelegt in:

Studie 4:

Schlegel G, Ringseis R, Keller J, Schwarz FJ, Windisch W, Eder K (2012) Expression of fibroblast growth factor 21 in the liver of dairy cows in the transition period and during

lactation. *Journal of Animal Physiology and Animal Nutrition.* doi: 10.1111/j.1439-0396.2012.01323.x. Reproduced with permission of John Wiley and Sons.

3. Originalarbeiten

Influence of a rumen-protected conjugated linoleic acid mixture on carcass traits and meat quality in young simmental heifers

G. Schlegel, R. Ringseis, M. Shibani, E. Most, M. Schuster, F. J. Schwarz and K. Eder

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Influence of a rumen-protected conjugated linoleic acid mixture on carcass traits and meat quality in young simmental heifers[1]

G. Schlegel,* R. Ringseis,* M. Shibani,* E. Most,* M. Schuster,† F. J. Schwarz,‡ and K. Eder*[2]

*Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Gießen, 35392 Gießen, Germany; †Department of Quality Assurance and Analysis, Bavarian State Research Center for Agriculture, 85586 Poing, Germany; and ‡Institute of Animal Nutrition, Technical University of Munich, 85354 Freising, Germany

ABSTRACT: The aim of the present study was to investigate the influence of feeding rumen-protected CLA during the early growing period on physical and chemical beef properties in young Simmental heifers. A total of 36 heifers (5 mo old; initial BW 185 ± 21 kg) were fed 250 g of different rumen-protected fats daily for 16 wk in 1 of 3 treatment groups: 250 g of a CLA-free control fat; 100 g of a CLA fat containing 2.4% of *cis*-9,*trans*-11 CLA and 2.1% of *trans*-10,*cis*-12 CLA and 150 g control fat; or 250 g of the CLA fat. Heifer growth performance variables as well as carcass weight, classification (conformation and fatness), and weights of organs and fat depots were not affected ($P > 0.05$) by CLA supplementation. Concentration of *trans*-10,*cis*-12 CLA in tissues (LM and subcutaneous fat) was dose-dependently increased ($P < 0.01$) by CLA supplementation, whereas that of *cis*-9,*trans*-11 CLA in these tissues did not differ ($P > 0.05$) between groups. The ratio of SFA to MUFA was increased ($P < 0.01$) in tissues of CLA-fed heifers compared with control heifers. Concentration of α-tocopherol in LM was greater ($P = 0.01$) in heifers of the 2 CLA groups than in control heifers. Other quality characteristics such as drip loss during storage, cooking loss, intramuscular fat content, and color variables in LM did not differ ($P > 0.05$) between groups. In conclusion, the present study demonstrates that feeding rumen-protected CLA during the early growing period changes tissue fatty acid composition but does not influence beef quality variables. Performance variables and carcass traits in young heifers, unlike in pigs and laboratory animals, are not influenced by CLA feeding.

Keywords: beef, cattle, conjugated linoleic acid, meat quality

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INTRODUCTION

Conjugated linoleic acids have attracted great scientific interest because they possess several beneficial properties, most of which have been observed in laboratory animals (Ha et al., 1987; Lee et al., 1994; Houseknecht et al., 1998). Research in laboratory animals moreover demonstrated that CLA supplementation affects growth and body composition of animals (Chin et al., 1994; Park et al., 1997, 1999). Similar results of CLA supplementation in swine (Dugan et al., 1997; Thiel-Cooper et al., 2001; Wiegand et al., 2001) and improvements of the quality of pork products were observed (Wiegand et al., 2001; Tischendorf et al., 2002; Larsen et al., 2009).

In contrast to information on laboratory animals and swine, limited information is available in the literature about the effects of CLA in beef cattle. The few published studies conducted with beef cattle focus mainly on the effects of rumen-protected CLA on cattle performance, fatty acid composition and CLA content of muscle and adipose tissue, lipid oxidation, and palatability of beef (Gillis et al., 2004a, 2007; Poulson et al., 2004). These studies revealed that feeding rumen-protected CLA did not alter cattle performance or fat and muscle accretion but did cause an increase in CLA content in tissues, altered fatty acid composition of tissues, and influenced sensory attributes of steaks and ground beef. In these recent studies with beef cattle, CLA was fed during the late finishing period and cattle

[1]G. Schlegel was supported by H. Wilhelm Schaumann-Stiftung (Hamburg, Germany).
[2]Corresponding author: klaus.eder@ernaehrung.uni-giessen.de
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were slaughtered at a finish BW typically seen in the US cattle industry. In contrast, no data are available regarding the influence of CLA on growing cattle fed CLA during the early growing period, in which ADG is greater than it is in the late growing period. Therefore, the aim of the present study was to investigate the hypothesis that feeding rumen-protected CLA during the early growing period influences physical and chemical beef properties in young heifers.

MATERIALS AND METHODS

All experimental procedures described followed established guidelines for the care and handling of cattle and were approved by the Animal Care and Use Committee of the Bavarian government.

Animals and Feeding System

A total of 36 Simmental heifers (~5 mo of age) with an initial BW of 185 ± 21 kg were divided into 3 treatment groups (n = 12/group): 250 g of a CLA-free control fat (control); 100 g of a CLA fat containing 2.4% of *cis*-9,*trans*-11 CLA and 2.1% of *trans*-10,*cis*-12 CLA and 150 g control fat (**CLA100**); or 250 g of the CLA fat (**CLA250**). Heifers in each group had similar mean BW. The heifers were allotted to a total of 6 pens; each pen provided 6 lying and 6 feeding places and fully slatted floors. Because of the limited capacity of 1 pen, the heifers of each group (n = 12) were housed in 2 pens with 6 heifers per pen. Heifers were individually granted access to feed by 6 electronically operated Calan gates (American Calan, New Hampshire, USA) per pen. By the use of transponder technique each heifer was matched to 1 individual feeding trough in which a defined amount of maize silage was provided every morning. Individual feed intake was calculated by reweighing the remaining feed. Every pen was equipped with 1 automatic drinking trough, thus ensuring that all heifers had free access to fresh water.

Throughout the experimental period, all heifers received a similar ration consisting of maize silage and concentrate. The concentrate was offered in the morning (0800 h) at a constant amount of 1.5 kg/d during the whole experiment. The 1.5-kg portion of concentrate provided 1,000 g of soybean meal, 250 g of rumen-protected fat, 110 g of wheat, 100 g of vitamin-mineral premix, and 40 g of lime. After consumption of the concentrate, the heifers were provided with their daily allotment of maize silage. The allocation of maize silage was increased during the experiment according to the increase of BW. In the first week of the experiment, the amount of maize silage offered was 3 kg of DM/d. Each 2 to 3 wk, the amount of maize silage was increased by approximately 0.35 kg of DM/d. The final allocation of maize silage was 5 kg of DM/d. The supply of the heifers with nutrients and energy was in accordance with the recommendations of the German Society of Nutrition Physiology (GfE, 1995). The nutrient composition and energy content of the maize silage and concentrate is reported in Table 1. Concentrations of crude nutrients were determined according to Naumann and Bassler (2003). Using the analyzed crude nutrients and the digestibility data of the single feedstuff (DLG, 1997), the ME was calculated (GfE, 1995). Mean intended calculated intakes throughout the trial were 5.38 kg/d for DM, 662 g/d for CP, 833 g/d for MP, 326 g/d for crude ash, 794 g/d for crude fiber, 483 g/d for crude fat, and 65 MJ of ME/d for energy.

Administration of CLA

Dietary fats were supplied by the daily portions of concentrate. The control group received 250 g of LactoPlus 2000 (Bewital, Südlohn, Germany)/d, the CLA100 group received 150 g of LactoPlus 2000 and 100 g of LactoPlus CLA 100 (Bewital)/d, and the CLA250 group received 250 g of LactoPlus CLA 100/d. The composition of the major fatty acids in the fat supplements is reported in Table 2. Absolute daily intake of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA were 2.2 and 1.9 g, respectively, in the CLA100 heifers and 5.5 and 4.8 g, respectively, in the CLA250 heifers. According to Bewital (2009), both CLA isomers of LactoPlus CLA 100 are protected against ruminal hydrogenation processes as evaluated by an in situ technique using duodenally cannulated sheep. Experiments using this technique revealed a bypass value of approximately 70% for both CLA isomers when testing the rumen stability of LactoPlus CLA 100 (Bewital, 2009).

Table 1. Nutrient composition and energy content of the basal feed components (maize silage and concentrates) fed to heifers (n = 12/treatment) during the early growing period[1]

Item	Maize silage	Concentrates
DM, %	36.5	92.1
CP, % of DM	6.9	28.0
MP, % of DM	13.5	21.2
Ash, % of DM	3.4	13.8
Crude fiber, % of DM	18.1	5.1
Crude fat, % of DM	3.9	23.7
ME, MJ/kg of DM	11.2	14.6

[1]The vitamin–mineral premix provided the following micronutrients per kilogram of concentrate: calcium, 12 g; phosphorus, 2.7 g; sodium, 5.3 g; magnesium, 3.3 g; zinc, 670 mg; manganese, 300 mg; copper, 67 mg; iodine, 6.7 mg; cobalt, 2 mg; selenium, 2.7 mg; vitamin A, 67,000 IU; vitamin D_3, 6,700 IU; vitamin E, 200 mg; vitamin B_1, 0.8 mg; vitamin B_2, 2.3 mg; vitamin B_6, 1.7 mg; vitamin B_{12}, 10 µg; nicotinic acid, 11.3 µg; pantothenic acid, 6.7 mg.

Individual feed intake was evaluated daily, whereas BW was determined every 14 d in the morning before feed was administered. Intakes of DM (kg/d) and CP (g/d), energy intake (MJ of ME/d), and ADG (kg) were calculated as measurements of animal performance.

Carcass Data Collection

After 16 wk of feeding the experimental diets, the heifers were slaughtered at the abattoir of the Bavarian State Research Center for Agriculture (Poing, Germany) at a final BW (mean ± SD) of 322 ± 13 kg, 328 ± 17 kg, and 325 ± 15 kg for control, CLA100, and CLA250 heifers, respectively (n = 12/treatment). Carcasses were trimmed and split into 2 halves according to EU legislation. Weights of visceral organs and fat depots were recorded. Weights of hot and chilled carcasses were determined to calculate dressing percentage and 24-h shrinkage percentage. Conformation and fatness scores for each carcass were evaluated by an authorized staff member of the abattoir according to the EUROP carcass classification system. The pH in LM at the 9th rib was determined 45 min and 24 h postmortem using a pH Star 6.05 (Matthäus, Nobitz, Germany).

Meat Quality

One day after slaughter, each left carcass half was ribbed to collect 1 steak (2.5-cm-thick) from LM at the 9th rib for color evaluation and determination of drip loss and cooking loss. Additional samples from LM and subcutaneous adipose tissue were taken at the 11th rib and stored at −20°C for determination of intramuscular fat content, fatty acid composition and α-tocopherol concentration. On the internal surface of the steak, the CIE L*a*b* color space (L* = lightness, a* = redness, b* = yellowness) was evaluated by surface reflectance using a ChromaMeter CM-508i (Minolta, Tokyo, Japan).

Table 2. Fatty acid composition (g/100 g of total fatty acids) of the fat supplements[1] administered to heifers during the early growing period

Item	LactoPlus 2000	LactoPlus CLA 100
C14:0	1.9	1.7
C16:0	59.7	51.4
C18:0	36.4	28.5
C18:1	0.8	9.7
C18:2 n-6	0.1	3.0
C18:3 n-3	0.1	0.2
cis-9,*trans*-11 CLA	—	2.4
trans-10,*cis*-12 CLA	—	2.1
C20:0	0.4	0.4
C20:1	0.6	0.6

[1]LactoPlus 2000 and LactoPlus CLA 100 (Bewital, Südlohn, Germany).

Five measurements of each attribute were taken across the surface of each sample and averaged. Greater L*, a*, and b* values correspond to lighter, redder, and more yellow meat, respectively. Chroma (color intensity) and hue angle (0° = red; 90° = yellow) were calculated as follows: chroma = $\sqrt{(a^{*2} + b^{*2})}$; hue angle = $\tan^{-1}(b^*/a^*)$ (Macdougall and Rhodes, 1972).

For the determination of drip loss, every steak was weighed, suspended in a plastic pouch to prevent contact with the released exudates, and stored at 4°C in a refrigerator. Samples were reweighed on d 14 postslaughter to determine drip loss during storage as a percentage of initial weight. After the 14-d storage period, the 2.5-cm-thick steak was placed in a polyethylene bag and cooked for 40 min to a core temperature of 70°C using a water bath. The core temperature was controlled using Testo 926 thermocouples (Testo, Lenzkirch, Germany). After cooking, the fluid was poured from the bags and, after being chilled, the samples were patted dry with a paper towel and reweighed to determine cooking loss, which was expressed as a percentage of uncooked sample weight. The intramuscular fat content was determined gravimetrically in homogenized LM samples after hydrolysis with 4 *N* HCl at 185°C for 45 min and subsequent Soxhlet extraction of the neutral hydrolysate with petroleum ether for 7 h.

Concentration of α-tocopherol in LM was determined by means of HPLC with fluorescence detection according to Catignani and Bieri (1983) and Cort et al. (1983). To saponify α-tocopherylacetate, LM samples were mixed with 1 mL of ethanol (absolute) containing 0.05% butylated hydroxy toluene and 150 μL of saturated sodium hydroxide solution. This mixture was heated for 30 min at 70°C, and tocopherols were extracted with n-hexane. The n-hexane phase was evaporated under a stream of nitrogen, and tocopherols were redissolved in methanol containing 0.05% butylated hydroxy toluene. Individual tocopherols of the extracts were separated isocratically using methanol as mobile phase at a flow rate of 1.7 mL/min and a C-18-reversed phase column (Purospher 100 RP-18; Merck-Hitachi, Darmstadt, Germany) and detected by fluorescence (excitation wavelength: 295 nm; emission wavelength: 325 nm). Concentration of α-tocopherol was calculated by an external calibration curve.

For determination of fatty acid composition of LM and subcutaneous adipose tissue lipids, total lipids from homogenized LM and subcutaneous adipose tissue were extracted with a mixture of hexane and isopropanol (3:2 vol/vol; Hara and Radin, 1978) and methylated with trimethylsulfonium hydroxide (Butte, 1983). Fatty acid methyl esters were separated by gas chromatography using a Chrompack 9000 system (Waldbronn, Germany) equipped with an automatic split injector, a polar capil-

Table 3. Performance variables of young Simmental heifers (n = 12/treatment) receiving 1 of 3 treatments

Item	Treatment[1]			SEM	*P*-value
	Control	CLA100	CLA250		
Total BW gain, kg	138	141	140	4.0	0.80
ADG, kg/d	1.18	1.21	1.21	0.031	0.75
DMI, kg/d	5.55	5.56	5.47	0.033	0.12
CP intake, g/d	798	798	791	2.6	0.10
MP intake, g/d	854	855	842	4.5	0.10
Energy intake, MJ of ME/d	68.3	68.4	67.4	0.37	0.10
Feed conversion, kg of DM/kg of BW gain	4.75	4.63	4.56	0.136	0.62

[1]Control = 250 g of a rumen-protected control fat/d; CLA100 = 150 g of rumen-protected control fat and 100 g of a rumen-protected CLA fat/d; CLA250 = 250 g of a rumen-protected CLA fat/d.

lary column (60 m free fatty acid phase, 0.25 mm i.d., 0.25 µm film thickness; Macherey and Nagel, Düren, Germany), and a flame ionization detector. Hydrogen was used as the carrier gas with a flow rate of 1 mL/min. Individual fatty acid methyl esters were identified by comparing their retention times with those of individually purified standards.

Statistical Analysis

Data were subjected to 1-way ANOVA using the Minitab Statistical Software Release 13.0 (Minitab, State College, PA) according to the 1-factorial design of the study with treatment (control vs. CLA100 vs. CLA250) as experimental factor. All data presented are means and SEM (n = 12 heifers/group). For statistically significant *F*-values, individual means of the treatment groups were compared by Fisher's multiple range test. Means were considered significantly different at $P < 0.05$.

RESULTS

Animal Performance, Carcass Traits, and Meat Quality

All heifer performance variables investigated, such as total BW gain, ADG, DMI, CP intake, MP intake, energy intake, and feed conversion, were not affected ($P > 0.05$) by CLA supplementation (Table 3). Hot and chilled carcass weight, dressing percentage, carcass classification scores, pH 45 min postmortem, LM area, and weights of organs and fat depots were not affected ($P > 0.05$) by CLA supplementation (Table 4). The pH 24 h postmortem was less ($P = 0.05$) in the 2 CLA groups than in the control group (Table 5).

Drip loss, cooking loss, intramuscular fat content, and color variables in LM did not differ ($P > 0.05$) between heifers of the control group and both CLA groups (Table 5). The concentration of α-tocopherol in LM was greater ($P = 0.014$) in heifers of the 2 CLA groups than in those of the control group. The concentration of α-tocopherol in LM did not differ ($P = 0.08$) between CLA100 and CLA250.

Fatty Acid Composition of LM

Inclusion of CLA in the diets increased ($P = 0.005$) the proportion of *trans*-10,*cis*-12 CLA in LM lipids in a dose-dependent manner. In contrast to *trans*-10,*cis*-12 CLA, the proportion of *cis*-9,*trans*-11 CLA in LM lipids was not influenced ($P = 0.13$) by inclusion of CLA in the diets (Table 6). The proportions of the individual SFA C18:0 and C20:0 in LM lipids were increased ($P < 0.05$) but the proportions of the individual MUFA C18:1 n-9 and total MUFA in LM lipids were decreased ($P < 0.05$) in heifers from the CLA250 group compared with those fed the control diet (Table 6). In the CLA100 heifers, the proportions of individual and total MUFA in LM lipids

Table 4. Carcass characteristics and weights of fat depots of selected visceral organs of young Simmental heifers (n = 12/treatment) receiving 1 of 3 treatments

Item	Treatment[1]			SEM	*P*-value
	Control	CLA100	CLA250		
HCW, kg	162	163	160	2.7	0.78
Chilled carcass weight, kg	159	159	157	2.6	0.77
Dressing percentage, %	52.4	51.8	51.2	0.38	0.10
Carcass classification					
Conformation[2]	3.17	3.42	3.58	0.137	0.11
Fatness[3]	2.25	2.25	2.08	0.153	0.68
pH 45 min postmortem	6.90	6.88	6.92	0.022	0.56
pH 24 h postmortem	5.63[a]	5.57[b]	5.58[b]	0.017	0.05
LM area, cm	35.2	34.5	34.8	1.39	0.95
Liver weight, kg	3.76	3.72	3.81	0.079	0.72
Kidney weight, kg	0.69	0.73	0.71	0.026	0.65
Kidney tallow, kg	4.24	3.54	4.64	0.336	0.08
Pelvic tallow, kg	0.43	0.41	0.46	0.039	0.62

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Control = 250 g of a rumen-protected control fat/d; CLA100 = 150 g of rumen-protected control fat and 100 g of a rumen-protected CLA fat/d; CLA250 = 250 g of a rumen-protected CLA fat/d.

[2]1 = excellent; 5 = poor.

[3]1 = very low fat; 5 = very high fat.

Table 5. Meat quality variables of LM of young Simmental heifers (n = 12/treatment) receiving 1 of 3 treatments

Item	Treatment[1]			SEM	*P*-value
	Control	CLA100	CLA250		
Drip loss, %	3.93	4.18	4.19	0.382	0.86
Cooking loss, %	23.6	24.5	25.1	1.03	0.60
Intramuscular fat, %	1.65	1.54	1.54	0.116	0.77
α-Tocopherol, mg/kg	0.55[b]	0.80[a]	0.82[a]	0.066	0.014
Color measurement value					
Lightness (L*)	39.5	39.9	40.0	0.34	0.61
Redness (a*)	8.75	8.44	8.37	0.308	0.66
Yellowness (b*)	3.97	4.03	3.83	0.206	0.78
Saturation	9.59	9.37	9.34	0.351	0.87
Hue angle	24.1	24.8	24.5	1.00	0.89

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Control = 250 g of a rumen-protected control fat/d; CLA100 = 150 g of rumen-protected control fat and 100 g of a rumen-protected CLA fat/d; CLA250 = 250 g of a rumen-protected CLA fat/d.

did not differ from those in the control heifers (Table 6). Proportions of all the individual PUFA and total PUFA in LM lipids did not differ ($P > 0.05$) between treatment groups. The Δ^9-desaturase index (total SFA:total MUFA) in LM lipids was greater ($P = 0.009$) in heifers from the CLA250 group than in control heifers. Other fatty acids did not differ ($P > 0.05$) between groups.

Fatty Acid Composition of Subcutaneous Adipose Tissue

As in LM lipids, inclusion of CLA in the diets increased ($P < 0.001$) the proportion of *trans*-10,*cis*-12 CLA in subcutaneous adipose tissue (Table 7). The increase in the proportion of *trans*-10,*cis*-12 CLA in subcutaneous adipose tissue by CLA was dose dependent. Heifers in the CLA250 treatment group had a greater ($P = 0.004$) proportion of *trans*-10,*cis*-12 CLA than the CLA100 heifers (Table 7). The proportion of *cis*-9,*trans*-11 CLA in subcutaneous adipose tissue did not differ ($P > 0.05$) between the 3 treatment groups. The proportions of the individual SFA C16:0 and total SFA in subcutaneous adipose tissue were increased ($P < 0.05$) but the proportions of the individual MUFA C14:1 and C18:1 n-9 and total MUFA in subcutaneous adipose tissue were decreased ($P < 0.05$) in heifers fed the CLA diets compared with heifers fed the control diet (Table 7). The proportions of these fatty acids in subcutaneous adipose tissue did not differ ($P > 0.05$) between CLA250 and CLA100 groups. Proportions of all the individual PUFA and total PUFA in subcutaneous adipose tissue did not differ ($P > 0.05$) between treatment groups. The Δ^9-desaturase index (total SFA:total MUFA) and the total SFA:total PUFA in subcutaneous adipose tissue were greater ($P < 0.05$) in heifers fed the CLA diets than in heifers fed the control diet (Table 7).

Table 6. Fatty acid composition of LM lipids of young Simmental heifers (n = 12/treatment) receiving 1 of 3 treatments

Item,[1] % of total fatty acid methyl esters	Treatment[2]			SEM	*P*-value
	Control	CLA100	CLA250		
C14:0	2.21	2.12	2.11	0.101	0.77
C14:1	0.41	0.40	0.35	0.026	0.28
C15:0	0.32	0.32	0.33	0.012	0.79
C15:1	0.13	0.13	0.15	0.008	0.26
C16:0	26.4	26.2	27.2	0.58	0.47
C16:1 n-7	3.16	2.94	2.82	0.100	0.08
C17:0	0.80	0.82	0.86	0.024	0.20
C17:1	0.48	0.48	0.46	0.017	0.62
C18:0	17.0[b]	17.8[ab]	19.5[a]	0.63	0.026
C18:1 n-9	34.7[a]	33.2[ab]	31.0[b]	0.70	0.006
C18:2 n-6	6.27	7.05	6.69	0.645	0.70
cis-9,*trans*-11 CLA	0.44	0.38	0.35	0.028	0.13
trans-10,*cis*-12 CLA	0.08[b]	0.13[a]	0.16[a]	0.014	0.005
C18:3 n-3	0.32	0.37	0.35	0.024	0.48
C20:0	0.15[b]	0.16[ab]	0.18[a]	0.009	0.023
C20:1	0.18	0.18	0.18	0.010	0.91
C20:3 n-6	0.75	0.76	0.72	0.100	0.97
C20:4 n-6	2.83	3.09	2.98	0.317	0.86
C22:1	0.13	0.16	0.16	0.025	0.74
C24:0	0.41	0.47	0.51	0.047	0.32
Total SFA	49.8	50.5	53.4	1.08	0.08
Total MUFA	39.3[a]	37.5[a]	35.2[b]	0.72	0.004
Total PUFA	10.9	12.0	11.5	1.11	0.79
Total SFA:total MUFA	1.29[b]	1.36[ab]	1.52[a]	0.047	0.009
Total SFA:total PUFA	5.36	4.50	5.17	0.518	0.49

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Fatty acids <0.1% of total fatty acid methyl esters are not reported.

[2]Control = 250 g of a rumen-protected control fat/d; CLA100 = 150 g of rumen-protected control fat and 100 g of a rumen-protected CLA fat/d; CLA250 = 250 g of a rumen-protected CLA fat/d.

DISCUSSION

The present study aimed to investigate the influence of feeding rumen-protected CLA during the early growing period on physical and chemical beef properties of young heifers. For this purpose, heifers were fed diets supplemented with 100 or 250 g of rumen-protected CLA supplements. For technical reasons, we were not able to determine the amounts of the CLA isomers reaching the small intestine. According to the supplier of this supplement, approximately 70% of both CLA isomers are protected against rumen biohydrogenation (Bewital, 2009). Assuming a bypass value of 70%, daily amounts reaching the small intestine were 1.5 and 3.9 g for *cis*-9,*trans*-10 CLA and 1.3 and 3.4 g for *trans*-10,*cis*-12 CLA in the groups receiving 100 or 250 g of rumen-protected CLA supplements, respectively. The duodenal flow of CLA isomer from the basal diet depends on the composition of the diet. It has been shown that an increase of the proportion of

Table 7. Fatty acid composition of subcutaneous adipose tissue lipids of young Simmental heifers (n = 12/treatment) receiving 1 of 3 treatments

Item,[1] % of total fatty acid methyl esters	Treatment[2]			SEM	*P*-value
	Control	CLA100	CLA250		
C14:0	3.00	3.10	3.21	0.131	0.53
C14:1	0.98[a]	0.82[ab]	0.69[b]	0.078	0.047
C15:0	0.29	0.31	0.33	0.012	0.11
C15:1	0.18	0.18	0.20	0.011	0.52
C16:0	25.5[b]	27.0[a]	27.6[a]	0.46	0.010
C16:1 n-7	5.18	4.83	4.91	0.246	0.59
C17:0	0.68	0.69	0.71	0.027	0.76
C17:1	0.65	0.59	0.61	0.025	0.36
C18:0	14.8	16.2	16.3	0.53	0.12
C18:1 n-9	45.2[a]	42.8[b]	42.1[b]	0.57	0.002
C18:2 n-6	1.67	1.70	1.52	0.086	0.28
cis-9,*trans*-11 CLA	0.46	0.33	0.31	0.025	0.06
trans-10,*cis*-12 CLA	0.02[b]	0.05[b]	0.08[a]	0.005	<0.001
C18:3 n-3	0.15	0.15	0.14	0.007	0.29
C20:0	0.11	0.13	0.13	0.009	0.28
C20:1	0.30	0.26	0.27	0.018	0.40
C24:0	0.18	0.20	0.19	0.017	0.71
Total SFA	45.0[b]	48.0[a]	48.9[a]	0.67	<0.001
Total MUFA	52.5[a]	49.6[b]	48.9[b]	0.64	<0.001
Total PUFA	2.49	2.42	2.23	0.117	0.28
Total SFA:total MUFA	0.86[b]	0.97[a]	1.00[a]	0.025	<0.001
Total SFA:total PUFA	18.8[b]	20.1[ab]	22.6[a]	1.14	0.049

[a,b]Means within a row without a common superscript differ ($P < 0.05$).

[1]Fatty acids <0.1% of total fatty acid methyl esters are not reported.

[2]Control = 250 g of a rumen-protected control fat/d; CLA100 = 150 g of rumen-protected control fat and 100 g of a rumen-protected CLA fat/d; CLA250 = 250 g of a rumen-protected CLA fat/d.

concentrate and supplementation of vegetable oils increases duodenal flow of CLA (Piperova et al., 2002; Sackmann et al., 2003; Loor et al., 2004). Nevertheless, duodenal flow of CLA from basal diets is generally low. Sackmann et al. (2003), using diets consisting of various amounts of forage, supplemented with sunflower oil, found that <0.23% of the total linoleic acid is converted into the rumen in beef steers regardless of dietary forage or oil content. In their study, duodenal flow of *cis*-9,*trans*-10 CLA and *trans*-10,*cis*-12 CLA was 0.22 and 0.07 g/d, respectively, at a forage content of 36% and a DMI of 5.5 kg/d. Similar low duodenal flows of these 2 CLA isomers from the basal diet were found in other studies in dairy cows or steers (Duckett et al., 2002; Piperova et al., 2002; Loor et al., 2004). Average DMI of the heifers in this study was similar to that in the study of Sackmann et al. (2003). However, because the proportion of concentrate in the total diet was low and no vegetable oil was supplemented, we assume that the duodenal flow of *cis*-9,*trans*-10 CLA and *trans*-10,*cis*-12 CLA in the control group of the present study might have been even less than in the study of Sackmann et al. (2003).

According to the experimental design, age and BW of heifers at slaughter were markedly less than that reported in recent studies in which cattle were slaughtered in the United States, where cattle are slaughtered at greater age (~12–24 mo of age) and greater BW (499–590 kg; Gillis et al., 2004b, 2007; Poulson et al., 2004). Thus, it was not surprising that dressing percentage, fatness, weights of kidney and pelvic tallow, and intramuscular fat content, which are known to be less in young cattle (Levi et al., 1967), were less than those reported in recent studies with beef cattle of heavy BW. The concentrations of *trans*-10,*cis*-12 CLA in LM were 63 and 100% greater ($P < 0.05$) in heifers from the CLA100 and CLA250 groups, respectively, than in control heifers. In subcutaneous adipose tissue, concentrations of *trans*-10,*cis*-12 CLA were 150 and 300% greater ($P < 0.05$) in heifers from the CLA100 and CLA250 groups, respectively, than in control heifers. Increased tissue concentrations of *trans*-10,*cis*-12 CLA were also observed in several other studies with cattle (Gillis et al., 2004a, 2007; Poulson et al., 2004), broilers (Suksombat et al., 2007), and pigs (Eggert et al., 2001; White et al., 2009) receiving supplemental CLA. Our findings therefore confirm these recent observations that CLA feeding is an effective strategy for increasing tissue CLA content in ruminants, like in nonruminants, provided that the CLA supplement is protected against ruminal biohydrogenation. Nevertheless, the absolute concentration of *trans*-10,*cis*-12 CLA in LM from CLA-fed heifers was insignificant, indicating that CLA uptake resulting from consumption of CLA-enriched beef is very limited when compared with CLA uptake from consumption of CLA supplements designated for human nutrition. It is therefore unlikely that consumption of such beef would result in any metabolic effects. In contrast to *trans*-10,*cis*-12 CLA, the concentration of *cis*-9,*trans*-11 CLA, which was the main CLA isomer in LM and subcutaneous adipose tissue of the heifers, was not increased ($P = 0.13$) by the CLA supplement. This is in contrast to findings in nonruminants (Eggert et al., 2001; Suksombat et al., 2007; White et al., 2009). However, the lack of increase of *cis*-9,*trans*-11 CLA after supplementation of CLA within the present study is in agreement with other studies with cattle (Gillis et al., 2004a; Poulson et al., 2004) demonstrating either no or marginal increases of tissue concentration of *cis*-9,*trans*-11 CLA by CLA supplementation. The *cis*-9,*trans*-11 CLA can be formed endogenously by Δ^9-desaturation of trans-vaccenic acid that is catalyzed by Δ^9-desaturase. This enzyme is present in several bovine tissues, including mammary gland, skeletal muscle, and adipose tissue (Griinari et al., 2000; Jiang et al., 2008), and is primarily responsible for the desaturation of stearic acid

(C18:0) to oleic acid (C18:1), the predominant fatty acid in bovine tissues. Research in dairy cattle has reported that up to 80% of the CLA in milk fat originates from desaturation of transvaccenic acid (Griinari et al., 2000; Corl et al., 2001). In beef cattle, approximately 90% of total CLA in tissues were estimated to originate from Δ^9-desaturation (Gillis et al., 2004a). Because CLA feeding was reported to inhibit Δ^9-desaturation, it is likely that endogenous formation of *cis*-9,*trans*-11 CLA in tissues was reduced in the CLA-fed heifers because of a decreased conversion of transvaccenic acid to *cis*-9,*trans*-11 CLA. This and other studies with cattle (Gillis et al., 2004a; Poulson et al., 2004) found that concentrations of *cis*-9,*trans*-11 CLA in tissues were either not or marginally altered by supplemental CLA. Probably, an increased uptake of *cis*-9,*trans*-11 CLA from the CLA supplement and its incorporation into the tissue lipids compensated for the reduced endogenous formation of *cis*-9,*trans*-11 CLA. A strong indication that Δ^9-desaturase in LM and adipose tissues of heifers was indeed reduced by CLA feeding was the observation that the proportions of C18:1 and total MUFA were reduced ($P < 0.05$) whereas those of C18:0 and total SFA were increased ($P < 0.05$) in LM and subcutaneous adipose tissue in CLA-fed heifers. Similar changes in fatty acid composition of tissue lipids by CLA feeding were also reported in several other studies with cattle (Gillis et al., 2004a), pigs (Bee, 2000; Eggert et al., 2001; Smith et al., 2002), broilers (Suksombat et al., 2007), and rats (Eder et al., 2005) and have been explained by inhibition of Δ^9-desaturase activity by CLA, which was reported in CLA-fed pigs (Smith et al., 2002). In the present study, the Δ^9-desaturase index (total SFA:total MUFA) in LM and subcutaneous adipose tissue was also greater ($P < 0.05$) in CLA-fed heifers than in control heifers, which is also indicative of inhibition of Δ^9-desaturase. Nevertheless, recent studies in steers revealed that indices of Δ^9-desaturase activity based on fatty acid concentrations do not accurately reflect Δ^9-desaturase enzyme activity (Archibeque et al., 2005). Thus, future studies have to demonstrate whether CLA feeding indeed inhibits Δ^9-desaturase activity in tissues of young heifers.

The main finding of the present study is that dietary CLA fed during the early growing period has limited to no influence on physical beef properties in young beef cattle, which is in contrast to findings in pigs (Wiegand et al., 2001; Joo et al., 2002; Larsen et al., 2009) and broilers (Suksombat et al., 2007). The only alteration in beef quality variables was related to pH 24 h postmortem. The reason for this, however, remains unexplained. Regardless, the pH 24 h postslaughter was within the normal range in all treatment groups. So far, comparable data regarding physical beef quality variables from CLA-fed beef cattle are not available in the literature. There are only data from sensory analysis of steaks and ground beef from CLA-fed heifers (Gillis et al., 2007). According to the latter study, juiciness and initial tenderness of ground beef were decreased by CLA supplementation (Gillis et al., 2007).

Drip loss is an indicator of the amount of free water in fresh meat, whereas cooking loss describes the extent to which bound water is lost after heating. It has been reported that water-holding capacity and intramuscular fat content of meat are positively correlated (Joo et al., 2000). Drip loss was reported to be decreased in highly marbled pork when compared with pork with less marbling (Joo et al., 2000). Our observation that intramuscular fat content was not influenced by feeding rumen-protected CLA supplement compared with the control fat is therefore in line with the unaltered water-holding capacity of LM in CLA-fed heifers. Similar findings were also reported in feeding experiments with pigs where CLA supplementation did not influence these measures of water-holding capacity in meat (Eggert et al., 2001; Janz et al., 2008; White et al., 2009). Thus, our findings suggest that CLA supplementation in young heifers, like in pigs, has no influence on the water-holding capacity of the meat.

In addition, the CLA supplement had no influence on 24 h-postslaughter beef color variables in the LM. This finding in young heifers is also consistent with observations in steer calves (Poulson et al., 2004) and pigs (Wiegand et al., 2002; Dugan et al., 2003; Janz et al., 2008), where meat color variables 24 h postslaughter were not influenced by CLA supplementation. However, in the study with steer calves (Poulson et al., 2004) it could be reported that supplementation of rumen-protected CLA during the finishing period decreased the red and yellow color of vacuum-packed steaks during a 15-d storage period at 3°C, indicating that that CLA supplementation influences color stability. The reduced yellow color stability could not be explained in a study conducted by Poulson et al. (2004); however, the decrease in red color by CLA supplementation during storage has been proposed to be attributable to increased intramuscular lipid oxidation (Poulson et al., 2004).

In the present study, concentrations of α-tocopherol in LM were increased ($P = 0.014$) in heifers fed rumen-protected CLA compared with control heifers. However, α-tocopherol concentrations in LM were less than in other studies (Arnold et al., 1993; Vega et al., 1997; Lynch et al., 1999). It is possible that the increased LM vitamin E concentrations in CLA-fed heifers in our study were attributable to an induction of hepatic α-tocopherol transfer protein as observed recently in mice (Chao et al., 2010) because this would protect α-tocopherol from degradation in the liver and thereby contribute to increased extrahepatic α-tocopherol concentrations. Future stud-

ies must clarify whether induction of α-tocopherol transfer protein in response to CLA also occurs in cattle. Regardless, the increase in LM vitamin E concentration of CLA-fed heifers indicates that protective mechanisms against lipid oxidation were improved by CLA supplementation. In addition, the proportions of PUFA in tissue lipids, which reduce oxidative stability of tissue lipids, were not altered by CLA supplementation.

Finally, the present study demonstrates that CLA supplementation has no influence on performance variables such as BW gain or feed conversion efficiency or on carcass traits such as conformation or fatness in young heifers. These findings in heifers are in strong contrast to observations in mice, rats, and pigs where CLA supplementation caused a marked improvement of feed efficiency, increased whole-body protein, and strongly reduced body fat accretion (Park et al., 1997; Azain et al., 2000; Sugano et al., 2001). However, our results are in line with those from other studies showing that rumen-protected CLA has either no or marginal effects on performance characteristics in beef cattle (Gassman et al., 2000; Gillis et al., 2004b).

In conclusion, the present study shows that feeding rumen-protected CLA to heifers during the early growing period alters fatty acid composition (increase of SFA, decrease of MUFA) of LM and subcutaneous adipose tissue. These CLA-induced changes in fatty acid composition were not accompanied by alterations of beef quality variables. Results indicated that performance variables and carcass traits in young heifers of light BW, like in older heifers of heavy BW, are not influenced by CLA feeding.

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Errata

The following articles were published with the incorrect DOI. The correct DOI numbers are listed below for each article. The DOI numbers have been corrected in the online version of the articles.

"Administration of estradiol, trenbolone acetate, and trenbolone acetate/estradiol implants alters adipogenic and myogenic gene expression in bovine skeletal muscle" (J. Anim. Sci 90:1421–1427). Correct DOI is as follows: doi:10.2527/jas2010-3496.

"Phosphorus requirements for 60 to 100 kg pigs selected for high lean deposition under different thermal environments" (J. Anim. Sci 90:1499–1505). Correct DOI is as follows: doi:10.2527/jas2010-3623.

"Effect of the administration program of two beta-adrenergic agonists on growth performance, carcass, and meat characteristics of feedlot ram lambs" (J. Anim. Sci 90:1521–1531). Correct DOI is as follows: doi:10.2527/jas2010-3513.

"Influence of a rumen-protected conjugated linoleic acid mixture on carcass traits and meat quality in young Simmental heifers" (J. Anim. Sci 90:1532–1540). Correct DOI is as follows: doi:10.2527/jas2010-3617.

doi:10.2527/jas2011-4571

"Obesity in dogs and cats: What is wrong with being fat?" (J. Anim. Sci. 90:1653–1662). There is an incorrect statement saying that obesity increases the risk for diabetes in cats 8 fold. This is stated in both the text on page 5, and in the abstract. The text and abstract should read that obesity increases the risk for diabetes in cats 4 fold, rather than 8 fold. The author regrets the error.

J. Dairy Sci. 95:3905–3918
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Effects of a rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows

G. Schlegel,* R. Ringseis,* W. Windisch,† F. J. Schwarz,† and K. Eder*[1]
*Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany
†Department of Animal Sciences, Chair of Animal Nutrition, Technical University of Munich, Liesel-Beckmann-Straße 6, D-85350 Freising-Weihenstephan, Germany

ABSTRACT

Supplementation of conjugated linoleic acids (CLA) reduces milk fat content in dairy cows and, thus, may be a useful dietary strategy to improve energy balance during early lactation. The present study was performed to investigate whether supplementation of CLA could have adverse effects on hepatic lipid metabolism such as observed in rodents. For this aim, 40 Holstein cows were allotted to 2 groups, which were fed daily 172 g of either a CLA-free, rumen-protected control fat (control group) or a rumen-protected CLA fat supplying 4.3 g of *cis*-9,*trans*-11 CLA and 3.8 g of *trans*-10,*cis*-12 CLA per day (CLA group). To identify potential changes of lipid metabolism, expression of several genes involved in lipid metabolism was determined in liver biopsy samples taken at wk 5 of lactation, using a whole-genome gene chip. In the CLA group, milk fat content and daily milk fat yield were lower than in the control group. Milk yield was higher, whereas fat-corrected milk and energy-corrected milk were lower in the CLA group than in the control group. The CLA group, moreover, had an improved energy balance. To study potential effects of CLA on hepatic lipid metabolism, we considered 6 genes encoding fatty acid transporters, 7 genes involved in intracellular fatty acid transport, 21 and 7 genes, respectively, involved in mitochondrial and peroxisomal β-oxidation, 6 genes of carnitine metabolism, 3 genes of ketogenesis, 21 genes involved in fatty acid and triacylglycerol synthesis, 17 genes involved in cholesterol metabolism, and 20 genes involved in lipoprotein metabolism. None of these genes was differentially regulated between the CLA group and the control group. Gene chip data were confirmed by quantitative PCR analysis, which revealed no difference in the expression of key enzymes of various pathways such as lipogenesis, β-oxidation, and ketogenesis between the 2 groups of cows. In line with those findings, concentrations of triacylglycerols and cholesterol in liver and plasma were not different between the 2 groups of cows. In conclusion, the present study shows that CLA supplementation at a dose effective for milk fat depression does not induce adverse effects on hepatic lipid metabolism in dairy cows.
Key words: conjugated linoleic acid, dairy cow, gene expression, hepatic lipid metabolism

INTRODUCTION

During early lactation, dairy cows are typically in a negative energy balance (**NEB**) as food intake capacity is limited and the amount of energy consumed does not meet the high energy requirement for maintenance and milk production (Drackley, 1999). As NEB in dairy cows is associated with metabolic disorders and reproductive failures, attempts are made to reduce the gap between energy intake and energy requirement. One of them is to increase energy intake by elevating the amounts of concentrates or by addition of fats. However, these dietary strategies have several drawbacks such as a reduction of DMI or an increased risk of rumen acidosis (Hayirli and Grummer, 2004; Kay et al., 2006). Thus, a more promising attempt to improve NEB is to lower energy output via the milk (e.g., by reduction of the content of fat, the nutrient which is most variable and moreover represents the major energy cost in milk production; Bauman and Davis, 1974; Kay et al., 2006). The use of dietary conjugated linoleic acid (**CLA**) supplements is well known to lower milk fat content, not only in dairy cows but also in several other species such as pigs or rats, with *trans*-10,*cis*-12 CLA being the isomer responsible for this effect (Bauman et al., 2008). It has been well established that *trans*-10,*cis*-12 CLA lowers milk fat content mainly by an inhibition of de novo FA synthesis in the mammary gland, and also by a reduction of uptake of FA from triacylglycerol-rich lipoproteins due to inhibition of lipoprotein lipase (Bauman et al., 2011). Recently, it has been shown that the use of dietary CLA supplements indeed improves NEB by decreasing energy output via the milk and,

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[1]Corresponding author: klaus.eder@ernaehrung.uni-giessen.de

thus, provides a promising dietary strategy to prevent health problems associated with NEB in dairy cows (Kay et al., 2006; Odens et al., 2007; Schwarz et al., 2007). However, for practical use, aspects of safety must be considered, particularly with respect to possible adverse side effects of CLA on metabolism.

Studies in laboratory animals such as rats and mice have shown that CLA exhibits several beneficial properties, such as anticarcinogenic, antiatherogenic, and antidiabetogenic effects (Ha et al., 1987; Lee et al., 1994; Houseknecht et al., 1998). However, besides these beneficial effects, some serious adverse side effects were observed. In mice, supplementation of *trans*-10,*cis*-12 CLA led to dramatic changes in hepatic lipid metabolism, resulting in the development of fatty liver (Belury and Kempa-Steczko, 1997; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002). Interestingly, this phenomenon was not observed in other species such as rats or hamsters (Stangl, 2000; Giudetti et al., 2005; Miranda et al., 2009), indicating that the response to CLA on hepatic lipid metabolism is species specific.

During early lactation, hepatic lipid metabolism is generally stressed by a great amount of FA released from adipose tissue and subsequently taken up into the liver. These FA cannot be completely oxidized or released into the blood as triacylglycerols (**TAG**) via very low-density lipoproteins (**VLDL**). Thus, during early lactation, TAG accumulate in the liver (Katoh, 2002). Therefore, a potential adverse effect of CLA on hepatic lipid metabolism during this critical phase requires particular attention in dairy cows. In dairy cows, many studies have been performed to investigate metabolic effects of dietary CLA. Most of these studies focused on the effects of CLA on the metabolism of mammary gland, particularly milk fat synthesis and fat content of milk (e.g., Baumgard et al., 2002b; Loor and Herbein, 2003; Moore et al., 2004; Piperova et al., 2004; Moore et al., 2005; Odens et al., 2007; Perfield et al., 2007; Medeiros et al., 2010; Weerasinghe et al., 2012) and the metabolism of adipose tissue (e.g., Baumgard et al., 2002a, Harvatine et al., 2009; Akter et al., 2011). In contrast, a few studies dealing with the effect of CLA on hepatic metabolism show that supplementation of CLA does not influence hepatic total lipid content (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005). However, as lipid metabolism is very complex and includes several pathways such as uptake, oxidation, and synthesis of FA, assembly and secretion of lipoproteins or ketogenesis, the finding of an unchanged hepatic TAG concentration does not necessarily mean that CLA has no adverse effects on specific pathways of lipid metabolism. The effect of CLA on hepatic lipid metabolism, however, has not yet been investigated in detail. Therefore, the present study aimed to investigate the effect of a rumen-protected CLA mixture at a level sufficient to achieve decrease in milk fat on hepatic lipid metabolism. For this purpose, the expression profile of several genes involved in hepatic lipid metabolism was considered, thus allowing us to detect potential changes of critical pathways of lipid metabolism.

MATERIALS AND METHODS

The animal experiment was conducted at the Agricultural Experimental Station Hirschau of the Technical University of Munich, Germany. It was approved by the Bavarian state animal care and use committee.

Animals

Forty primi- and multiparous Holstein cows (2.6 ± 1.1 parities, mean ± SD) were used as experimental animals for this study. The experimental period ranged from wk 3 prepartum to wk 14 postpartum. The lactating herd was housed in a freestall barn with individual feeding places. From 7 d before expected calving until 5 d postpartum, animals were fed individually in calving pens with straw bedding. The animals were allocated to 2 experimental groups. The control group was composed of 4 primiparous and 16 multiparous (2.7 ± 1.1 parities) dairy cows; the treatment group (CLA) included 5 primiparous and 15 multiparous (2.5 ± 1.2 parities) dairy cows.

Feeding Regimen

Animals received a partial mixed ration (**PMR**) for ad libitum intake of basic feed with separate and limited intake of concentrates. The PMR was calculated to meet the demands for energy and protein of a cow (650 kg of BW) producing 21 kg of milk/d with an assumed DMI of 16 kg of DM/d. The PMR consisted of 33.7% grass silage, 44.9% maize silage, 6.4% hay, and 14.9% concentrate (DM basis). Partial mixed ration intake was recorded individually by using feed bins that were connected to electronic balances. In addition to the PMR, concentrate (**CONC**) was offered individually in transponder-access feeding stations by an automatic feeding program (Alpro; DeLaval GmbH, Glinde, Germany). Concentrate was composed of 24.8% maize, 21.8% wheat, 20.1% soybean meal, 15.2% dried sugar beet pulp with molasses, 14.9% barley, and 3.2% vitamin-mineral premix (R-Lactol, Mineralfutter 2 für Rinder, RKW Süd, Würzburg, Germany) including limestone (DM basis). In the first 5 d of lactation, the cows received 1.2 kg of CONC DM/d. On d 6 postpartum, cows were fed 1.8 kg of CONC DM/d, which

Table 1. Energy and nutrient concentrations of partial mixed ration (PMR), supplemental concentrate (SUPP), and individually allocated concentrate (CONC)

Item	PMR	SUPP	CONC
Energy[1] (MJ of NE_L/kg of DM)	6.45	12.8	8.00
Crude fiber (g/kg of DM)	214	69	67
Crude ash (g/kg of DM)	81	49	72
Crude fat (g/kg of DM)	32	303	20
CP (g/kg of DM)	129	140	184
Available CP[1] (g/kg of DM)	142	151	187

[1]Calculated values.

was increased up to 6.2 kg of DM/d in the primiparous cows or 8.0 kg of DM/d in the multiparous cows in the following 35 d. Thereafter, CONC was fed according to individual extra requirements for milk production. Nutrient concentrations and energy content of the feed components are shown in Table 1. Calculations for energy and protein supply followed the recommendations of the German Society of Nutrition Physiology (**GfE**, 2001).

Administration of Control Fat or CLA

The dietary fats (control fat or CLA) were supplied via an extra portion of concentrate (supplemental concentrate, **SUPP**), which consisted (DM basis) of 24.4% soybean meal, 48.3% maize, and 27.3% of rumen-protected fat supplements (control fat or CLA) for both groups. Nutrient concentrations and energy content of SUPP are shown in Table 1. Supplemental concentrate was administered during the whole experimental period from 3 wk prepartum to 14 wk postpartum at a constant amount of 0.63 kg of DM/d for each cow by hand once daily. The cows were separated after milking, fixed in the self-locking feed fence and offered the weighed amount of SUPP. The daily portion of SUPP offered 172 g of rumen-protected fats per day. The fat of the control group (LactoPlus 2000; Bewital GmbH & Co. KG, Südlohn, Germany) was free of CLA. The fat of the CLA group (LactoPlus CLA 100; Bewital GmbH & Co. KG) contained 25 and 22 g of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA, respectively, per kilogram, resulting in a daily intake of 4.3 g of *cis*-9,*trans*-11 CLA and 3.8 g of *trans*-10,*cis*-12 CLA per animal and day. With the exception of the 2 CLA isomers, the contents of the major FA in the 2 fats were largely similar, with palmitic and stearic acid being the major FA (Table 2). The amount of CLA supplemented was selected in accordance with a recent study in dairy cows (Schwarz et al., 2007). In that study, supplementation of 4 g of *trans*-10,*cis*-12 CLA per animal decreased milk fat by 0.7% points.

Feed Sampling and Analysis

All used forages, the PMR, and CONC were sampled once per week, whereas SUPP was sampled once in the experimental period. First, DM content was analyzed by weighing fresh material, drying for 24 h at 60°C, and reweighing. Then samples were ground (Brabender GmbH & Co. KG, Duisburg, Germany; filter width 1.1 mm) and PMR samples were combined to 2-wk pools and CONC samples to 4-wk pools. In these pools, the concentrations of crude nutrients crude ash, crude fiber, and crude fat were determined according to Naumann et al. (2000), whereas CP was analyzed using the Dumas method. The net energy content (MJ of NE_L) and the available CP at the duodenum were calculated according to GfE (2001).

Sample Collection

Lactating cows were milked twice daily (0500 and 1500 h) in a 2 × 6 herringbone milking parlor (DeLaval GmbH). Milk yields of each cow were recorded automatically and stored in data files. Twice weekly, representative milk samples (50 mL) from every individual cow were collected by using the sampling device of the milking parlor. One sampling comprised 2 consecutive milking procedures (one evening and the next morning milking). Milk that could not be sold (colostrum, milk from ill cows) was collected in buckets with a scale with which the exact yield could be read.

At wk 1, 5, and 14 of lactation, blood samples of the dammed vena jugularis were taken using sterile 20G cannulas and lithium heparin tubes (Greiner Bio-One GmbH, Kremsmünster, Austria). Blood sampling took place before morning feeding between 0730 and 0900 h. Tubes were kept on ice until subsequent centrifugation (2,000 × *g* for 15 min). Plasma was pipetted into 1.5-mL tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) and stored in aliquots at −20°C until analysis.

Table 2. Contents of major FA in the experimental fat supplements (g/kg)

FA	Control fat	CLA fat[1]
C14:0	17	16
C16:0	543	467
C18:0	331	259
C18:1	7	88
C18:2 n-6	1	27
C18:3 n-3	1	2
cis-9,*trans*-11 CLA	—	25
trans-10,*cis*-12 CLA	—	22
C20:0	4	4
C20:1	6	6

[1]CLA = conjugated linoleic acid.

At wk 1, 5, and 14 of lactation, liver biopsies were taken. Biopsies were conducted before feeding between 0700 and 0900 h. Cows were separated and fixed. After shaving and disinfecting the liver biopsy site on the right side of the cow between the 11th and 12th ribs on a line between the olecranon and the tuber coxae, a local subcutaneous anesthesia with 5 mL of Isocaine 2% (procaine hydrochloride/epinephrine; Selectavet Dr. Otto Fischer GmbH, Weyarn/Holzolling, Germany) was performed. Biopsies were taken with sterile 14G biopsy needles (Dispomed Witt oHG, Gelnhausen, Germany) after introducing an autoclaved cannula as a duct for the needle. About 50 mg of liver tissue were immediately snap frozen in liquid nitrogen. Storage followed at −80°C until further analysis. The biopsy site was treated with wound spray and animals were kept separately until the next day.

Sample Analysis

Milk samples were analyzed for protein, fat, and lactose by infrared spectrophotometry (MilkoScan FT 6000; Foss Analytical A/S, Hillerød, Denmark) at the laboratory of Milchprüfring Bayern e.V., Wolnzach, Germany. Plasma samples were thawed and analyzed for NEFA, BHBA, TAG, and cholesterol content using commercial available kits [NEFA-HR(2) and Autokit 3-HB, obtained from Wako Chemicals GmbH, Neuss, Germany; Fluitest TG and Fluitest CHOL, obtained from Analyticon Biotechnologies AG, Lichtenfels, Germany]. Lipids from liver biopsy samples were extracted with a mixture of n-hexane and isopropanol (3:2, vol/vol; Hara and Radin, 1978). For lipid analyses, aliquots of the lipid extracts were dried and the lipids were dissolved using a 1:1-mixture of chloroform and Triton X-100 (De Hoff et al., 1978). Liver TAG and cholesterol content were measured using enzymatic reagent kits (Fluitest TG and Fluitest CHOL, respectively; Analyticon Biotechnologies AG).

Energy Balance

Average daily energy balance of every individual cow was calculated. For this purpose, energy intake was calculated from the mean daily intake of PMR, SUPP, and CONC and the corresponding energy contents (MJ of NE_L). Energy requirements for maintenance were calculated using the mean BW of the cows according to GfE (2001). Body weights were automatically recorded daily by electronic scales installed in the feeding stations and used to calculate the weekly mean BW of every individual cow. Energy requirements for milk production were calculated based on weekly means of daily milk yield, milk protein content, and milk fat content according to GfE (2001). Changes in body composition were not considered in energy balance evaluation.

RNA Isolation and Quality Control

Total RNA was isolated from liver biopsies using Trizol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Ribonucleic acid from 15 mg of each sample was isolated within 1 wk after finishing the trial. Isolated RNA was preserved at −80°C until use. Ribonucleic acid concentration and purity were estimated from the optical density at 260 and 280 nm, respectively, using an Infinite 200M microplate reader and a NanoQuant Plate (both from Tecan Group Ltd., Männedorf, Switzerland). The A260/A280 ratios were 2.02 ± 0.05. The integrity of the total RNA was checked by 1% agarose gel electrophoresis. Ribonucleic acid was judged as suitable for array hybridization only if the samples exhibited intact bands corresponding to the 18S and 28S ribosomal RNA subunits. Subsequently, 5 RNA pools each for the control and for the CLA group, were prepared. Each RNA pool comprised RNA from 4 animals.

Microarray Analysis

For microarray analyses, the RNA pools were sent to the Center of Excellence for Fluorescent Bioanalytics (KFB) at the University of Regensburg (Regensburg, Germany) for hybridization to the Affymetrix GeneChip bovine genome arrays (Affymetrix UK Ltd., High Wycombe, UK). The Affymetrix GeneChip bovine genome array is based on content from UniGene (http://www.ncbi.nlm.nih.gov/unigene) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/) mRNA. It contains 24,027 probe sets representing more than 23,000 transcripts and includes approximately 19,000 UniGene clusters. In brief, total RNA was checked for quality and quantity using an Agilent Bioanalyzer 2100 instrument (Agilent Technologies, Waldbronn, Germany) and total RNA was transcribed to first- and second-strand cDNA. After purification and testing on an Agilent Bioanalyzer 2100, the double-stranded cDNA served as a template for the in vitro transcription reaction for cRNA synthesis. The cRNA was labeled with biotin using the Affymetrix GeneChip labeling kit. After checking the quality and quantity of the labeled cRNA, cRNA was fractionated and hybridized with Affymetrix GeneChips. The GeneChips were washed and stained with the Affymetrix GeneChip Fluidics Station 450. The GeneChips were then scanned with an Affymetrix GeneChip Scanner 3000. All procedures were performed according to Affymetrix protocols (GeneChip expression analysis, technical manual from

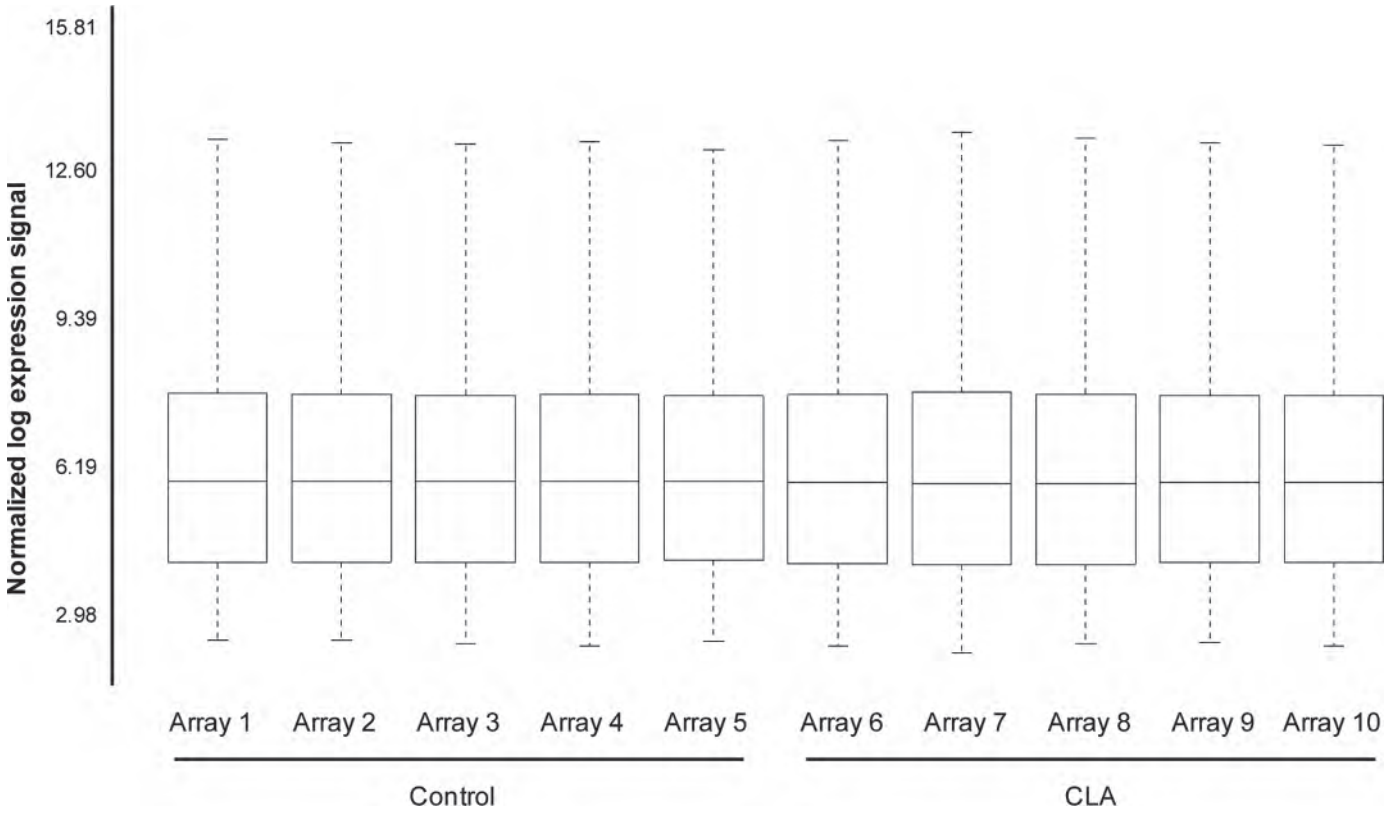

Figure 1. Box plots displaying the average normalized log (base 2) expression signal (y-axis) of all 10 Affymetrix GeneChip (Affymetrix UK Ltd., High Wycombe, UK) bovine genome arrays.

Affymetrix). The quality of hybridization was assessed in all samples following the manufacturer's recommendations. After scanning the arrays, cell intensity files containing a single-intensity value for each probe cell were computed from the image data with the Affymetrix GeneChip Operating Software (GCOS). Probe cell intensity data were further analyzed in the Affymetrix Expression Console 1.1 software using the Affymetrix Microarray Suite Version 5.0 (MAS 5.0) algorithm to create chip files. In the MAS 5.0 algorithm, a global scaling strategy is applied for normalization where the average signal intensity of all probe sets is scaled to a default target signal. The box plots of log (base 2) expression signals of the 10 arrays show that GeneChips were adequately normalized (Figure 1). The fold changes (**FC**) in gene expression were calculated from the signal log ratios (**SLR**) using the following equation: FC = 2SLR for SLR $\geq$ 0 and FC = $(-1) \times 2^{-(\mathrm{SLR})}$ for SLR $<$ 0. An SLR of zero means no change. The SLR algorithm estimates the magnitude and direction of change of a gene when 2 arrays are compared (CLA versus control). It is calculated by comparing each gene on the CLA array to the corresponding gene on the control array. The log scale used is base 2, making it intuitive to interpret the SLR in terms of multiples of 2. Thus, an SLR of 1 indicates a 2-fold increase of the transcript level, and an SLR of −1.0 indicates a 2-fold decrease. Correspondingly, an FC of 2.0 indicates an increase in the transcript level of 100%, and an FC of −2.0 indicates a decrease of 50%.

Real-Time Quantitative PCR

Differential expression data of selected genes obtained from Affymetrix GeneChip analysis were validated by real-time quantitative PCR (**qPCR**). For this purpose, cDNA was synthesized in less than a week after RNA extraction from 1.2 μg of total RNA from all individual samples (n = 20/group) contributing to the RNA pools for microarray analysis using 100 pmol of dT18 primer (Eurofins MWG Operon, Ebersberg, Germany), 1.25 μL 10 mmol/L of dNTP mix (GeneCraft GmbH, Lüdinghausen, Germany), 5 μL of buffer (Fermentas GmbH, St. Leon-Rot, Deutschland), and 60 units of M-MuLV Reverse Transcriptase (MBI Fermentas GmbH, St. Leon-Rot, Germany) at 42°C for 60 min, and a final inactivating step at 70°C for 10 min in a Biometra Thermal Cycler (Whatman Biometra GmbH, Göttingen,

Germany). Subsequently, cDNA was stored in aliquots at −20°C. For the standard curve, a cDNA pool of all samples was made. Quantitative PCR was performed using 2 μL of cDNA combined with 18 μL of a mixture composed of 10 μL of KAPA SYBR FAST qPCR Universal Master Mix (Peqlab Biotechnologie GmbH, Erlangen, Germany), 0.4 μL each of 10 μ*M* forward and reverse primers, and 7.2 μL of DNase/RNase-free water in 0.1-mL tubes (LTF Labortechnik GmbH & Co. KG, Wasserburg, Germany). Gene-specific primer pairs obtained from Eurofins MWG Operon were designed using Primer3 (http://frodo.wi.mit.edu/) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Features of primer pairs are listed in Table 3. All primer pairs were designed to have melting temperatures of about 60°C and, if possible, both primers of a primer pair were designed to be located in different exons. Quantitative PCR runs were performed using a Rotor-Gene 2000 system (Corbett Research Pty Ltd., Mortlake, Australia), and included all samples and a 5-point relative standard curve plus the nontemplate control (NTC). The qPCR protocol was as follows: 3 min at 95°C, followed by 40 cycles of a 2-step PCR consisting of 5 s at 95°C (denaturation) and 20 s at 60°C (annealing and extension). Subsequently, melting curve analysis was performed from 50 to 95°C to verify the presence of a single PCR product. In addition, the amplification of a single product of the expected size was confirmed using 2% agarose gel electrophoresis stained with GelRed nucleic acid gel stain (Biotium Inc., Hayward, CA). Data on qPCR performance for each genes measured are also shown in Table 3. For determination of relative expression levels relative quantities were calculated using GeNorm normalization factor. To calculate the normalization factor, all cycle threshold (Ct) values were transformed into relative quantification data by using the $2^{-\Delta Ct}$ equation (Livak and Schmittgen, 2001), and the highest relative quantities for each gene were set to 1. From these values the normalization factor was calculated as the geometric mean of expression data of the 3 most stable out of 6 tested potential reference genes. Reference gene stability across samples was determined by performing GeNorm analysis (Vandesompele et al., 2002), which is based on calculation of a reference gene-stability measure *M*. Genes with the lowest *M* values have the most stable expression. After normalization of gene expression data using the calculated GeNorm normalization factor, means and SD were calculated from normalized expression data for samples of the same treatment group. The mean of the control group was set to 1 and mean and SD of the CLA group was scaled proportionally. After normalization, means and SD were calculated from normalized expression data for samples of the same treatment group. Relative expression ratios of the CLA group are expressed as fold changes compared with the control group.

Statistics

Data are presented as means ± standard errors of the means. The individual cow serves as experimental unit. Statistical analysis of performance and metabolic data was performed using the MIXED procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC). For each performance parameter, the model included lactation week, treatment group, parity of the cow (primiparous and multiparous), and the lactation week × treatment group interaction. The repeated subject was the individual cow. The significances of differences between the groups over time were analyzed using the Bonferroni *t*-test. Differences were regarded as significant at $P < 0.05$.

RESULTS

Feed Intake, Energy Balance, Milk Yield, and Milk Composition

As expected, the DMI of the cows rose from wk 1 to 14. Dry matter intake was not significantly different between the CLA group and the control group, but a tendency ($P = 0.075$) existed toward an increase in DMI (+ 0.4 kg) in the cows supplemented with CLA during the period from wk 1 to 14 (Table 4). Daily milk yield during the whole period was higher in the CLA group than in the control group ($P < 0.001$). In wk 1 of lactation, milk fat was not different between the 2 groups of cows. However, during the whole period, milk fat and daily amount of milk fat were 0.6 percentage points and 0.17 kg, respectively, lower in the CLA group than in the control group ($P < 0.001$; Table 4). For milk protein concentration, an interaction between treatment and time ($P = 0.028$) was observed: in wk 1 and 5, no difference in milk protein content was observed; in wk 14, milk protein content was decreased in the CLA group compared with the control group ($P < 0.05$). During the whole period, milk protein content was decreased by 0.15 percentage points in the CLA group ($P < 0.001$), whereas daily milk protein yield was not different between the 2 groups of cows (Table 4). The concentration of lactose in the milk was not different between the 2 groups of cows. Due to the decreased milk fat content, amounts of FCM and ECM were lower in the CLA group than in the control group ($P = 0.002$ and $P = 0.005$, respectively; Table 4). The energy bal-

Table 3. Characteristics and performance data of the primers used for quantitative PCR analysis and reference gene-stability measure *M*

Gene	Forward primer (from 5′ to 3′); Reverse primer (from 5′ to 3′)	PCR product size (bp)	NCBI GenBank accession number	Slope	R^2	Efficiency	*M*
Reference gene							
ACTB	ACTTGCGCAGAAAACGAGAT CACCTTCACCGTTCCAGTTT	120	AY141970	−0.29	0.99	1.94	0.135
SDHA	GCAGAACCTGATGCTTTGTG CGTAGGAGAGCGTGTGCTT	185	NM_174178	−0.23	0.99	1.69	0.085
ATP5B	GGACTCAGCCCTTCAGCGCC GCCTGGTCTCCCTGCCTTGC	229	NM_175796	−0.24	0.93	1.74	0.135
RPS9	GTGAGGTCTGGAGGGTCAAA GGGCATTACCTTCGAACAGA	108	BC148016	−0.30	0.99	2.00	0.080
PPIA	GGCAAATGCTGGCCCCAACACA AGTACCACGTGCTTGCCATCCA	87	NM_178320	−0.29	0.99	1.95	0.091
RPL12	CACCAGCCGCCTCCACCATG CGACTTCCCCACCGGTGCAC	84	NM_205797	−0.35	0.99	2.25	0.078
Target gene							
SREBP-1	CCGAGGCCAAGTTGAATAAA TTCAGCGATTTGCTTTTGTG	136	AB355703	−0.29	0.99	1.93	—
FASN	GCTGAGCCTGATGCGTCTGAGC GGATGGCAGTGAGGCTCACGAA	138	NM_001012669	−0.30	0.99	1.97	—
ACACA	CTCTTCCGACAGGTTCAAGC AGTCCCCGCACTCACATAAC	270	NM_174224	−0.22	−0.22	1.000	—
SREBP-2	CTGGCTCCAGGGAGATGAC GCTCTGCAGGTGTGGAAGAC	120	XM_583656	−0.31	−0.31	0.995	—
HMGCR	GAGTGGCAGGACCTCTGTGC GCACCTCCACCAAGGCCTAT	121	BC153262	−0.29	−0.29	0.999	—
CPT1A	CAAAACCATGTTGTACAGCTTCCA GCTTCCTTCATCAGAGGCTTCA	111	FJ415874	−0.31	−0.31	0.928	—
HMGCS2	GCCCAATATGTGGACCAAAC ATGGTCTCAGTGCCCACTTC	209	NM_001045883	−0.29	−0.29	0.997	—
SLC27A1	CTGAAGGAGACCTCCACAGC GTGGTACAGGGGCAGACAGT	208	NM_001033625	−0.24	−0.24	0.882	—
CD36/FAT	GCATTCTGAAAGTGCGTTGA CGGGTCTGATGAAAGTGGTT	179	BC103112	−0.31	−0.31	0.928	—

[1]*ACTB* = actin β; *SDHA* = succinate dehydrogenase complex, subunit A, flavoprotein (Fp); *ATP5B* = ATP synthase, H^+ transporting, mitochondrial F1 complex, β polypeptide; *RPS9* = ribosomal protein S9; *PPIA* = peptidylprolyl isomerase A (cyclophilin A); *RPL12* = ribosomal protein L12; *SREBP-1* = sterol regulatory element-binding protein 1; *FASN*= FA synthase; *ACACA* = acetyl-CoA carboxylase α; *SREBP-2* = sterol regulatory element-binding protein 2; *HMGCR* = 3-hydroxy-3-methylglutaryl-CoA reductase; *CPT1A* = carnitine palmitoyltransferase 1A (liver); *HMGCS2* = 3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial); *SLC27A1* = solute carrier family 27 (FA transporter), member 1; *CD36/FAT* = CD36/FA translocase; NCBI = National Center for Biotechnology Information.

ance during the whole period was improved in the CLA group compared with the control group ($P = 0.024$).

Concentrations of NEFA and BHBA in Plasma

In wk 1, plasma concentration of NEFA in the CLA group was lower than in the control group ($P < 0.05$; Table 5). In wk 5 and 14, no difference was observed in plasma NEFA concentration between both groups of cows. Plasma BHBA concentration was not different between the 2 groups of cows during the whole period (Table 5).

Concentrations of TAG and Cholesterol in Liver and Plasma

Hepatic TAG showed the expected dependence on lactation week, being highest in wk 5 of lactation. Conjugated linoleic acid supplementation, however, did not change hepatic TAG concentration (Table 5). Hepatic cholesterol concentration was also not different between the 2 groups of cows. Plasma TAG and cholesterol concentrations at wk 5 were not different between the 2 groups of cows (Table 5).

Expression of Genes Involved in Hepatic FA Uptake and Intracellular FA Transport

To assess the effect of CLA on hepatic FA uptake and intracellular transport, we considered the expression of a total of 6 genes encoding FA transporters (*SLC27A1*, *SLC27A2*, *SLC27A4*, *SLC27A5*, *SLC27A6*, and *CD36/FAT*) and 7 genes encoding FA-binding proteins (*FABP1* to *7*; Table 6). The expression of these genes was not differentially regulated between the CLA group and the control group, indicating that CLA did not affect FA uptake into the liver and FA binding and transport in the liver. This indication is supported by

Table 4. Effect of conjugated linoleic acid (CLA) on performance parameters in Holstein cows at wk 1, 5, and 14 of lactation and during the whole period (wk 1 to 14)

	wk 1		wk 5		wk 14		wk 1 to 14			*P*-value		
Variable	Control	CLA	Control	CLA	Control	CLA	Control	CLA	SEM	CLA	Time	CLA × time
DMI (kg/d)	13.4	16.0	18.4	19.1	20.4	21.3	20.2	20.6	0.17	0.075	<0.0001	0.997
Milk yield (kg/d)	28.6	28.5	37.5	38.6	32.0[b]	35.6[a]	35.6[b]	37.1[a]	0.23	<0.0001	<0.0001	0.072
FCM[1] (kg/d)	39.2	38.8	40.9	38.6	33.0	31.9	38.2[a]	36.3[b]	0.28	0.002	<0.0001	0.968
ECM[2] (kg/d)	38.3	37.8	38.9	37.1	32.3	31.6	36.9[a]	35.4[b]	0.25	0.005	<0.0001	0.960
Milk fat (%)	6.40	6.21	4.51[a]	3.85[b]	4.11[a]	3.19[b]	4.40[a]	3.81[b]	0.05	<0.0001	<0.0001	0.743
Milk fat yield (kg/d)	1.78	1.75	1.68[a]	1.47[b]	1.31	1.12	1.56[a]	1.39[b]	0.02	<0.0001	<0.0001	0.991
Milk protein (%)	4.09	4.15	2.85	2.76	3.13[a]	2.87[b]	3.11[a]	2.96[b]	0.02	<0.0001	<0.0001	0.028
Milk protein yield (kg/d)	1.22	1.14	1.06	1.05	1.00	1.00	1.10	1.08	0.01	0.193	0.0002	0.842
Milk lactose (%)	4.38	4.36	4.76	4.80	4.75	4.75	4.75	4.74	0.01	0.356	<0.0001	0.984
Energy balance[3] (MJ of NE_L/d)	−64.9	−55.4	−30.4[b]	−20.7[a]	3.9[b]	5.9[a]	−10.9[b]	−5.59[a]	1.40	0.024	<0.0001	0.440

[a,b]Means within rows within the same lactation week with different superscripts differ ($P < 0.05$).

[1]Corrected for 4% milk fat content.

[2]Corrected for 4% milk fat content and 3.4% milk protein content.

[3]Calculated value.

Table 5. Effect of conjugated linoleic acid (CLA) on metabolic parameters in Holstein cows at wk 1, 5, and 14 of lactation and in average of wk 1, 5, and 14

	wk 1		wk 5		wk 14		wk 1, 5, 14			*P*-value		
Variable	Control	CLA	Control	CLA	Control	CLA	Control	CLA	SEM	CLA	Time	CLA × time
Plasma NEFA (μmol/L)	867[a]	731[b]	283	284	171	148	440	388	24.4	0.535	<0.0001	0.014
Plasma BHBA (μmol/L)	707	747	724	723	521	531	651	667	14.9	0.317	<0.0001	0.910
Plasma TAG[1,2] (mmol/L)	—	—	0.13	0.14	—	—	—	—	0.003	0.175	—	—
Plasma cholesterol[2] (mmol/L)	—	—	4.55	4.52	—	—	—	—	2.91	0.08	—	—
Liver TAG (μmol/g of liver)	25.1	20.0	59.7	54.9	3.4	3.4	28.2	23.7	2.41	0.610	<0.0001	0.841
Liver cholesterol (μmol/g of liver)	15.4	13.9	12.1	10.9	6.7	7.1	11.0	10.0	0.03	0.375	<0.0001	0.094

[a,b]Means within the same lactation week with different superscripts differ ($P < 0.05$).

[1]TAG = triacylglycerol.

[2]Plasma TAG and plasma cholesterol were determined only at wk 5.

Table 6. Effect of conjugated linoleic acid (CLA) on the expression of genes involved in hepatic FA uptake, intracellular FA transport, mitochondrial and peroxisomal β-oxidation, carnitine metabolism, and ketogenesis in Holstein cows at wk 5

Gene	Description	FC[1]
FA uptake		
SLC27A1	Solute carrier family 27 (FA transporter), member 1	1.03
SLC27A2	Solute carrier family 27 (FA transporter), member 2	1.15
SLC27A4	Solute carrier family 27 (FA transporter), member 4	1.20
SLC27A5	Solute carrier family 27 (FA transporter), member 5	−1.05
SLC27A6	Solute carrier family 27 (FA transporter), member 6	1.04
CD36/FAT	CD36/FA translocase	−1.01
Intracellular FA transport		
FABP1	FA-binding protein 1	1.00
FABP2	FA-binding protein 2	1.02
FABP3	FA-binding protein 3	−1.03
FABP4	FA-binding protein 4	1.31
FABP5	FA-binding protein 5	−1.01
FABP6	FA-binding protein 6	−1.07
FABP7	FA-binding protein 7	1.02
Mitochondrial FA oxidation		
CPT1A	Carnitine palmitoyltransferase 1A	−1.04
CPT1B	Carnitine palmitoyltransferase 1B	−1.03
CPT1C	Carnitine palmitoyltransferase 1C	−1.15
CPT2	Carnitine palmitoyltransferase 2	1.05
CACT	Carnitine/acylcarnitine translocase	1.05
MLYCD	Malonyl-CoA decarboxylase	1.07
ACADS	Acyl-CoA dehydrogenase, C-2 to C-3 short chain	1.01
HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/ enoyl-CoA hydratase (trifunctional protein), α subunit	−1.17
HADHB	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/ enoyl-CoA hydratase (trifunctional protein), β subunit	1.06
ACADVL	Acyl-CoA dehydrogenase, very long chain	−1.02
ACADM	Acyl-CoA dehydrogenase, C-4 to C-12 straight chain	1.11
ACADL	Acyl-CoA dehydrogenase, long chain	−1.09
ACADSB	Acyl-CoA dehydrogenase, short/branched chain	1.00
ACAD9	Acyl-CoA dehydrogenase family, member 9	−1.07
ACAD10	Acyl-CoA dehydrogenase family, member 10	−1.04
ACAD11	Acyl-CoA dehydrogenase family, member 11	1.07
ECHS1	Enoyl CoA hydratase, short chain, 1, mitochondrial	1.05
ACAA2	Acetyl-CoA acyltransferase 2	1.15
PCCA	Propionyl CoA carboxylase, α polypeptide	1.04
PCCB	Propionyl CoA carboxylase, β polypeptide	1.03
MUT	Methylmalonyl CoA mutase	1.12
Peroxisomal FA oxidation		
ACOX1	Acyl-CoA oxidase 1, palmitoyl	1.06
ACOX2	Acyl-CoA oxidase 2, branched chain	−1.04
ACOX3	Acyl-CoA oxidase 3, pristanoyl	−1.08
ECH1	Enoyl CoA hydratase 1, peroxisomal	1.03
ECHDC1	Enoyl CoA hydratase domain containing 1	1.27
ECHDC2	Enoyl CoA hydratase domain containing 2	1.00
ECHDC3	Enoyl CoA hydratase domain containing 3	−1.09
Carnitine synthesis, transport, and esterification		
ALDH9A1	Aldehyde dehydrogenase 9 family, member A1	1.07
BBOX1	Butyrobetaine (γ), 2-oxoglutarate dioxygenase	1.14
TMLHE	Trimethyllysine hydroxylase, ε	1.06
SLC22A5	Organic cation/carnitine transporter 2	1.11
CRAT	Carnitine *O*-acetyltransferase	−1.07
CROT	Carnitine *O*-octanoyltransferase	1.14
Ketogenesis		
HMGCL	3-Hydroxymethyl-3-methylglutaryl-CoA lyase	1.04
HMGCS2	3-Hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	1.07
ACAT1	Acetyl-CoA acetyltransferase 1	1.20

[1]Fold change in the CLA group versus the control group. For example, an FC of 2.0 indicates an increase in the transcript level of 100%, and an FC of −2.0 indicates a decrease of 50%.

qPCR analysis of *CD36* and *FATP1*, 2 important FA transporters, which revealed no difference in relative mRNA concentrations of these genes (CLA group vs. control group: *CD36*: 0.73 ± 0.64 vs. 1.00 ± 0.69; *FATP1*: 0.85 ± 0.51 vs. 1.00 ± 0.57; $P > 0.05$ for both genes).

Expression of Genes Involved in Mitochondrial and Peroxisomal β-Oxidation of FA, Carnitine Metabolism, and Ketogenesis

To assess the effect of CLA on hepatic mitochondrial and peroxisomal β-oxidation, we considered the expression of 21 and 7 genes, respectively, involved in those pathways (Table 6). None of the genes considered was differentially regulated in the CLA group compared with the control group. Carnitine is required for the transport of acyl-CoA into the mitochondrion and, thus, is an essential cofactor in β-oxidation. To assess whether CLA influenced carnitine metabolism, we determined the expression of 5 genes involved in synthesis (*ALDH9A1*, *BBOX1*, and *TMLHE*), transport (*SLC22A5*), and esterification (*CRAT* and *CROT*) of carnitine (Table 6). The expression of all of these genes was not different between the 2 groups of cows. Moreover, expression of 3 genes involved in ketogenesis was not different between cows supplemented with CLA and cows of the control group. The consideration of all of the genes involved in β-oxidation, carnitine metabolism, and ketogenesis indicates that hepatic mitochondrial and peroxisomal β-oxidation, metabolism of carnitine, and ketogenesis were not influenced by supplementation of CLA. This indication was confirmed by determining relative mRNA concentrations of *CPT1A* and *HMGCS2*, key enzymes of mitochondrial β-oxidation and ketogenesis, respectively, by qPCR (CLA group vs. control group: *CPT1A*: 0.93 ± 0.68 vs. 1.00 ± 0.56; *HMGCS2*: 0.84 ± 0.41 vs. 1.00 ± 0.57; $P > 0.05$ for both genes).

Expression of Genes Involved in Lipid Synthesis

To assess the effect of CLA on lipid synthesis, we considered the expression of 7 genes involved in FA synthesis, 4 genes involved in TAG synthesis, 5 genes involved in the synthesis of unsaturated FA (desaturases), 5 genes involved in elongation of FA (elongases), and 17 genes involved in cholesterol synthesis (Table 7). None of these genes was differentially regulated in the CLA group in comparison to the control group, indicating that CLA had no influence on hepatic lipid synthesis. This indication was confirmed by determining relative mRNA concentrations of *ACC* and *FASN* (2 enzymes of FA synthesis), *HMGCR* (the key enzyme of cholesterol synthesis), and *SREBP-1* and *SREBP-2* (2 transcription factors controlling the transcription of genes involved in lipogenesis and cholesterol synthesis). Relative mRNA concentrations of these genes in the CLA group versus control group were as follows: *ACC*: 1.25 ± 0.66 vs. 1.00 ± 0.56; *FASN*: 0.73 ± 0.64 vs. 1.00 ± 0.69; *HMGCR*: 1.04 ± 0.62 vs. 1.00 ± 0.51; *SREBP-1*: 0.89 ± 0.59 vs. 1.00 ± 0.52; *SREBP-2*: 0.90 ± 0.57 vs. 1.00 ± 0.52 ($P > 0.05$ for all genes).

Expression of Genes Involved in Lipoprotein Uptake and Metabolism

To assess the effect of CLA on hepatic lipoprotein metabolism, we considered the expression of 2 genes involved in lipoprotein uptake (*VLDLR* and *LDLR*), 6 genes involved in lipoprotein metabolism, and 12 genes involved in lipoprotein secretion (Table 7). All of these genes were not differentially regulated between cows of the CLA group and cows of the control group, indicating that uptake, metabolism, as well as assembly and secretion of lipoproteins were not affected by supplementation of CLA.

DISCUSSION

The present study aimed to investigate the influence of feeding rumen-protected CLA on liver lipid metabolism in dairy cows. For this purpose, dairy cows received a rumen-protected CLA mixture supplying 3.8 g of *trans*-10,*cis*-12 CLA per day as milk fat-depressing compound from late pregnancy to wk 14 of lactation. The finding that such a moderate amount of *trans*-10,*cis*-12 CLA caused a considerable decrease in milk fat of around 0.6% points from wk 1 to 14 of lactation agrees with several other studies (Giesy et al., 2002; Piperova et al., 2004; Odens et al., 2007; Schwarz et al., 2007), confirming that *trans*-10,*cis*-12 CLA is highly efficient in decreasing milk fat. The finding that milk fat was not decreased in wk 1 of lactation by CLA agrees with several other studies that used moderate doses of CLA (Bernal-Santos et al., 2003; Selberg et al., 2004; Kay et al., 2006; Pappritz et al., 2011). Kay et al. (2006) hypothesized that the lack of effect in the first week of lactation could be due to a decreased uptake of CLA into the mammary gland immediately postpartum. During the transition period, plasma NEFA concentrations are very high, and these NEFA deriving from adipose tissue might compete with CLA for their uptake into mammary gland epithelial cells.

In the present study, CLA supplementation tended to increase DMI ($P = 0.075$) and increased milk yield but lowered energy output via milk due to the decreased milk fat content, resulting in an improved energy balance during lactation. The finding of an improved energy balance agrees with some other studies and confirms that supplementation of CLA may be a dietary strategy to prevent health problems associated with NEB in dairy cows (Kay et al., 2006; Odens et al., 2007; Schwarz et al., 2007). It has been shown that plasma NEFA concentration is a reliable index of the magnitude of adipose fat mobilization (Bauman et al., 1988). Thus, an improvement of NEB is expected to lower plasma NEFA concentration. Indeed, CLA-

Table 7. Effect of conjugated linoleic acid (CLA) on the expression of genes involved in hepatic FA synthesis, triacylglycerol (TAG) synthesis, synthesis of unsaturated FA, elongation of FA, cholesterol synthesis, lipoprotein uptake, lipoprotein metabolism, and lipoprotein secretion in Holstein cows at wk 5

Gene	Description	FC[1]
FA synthesis		
SREBP-1	Sterol regulatory element-binding protein-1	−1.07
FASN	FA synthase	1.01
ACLY	ATP citrate lyase	1.00
ME1	Malic enzyme 1, $NADP^+$-dependent, cytosolic	−1.06
ME2	Malic enzyme 2, NAD^+-dependent, mitochondrial	1.04
ME3	Malic enzyme 3, $NADP^+$-dependent, mitochondrial	−1.03
ACACA	Acetyl-CoA carboxylase α	−1.11
TAG synthesis		
DGAT2	Diacylglycerol *O*-acyltransferase homolog 2	1.04
AGPAT2	1-Acylglycerol-3-phosphate *O*-acyltransferase 2	−1.11
AGPAT3	1-Acylglycerol-3-phosphate *O*-acyltransferase 3	1.05
LPIN2	Lipin 2	1.11
Synthesis of unsaturated FA		
FADS6	FA desaturase domain family, member 6	−1.02
FADS3	FA desaturase 3	1.14
FADS2	FA desaturase 2	−1.15
SCD	Stearoyl-CoA desaturase (Δ^9-desaturase)	−1.01
SCD5	Stearoyl-CoA desaturase 5	1.08
FA elongation		
ELOVL1	Elongation of very long chain FA-like 1	−1.09
ELOVL4	Elongation of very long chain FA-like 4	−1.09
ELOVL5	ELOVL family member 5, elongation of long chain FA	1.05
ELOVL6	ELOVL family member 6, elongation of long chain FA	1.11
ELOVL7	ELOVL family member 7, elongation of long chain FA	1.10
Cholesterol synthesis		
SREBP-2	Sterol regulatory element-binding protein-2	−1.08
SCAP	SREBP chaperone	−1.07
MVK	Mevalonate kinase	1.07
PMVK	Phosphomevalonate kinase	−1.04
MVD	Mevalonate (diphospho) decarboxylase	1.01
FDPS	Farnesyl diphosphate synthase	−1.10
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	−1.10
IDI1	Isopentenyl-diphosphate Δ-isomerase 1	1.05
SQLE	Squalene epoxidase	1.03
LSS	Lanosterol synthase	1.14
CYP51A1	Cytochrome P450, family 51, subfamily A, polypeptide 1	−1.03
SC4MOL	Sterol-C4-methyl oxidase-like	−1.08
NSDHL	NAD(P)-dependent steroid dehydrogenase-like	1.00
DHCR7	7-Dehydrocholesterol reductase	1.02
INSIG1	Insulin induced gene 1	1.27
HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase	1.12
INSIG2	Insulin induced gene 2	1.16
Lipoprotein uptake		
VLDLR	Very low density lipoprotein receptor	−1.08
LDLR	Low density lipoprotein receptor	−1.02
Lipoprotein metabolism		
LIPC	Lipase, hepatic	1.14
LIPG	Lipase, endothelial	1.08
LPL	Lipoprotein lipase	1.12
PCTP	Phosphatidylcholine transfer protein	1.05
HDLBP	High density lipoprotein-binding protein	−1.02
ANGPTL4	Angiopoietin-like 4	1.19
Lipoprotein secretion		
MTTP	Microsomal triglyceride transfer protein	1.07
APOA1	Apolipoprotein A-I	1.07
APOA2	Apolipoprotein A-II	−1.01
APOA4	Apolipoprotein A-IV	1.00
APOA5	Apolipoprotein A-V	1.13
APOB	Apolipoprotein B	−1.06
APOC2	Apolipoprotein C-II	1.05
APOC3	Apolipoprotein C-III	1.04
APOC4	Apolipoprotein C-IV	1.00
APOM	Apolipoprotein M	1.06
APON	Apolipoprotein N	−1.02
SAA4	Serum amyloid A4, constitutive	1.12

[1]Fold change in the CLA group versus the control group. For example, an FC of 2.0 indicates an increase in the transcript level of 100%, and an FC of −2.0 indicates a decrease of 50%.

supplemented cows exerted a decreased plasma NEFA concentration in wk 1 of lactation, the time point of the strongest improvement of NEB by CLA. In contrast, NEFA concentrations at wk 5 and 14 were not different between both groups of cows, although energy balance was also improved at those time points. We assume that the differences in energy balance at those time points were not great enough to cause a significant alteration in plasma NEFA concentration. Notably, our study disagrees with a study of Selberg et al. (2004) who found an increased plasma NEFA concentration in cows supplemented with CLA in wk 1 of lactation. Their finding indicated that CLA supplementation transiently enhances lipolysis shortly after calving. The observation of unchanged plasma NEFA concentrations in wk 5 and 14 of lactation agrees with most other studies showing that CLA does not influence plasma NEFA concentration (Perfield et al., 2002; Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005; Pappritz et al., 2011).

The primary aim of this study was to elucidate whether CLA could exert adverse effects on hepatic lipid metabolism. Therefore, we determined hepatic concentrations of TAG and cholesterol in liver biopsy samples and performed a GeneChip analysis in samples obtained at wk 5 of lactation, representing the phase in which hepatic lipid concentrations are highest during lactation. Although GeneChip analysis generates rather semiquantitative data, the use of this technique has the great advantage that a great number of genes involved in several pathways of lipid metabolism can be detected simultaneously in a small sample amount. This is especially important when using small biopsy samples, such as in the present study. Nevertheless, we also determined mRNA concentrations of key enzymes of the relevant pathways by means of qPCR to confirm the validity of GeneChip data.

Fatty acid uptake, FA binding, FA oxidation, ketogenesis, as well as carnitine synthesis and uptake are pathways that are mainly controlled by peroxisome proliferator-activated receptor (***PPAR***)α, a transcription factor that acts as an important regulator of lipid metabolism and energy homeostasis (Desvergne and Wahli, 1999). It has been found that CLA are able to bind to and activate *PPARα* (Moya-Camarena et al., 1999a). In addition, several feeding studies in rodents have shown that feeding of CLA causes an activation of *PPARα* in vivo and, thus, stimulates pathways controlled by *PPARα* such as mitochondrial and peroxisomal β-oxidation (Belury et al., 1996; Moya-Camarena et al., 1999b; Ringseis et al., 2004). The finding that mRNA concentrations of many genes directly controlled by *PPARα*, such as *ACO*, *CPT1A*, or *HMGCS2*, were unchanged in cows supplemented with CLA clearly indicates that CLA supplementation did not cause an activation of *PPARα* in the liver. It is well known that NEFA deriving from adipose tissue, taken up into the liver, are acting as endogenous ligands of *PPARα*. Therefore, increased plasma concentrations of NEFA cause an activation of *PPARα* (Mandard et al., 2004). Thus, the finding that plasma NEFA concentrations were not influenced by CLA is in line with the observation that no activation of *PPARα* occurred in the cows supplemented with CLA. The observation that expression of *HMGCS2,* the key enzyme of hepatic ketogenesis, was not altered is in agreement with the finding of an unchanged concentration of plasma BHBA. Several other studies in dairy cows also observed that supplementation of CLA does not change plasma BHBA concentration in dairy cows (Perfield et al., 2002; Bernal-Santos et al., 2003; Selberg et al., 2004; Pappritz et al., 2011).

Synthesis of FA (including FA elongation and desaturation) and cholesterol is mainly controlled by *SREBP-1c* and *SREBP-2*, respectively. In the liver of mice, supplementation of CLA caused an activation of *SREBP-1c* associated with an upregulation of several enzymes involved in de novo synthesis of FA and TAG (Clément et al., 2002). In the present study, genes involved in FA and cholesterol synthesis were not different in their expression between CLA and control cows. This indicates that both *SREBP-1c* and *SREBP-2* were not activated by supplementation of CLA in dairy cows.

Hepatic TAG concentration is the result of various pathways of lipid metabolism, including uptake of FA from plasma, oxidation of FA, de novo synthesis of FA, and secretion of TAG via VLDL (Katoh, 2002). Early lactation is a critical phase with respect to hepatic lipid metabolism as the amount of FA taken up from plasma into the liver normally exceeds the capacity of FA oxidation and secretion of TAG via VLDL. For this reason, hepatic TAG concentration in dairy cows is normally highest in early lactation. Accordingly, hepatic TAG concentration in the present study was highest in wk 5 and decreased until wk 14. The finding that genes involved in uptake, oxidation, and de novo synthesis of FA as well as secretion of TAG via VLDL were not differentially regulated by CLA matches with the unchanged hepatic TAG concentration in cows supplemented with CLA compared with control cows. The finding that CLA does not influence hepatic TAG concentration in dairy cows is in agreement with 3 other studies (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005). Our study, moreover, shows that CLA does not influence hepatic cholesterol concentration. This observation is in line with the findings of unchanged expression of genes in-

volved in cholesterol synthesis, cholesterol uptake via LDL, and cholesterol secretion via VLDL.

Concentrations of lipids in plasma are the result of 1) assembly of lipoproteins in tissues and secretion into the blood, 2) decomposition of circulating lipoproteins, and 3) uptake of lipoproteins into tissues by receptors. The GeneChip analysis showed that CLA did not alter the expression of genes involved in hepatic uptake of lipoproteins such as VLDL or LDL receptor, genes involved in decomposition of lipoproteins such as hepatic lipase or lipoprotein lipase, or genes involved in hepatic assembly of VLDL such as microsomal triglyceride transfer protein (*MTTP*) and several apolipoproteins. According to these observations, indicating that CLA had no effect on hepatic lipoprotein metabolism, it was not unexpected that plasma concentrations of TAG and cholesterol were not altered in cows supplemented with CLA.

Notably, the effects observed in this study are similar to those of a recent study performed with growing beef cattle. In that study, daily supplementation of 5 g of rumen-protected *trans*-10,*cis*-12 CLA in cattle also had less effect on expression of genes involved in hepatic lipid metabolism and did not change plasma and liver concentrations of TAG and cholesterol (Shibani et al., 2011). However, the findings of the present study strongly differ from those observed in rodents. In mice, *trans*-10,*cis*-12 CLA caused the development of severe fatty liver. The differences between the 2 species can be either due to species specificity of the effects or to differences in supplementation levels. In mice, development of fatty liver was observed in studies using diets with 0.25 to 1% *trans*-10,*cis*-12 CLA (Belury and Kempa-Steczko, 1997; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002). In contrast, the daily supplementation of 3.8 g of *trans*-10,*cis*-12 CLA per day in dairy cows was equivalent to a concentration of only 0.02% of total DMI.

CONCLUSIONS

The present study confirms previous studies in showing that supplementation of a rumen-protected CLA mixture is an effective way to lower milk fat concentration and to improve energy balance during early lactation in dairy cows. The study moreover shows, using a GeneChip as a tool to detect the expression of a broad range of genes involved in various pathways, that a dose of CLA effective for milk fat depression does not influence the metabolism of lipids (uptake, oxidation, and synthesis of FA, including synthesis of carnitine; uptake, synthesis, and secretion of cholesterol; metabolism and secretion of lipoproteins; or ketogenesis) and hepatic and plasma concentrations of TAG and cholesterol. Thus, based on these data, adverse effects of CLA—at a level sufficient for depression of milk fat—on hepatic lipid metabolism in dairy cows can be excluded.

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Expression of genes involved in hepatic carnitine synthesis and uptake in dairy cows in the transition period and at different stages of lactation

Gloria Schlegel[1], Janine Keller[1], Frank Hirche[2], Stefanie Geißler[2], Frieder J Schwarz[3], Robert Ringseis[1], Gabriele I Stangl[2] and Klaus Eder[1*]

Abstract

Background: In rodents and pigs, it has shown that carnitine synthesis and uptake of carnitine into cells are regulated by peroxisome proliferator-activated receptor α (PPARA), a transcription factor which is physiologically activated during fasting or energy deprivation. Dairy cows are typically in a negative energy balance during early lactation. We investigated the hypothesis that genes of carnitine synthesis and uptake in dairy cows are enhanced during early lactation.

Results: mRNA abundances of PPARA and some of its classical target genes and genes involved in carnitine biosynthesis [trimethyllysine dioxygenase (TMLHE), 4-N-trimethylaminobutyraldehyde dehydrogenase (ALDH9A1), γ-butyrobetaine dioxygenase (BBOX1)] and uptake of carnitine [novel organic cation transporter 2 (SLC22A5)] as well as carnitine concentrations in liver biopsy samples of 20 dairy cows in late pregnancy (3 wk prepartum) and early lactation (1 wk, 5 wk, 14 wk postpartum) were determined. From 3 wk prepartum to 1 wk postpartum, mRNA abundances of PPARA and several PPARA target genes involved in fatty acid uptake, fatty acid oxidation and ketogenesis in the liver were strongly increased. Simultaneously, mRNA abundances of enzymes of carnitine synthesis (TMLHE: 10-fold; ALDH9A1: 6-fold; BBOX1: 1.8-fold) and carnitine uptake (SLC22A5: 13-fold) and the concentration of carnitine in the liver were increased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$). From 1 wk to 5 and 14 wk postpartum, mRNA abundances of these genes and hepatic carnitine concentrations were declining ($P < 0.05$). There were moreover positive correlations between plasma concentrations of non-esterified fatty acids (NEFA) and hepatic carnitine concentrations at 1 wk, 5 wk and 14 wk postpartum ($P < 0.05$).

Conclusions: The results of this study show for the first time that the expression of hepatic genes of carnitine synthesis and cellular uptake of carnitine is enhanced in dairy cows during early lactation. These changes might provide an explanation for increased hepatic carnitine concentrations observed in 1 wk postpartum and might be regarded as a physiologic means to provide liver cells with sufficient carnitine required for transport of excessive amounts of NEFA during a negative energy balance.

Background

Carnitine (3-hydroxy-4-*N, N, N*-trimethylaminobutyric acid) is an essential metabolite that has a number of indispensable functions in intermediary metabolism. The most important function lies in its role in the transport of activated long-chain fatty acids (acyl groups) from the cytosol into the mitochondrial matrix where β-oxidation takes place [1]. Carnitine is derived from dietary sources and synthesized endogenously from trimethyllysine (TML), which is released upon protein degradation. The released TML is further oxidized to γ-butyrobetaine (BB) by the action of trimethyllysine dioxygenase (TMLHE), 3-hydroxy-N-TML aldolase and 4-N-trimethylaminobutyraldehyde dehydrogenase (ALDH9A1). In the final biosynthetic step, BB is hydroxylated by γ-butyrobetaine dioxygenase (BBOX1) to form carnitine. In cattle this last step occurs only in liver and kidney [2]. Tissues which are not capable of producing

* Correspondence: klaus.eder@ernaehrung.uni-giessen.de
[1]Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany
Full list of author information is available at the end of the article

carnitine depend on the uptake of carnitine from blood by novel organic cation transporters (OCTN), particularly novel organic cation transporter 2 (SLC22A5) which is the physiologically most important carnitine transporter [3,4]. Studies in rodents and pigs demonstrated that carnitine biosynthesis and uptake of carnitine from blood into cells by SLC22A5 are directly regulated by peroxisome proliferator-activated receptor α (PPARA), a transcription factor which plays a central role in the adaptation of metabolism to energy deficiency [5]. In these species, activation of PPARA such as induced by fasting or treatment with synthetic agonists led to increased tissue carnitine concentrations due to an increased rate of biosynthesis and an increased uptake of carnitine from blood into tissues [6-10].

In dairy cows, the transition from late pregnancy to early lactation is associated with severe metabolic adaptations. Production of milk leads to a strong increase of the energy requirement, which however cannot be met as the food intake capacity is limited. Thus, during early lactation, dairy cows are typically in a negative energy balance which is compensated by the mobilization of non-esterified fatty acids (NEFA) from adipose tissue. NEFA are transported by binding with serum albumin and are taken up by fatty acid transporters into tissues, mainly the liver [11]. Studies in rodents have clearly established that NEFA taken up into the liver are able to bind to and activate PPARA [12,13]. In contrast to the large body of literature in non-ruminants, very little work has been conducted to define the specific effects or mechanisms of PPARA in cattle liver so far. However, a recent study using clofibrate as a synthetic agonist in weaned calves showed that PPARA is functional in cattle liver [14]. Moreover, it has been shown that long chain fatty acids are able to activate PPARA also in bovine cells [15]. In accordance with this finding, a negative energy balance in dairy cattle, either occurring physiologically during early lactation or induced by feed restriction, was associated with an up-regulation of several PPARA target genes involved in fatty acid oxidation or ketosis in the liver, indicative of an activation of PPARA [16,17].

To our knowledge, the regulation of carnitine homeostasis in dairy cattle has been less investigated. However, it has been found that hepatic carnitine concentration in dairy cows is increasing during the transition from late pregnancy to early lactation [18,19]. That finding and the assumption that genes involved in carnitine homeostasis in dairy cows might be regulated by PPARA such as in other species, prompted us to the hypothesis that hepatic genes of carnitine synthesis and uptake of carnitine are up-regulated during early lactation in dairy cows. To investigate this hypothesis, we determined mRNA abundances of the relevant genes involved in carnitine synthesis as well as carnitine concentrations in liver biopsy samples of dairy cows in late pregnancy and early lactation.

Methods

The animal experiment was conducted at the Agricultural Experimental Station Hirschau of the Technical University of Munich, Germany. It was approved by the Bavarian state animal care and use committee.

Animals and feeding

This study included twenty Holstein cows (four primi- and sixteen multiparous, 2.7 ± 0.3 parities, mean ± SE) as experimental animals with an experimental period from 3 wk prepartum until 14 wk postpartum. The animals were housed in a playpen. Feeding was composed of a partial mixed ration (PMR) for ad libitum intake of basic feed and separately allocated concentrates [supplemental concentrate (SUPP), 0.63 kg DM/d for each cow; individual concentrate (CONC), individual access]. PMR consisted (dry matter, DM, basis) of 33.7% grass silage, 44.9% maize silage, 6.4% hay and 14.9% concentrate while SUPP contained (DM basis) 24.4% soybean meal, 48.3% grain maize and 27.3% rumen-protected fat supplement. With an assumed dry matter intake of 16 kg of PMR/d and the alloted amount of SUPP, the calculated nutrient supply covered the energy and protein requirements for 23 kg of milk/d. CONC was individually allocated at four computer-operated feeding stations with an automatic feeding program (DeLaval Alpro, Glinde, Germany). CONC was composed of 24.8% grain maize, 21.8% wheat, 20.1% soybean meal, 15.2% dried sugar beet pulp with molasses, 14.9% barley and 3.2% vitamin-mineral premix including limestone (DM basis). The allocation of CONC was increased from 1.2 to 8.0 kg of DM/d during the first 42 d of lactation, and thereafter, it was dependent on the milk performance of the individual cow. Daily intakes of PMR and CONC were recorded for each individual cow. The cows were generally in a good health condition, although four cows had slight metabolic diseases (subclinical ketosis, subclinical acidosis) and nine cows suffered temporarily from mastitis. All used feed components were sampled and analyzed for DM content, for crude nutrients, crude ash, crude fibre and crude fat according to [20], and crude protein by Dumas method. According to the German Society of Nutrition Physiology [21], the net energy content (MJ NEL) and the available CP at the duodenum were calculated. Nutrient concentrations and energy content of all feed components are shown in Table 1.

Sample collection

Milking of lactating cows occured twice daily (0500 and 1500 h) in a 2 × 6 herringbone milking parlor (DeLaval).

Table 1 Nutrient values of experimental feedstuff

	PMR	CONC	SUPP
Energy* (MJ NE_L/kg of DM)	6.45	8.00	12.8
Crude fibre (g/kg of DM)	214	67	69
Crude ash (g/kg of DM)	81	72	49
Crude fat (g/kg of DM)	32	20	303
CP (g/kg of DM)	129	184	140
Available CP (g/kg of DM)*	142	187	151

*calculated values

Milk yields of each cow were recorded automatically and stored in data files. Representative milk samples (50 mL) from every individual cow comprised two consecutive milking procedures (one evening and next morning milking) and were collected twice weekly. Milk sampling at 1, 5 and 14 wk postpartum occurred at days 5.1 ± 1.6, 29.7 ± 1.9, and 92.7 ± 1.9 (means ± SE), respectively. At 3 wk prepartum (21.1 ± 6.0 d prepartum) and 1, 5 and 14 wk postpartum (3.7 ± 1.5, 30.9 ± 1.9 and 94.2 ± 2.6 d postpartum), blood samples of the dammed vena jugularis were drawn using sterile 20 G canulas and lithium heparin tubes (Greiner bio-one, Kremsmunster, Austria). Blood sampling happened before morning feeding between 0730 and 0900 h. Tubes were kept on ice until subsequent centrifugation (2000 × *g*; 15 min). Then, plasma was transferred into 1.5 mL tubes (Greiner bio-one, Frickenhausen, Germany) and stored in aliquots at -20°C until analysis. Furthermore, liver biopsies were taken at 3 wk prepartum (20.4 ± 5.8 d prepartum), and 1, 5 and 14 wk postpartum (3.8 ± 1.4, 31.5 ± 2.1 and 94.9 ± 2.9 d postpartum). For this purpose, cows were separated and fixed after morning milking before feeding between 0700 and 0900 h. The liver biopsy site on the right side of the cow between the 11th and 12th ribs on a line between the olecranon and the tuber coxae was shaved and disinfected before a local subcutaneous anesthesia with 5 mL Isocaine 2% (Procainhydrochloride/Epinephrin, Selectavet, Weyarn/Holzolling, Germany) was performed. Then an autoclaved canula was introduced as a duct for the sterile 14 G biopsy needle (Dispomed Witt oHG, Gelnhausen, Germany) and about 50 mg of liver tissue were removed and immediately snap-frozen in liquid nitrogen. Samples were stored at -80°C until further analysis. The biopsy site was treated with wound spray and animals were kept separated for one day.

Sample analysis

Milk protein and milk fat contents were analyzed by infrared spectrophotometry (MilkoScan-FT-6000, Foss Analytical A/S, Hillerod, Denmark) at the laboratory of Milchprüfring Bayern e.V., Wolnzach, Germany. NEFA and BHBA were determined in the thawed plasma samples using commercial available kits [NEFA-HR(2) and Autokit 3-HB, obtained from Wako Chemicals GmbH, Neuss, Germany]. Lipids from liver biopsy samples were extracted with a mixture of n-hexane and isopropanol (3:2, vol/vol) [22]. An aliquot of the extracts containing 25-50 nmoles of TAG was pipetted into a glass vial (1.5 ml), and the solvent was evaporated by vacuum. The lipids were resolved in a 20 μl portion of a 1:1-mixture of chloroform and Triton X-100 [23], and again the solvent was evaporated. Then 1 ml of commercially available enzymatic TAG kit reagent (Fluitest TG, Analyticon Biotechnologies AG, Lichtenfels, Germany) was added, and after incubation-according to the instruction of the manufacturer-the TAG content was determined by colorimetry.

Energy balance

For calculation of the average daily energy balance of every individual cow, energy intake was calculated from the mean daily intake of PMR, SUPP and CONC and the corresponding energy contents (MJ NEL). Body weights (BW) of the cows were automatically recorded daily by electronic scales installed in the feeding stations. Using the weekly mean BW of the cows, energy requirements for maintenance were calculated according to the German Society of Nutrition Physiology [21]. Those for milk production were calculated on the basis of weekly means of daily milk yield, milk protein content and milk fat content [21]. Changes in body composition were not considered in energy balance evaluation.

Carnitine analysis

Concentrations of total carnitine, free carnitine, acetylcarnitine and propionylcarnitine in plasma, milk and liver tissue were determined by tandem mass spectrometry [24,25]. In brief, freeze dried tissue samples were extracted with methanol:water (2:1 v/v) by homogenization (Tissue Lyser, Qiagen, Hilden, Germany), followed by sonification for 20 min and incubation at 50°C for 30 min in a shaker. After centrifugation (13000 × *g*, 10 min) 20 μL of the supernatant were added with 100 μL methanol containing the internal standards, mixed, incubated for 10 min, and centrifuged (13000 × *g*, 10 min). Plasma and milk samples were handled at 4°C in the same manner as the supernatant after tissue extraction. The final supernatants were used for quantification of the compounds by a 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a Kromasil 100 column (125 mm × 2 mm, 5 μm particle size, CS-Chromatographie Service Langerwehe, Germany) and an API 2000 LC-MS/MS-System (Applied Biosystems, Darmstadt, Germany). As eluents, methanol and a methanol:water:ACN:acetic acid mixture (100:90:9:1 v/v/v/v) were used.

RNA isolation and quantitative real-time PCR (qPCR)

Total RNA was isolated from liver biopsies using Trizol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. RNA from 10 mg of each sample was isolated within one week after finishing the trial. Isolated RNA was preserved at -80°C until use. To estimate RNA concentration and purity, the optical density at 260 and 280 nm, respectively, was determined using an Infinite 200 M microplate reader and a NanoQuant Plate (both from Tecan, Mannedorf, Switzerland). The A260/A280 ratios were 1.96 ± 0.05. In addition, the optical density at 230 nm was determined and the A260/A230 ratios were calculated to control for the presence of contaminations such as guanidine thiocyanate. Although the A260/A230 ratio of some samples was below 2.0 indicating the presence of guanidine thiocyanate, it has been shown that guanidine thiocyanate has no measurable effect on downstream applications such as RT-qPCR until concentrations of more than 100 mM [26] Moreover, RNA quality was assessed by 1% agarose gel electrophoresis. RNA was judged as suitable for only if the samples exhibited intact bands corresponding to the 18S and 28S ribosomal RNA subunits. cDNA was synthesized after RNA extraction from 1.2 μg of total RNA using 100 pmol dT18 primer (Eurofins MWG Operon, Ebersberg, Germany), 1.25 μL 10 mmol/L dNTP mix (GeneCraft, Ludinghausen, Germany), 5 μL buffer (Fermentas, St. Leon-Rot, Deutschland), and 60 units M-MuLV Reverse Transcriptase (MBI Fermentas, St. Leon-Rot, Germany) at 42°C for 60 min, and a final inactivating step at 70°C for 10 min in Biometra Thermal Cycler (Whatman BiometraR, Göttingen, Germany). Subsequently, cDNA was stored in aliquots at -20°C. For the standard curve a cDNA pool of all samples was made. qPCR was performed using 2 μL cDNA combined with 18 μL of a mixture composed of 10 μL KAPA SYBR FAST qPCR Universal Mastermix (Peqlab, Erlangen, Germany), 0.4 μL each of 10 μM forward and reverse primers and 7.2 μL DNase/RNase free water in 0.1 mL tubes (Ltf Labortechnik, Wasserburg, Germany). Gene-specific primer pairs obtained from Eurofins MWG Operon (Ebersberg, Germany) were designed using Primer3 and BLAST. Features of primer pairs are listed in Table 2. All primer pairs were designed to have annealing temperatures of about 60°C, and, if possible, both primers of a primer pair were designed to be located in different exons. qPCR runs were performed with a Rotorgene 2000 system (Corbett Research, Mortlake, Australia), and included all samples and a 5 point relative standard curve plus the non-template control (NTC). The qPCR protocol was as follows: 3 min at 95°C, followed by 40 cycles of a two-step PCR consisting of 5 sec at 95°C (denaturation) and 20 sec at 60°C (annealing and extension). Subsequently, melting curve analysis was performed from 50°C to 95°C to verify the presence of a single PCR product. In addition, the amplification of a single product of the expected size was confirmed using 2% agarose gel electrophoresis stained with GelRedTM nucleic acid gel stain (Biotium, California, USA). Data on qPCR performance for each genes measured are also shown in Table 2. Reference gene stability was determined by performing GeNorm analysis [27] which is based on calculation of a reference gene-stability measure *M*. Out of six tested potential reference genes, the three reference genes with the lowest *M* values have the most stable expression and are used to calculate the GeNorm normalization factor. Therefore, all Ct values were transformed into relative quantification data by using the $2^{-\Delta\Delta Ct}$ equation [28]. Using the GeNorm normalization factor, relative expression levels were calculated, and from normalized expression data, means and SE were computed for samples of the same lactation week. The mean of 3 wk prepartum was set to 1 and relative expression ratios of 1, 5 and 14 wk postpartum are expressed as fold changes compared to 3 wk prepartum.

Statistics

Data were statistically evaluated by a generalized linear model, including the factors time point of sampling, animal, parity number and the interactions between these factors, using the Minitab Statistical Software Release 13.0 (Minitab, State College, PA, USA). Prior to statistical analysis, all data were checked for normality and outliers before statistical analysis. As there was no significant effect of animal and parity number on the parameters investigated, only the effects of time point of sampling are reported in the results section. Linear regression models for relationships of carnitine concentrations in liver tissue with metabolic parameters at the different time points were subjected to analysis by fitted line plots. The significances of differences between the groups over time were analyzed by Fisher's multiple range test. Differences were regarded as significant for $P < 0.05$.

Results

Dry matter intake, performance and energy balance in dairy cows in the transition period and at different stages of lactation

Dry matter intake of the cows 1 wk postpartum was similar to that 3 wk prepartum and increased thereafter towards 5 and 14 wk postpartum (Table 3). The onset of lactation led to a strong negative energy balance (Table 3). Energy balance that was strongest negative in 1 wk postpartum was then improving. At 14 wk postpartum, the cows returned to a slight positive energy balance (Table 3). Milk yield was increasing from 1 to 5

Table 2 Characteristics and performance data of the primers used for reference gene-stability measure *M* and quantitative real-time PCR analysis

Gene	Forward primer (from 5′ to 3′) Reverse primer (from 5′ to 3′)	PCR product	NCBI GenBank	Slope	R^2	Efficiency	*M*
Reference genes							
ACTB	ACTTGCGCAGAAAACGAGAT CACCTTCACCGTTCCAGTTT	120	AY141970	-0.30	0.99	1.99	0.039
SDHA	GCAGAACCTGATGCTTTGTG CGTAGGAGAGCGTGTGCTT	185	NM_174178	-0.24	0.99	1.74	0.048
ATP5B	GGACTCAGCCCTTCAGCGCC GCCTGGTCTCCCTGCCTTGC	229	NM_175796.2	-0.16	0.99	1.44	0.039
RPS9	GTGAGGTCTGGAGGGTCAAA GGGCATTACCTTCGAACAGA	108	BC148016	-0.31	0.99	2.04	0.040
PPIA	GGCAAATGCTGGCCCCAACACA AGTACCACGTGCTTGCCATCCA	87	NM_178320.2	-0.34	0.99	2.13	0.034
RPL12	CACCAGCCGCCTCCACCATG CGACTTCCCCACCGGTGCAC	84	NM_205797.1	-0.35	0.99	2.25	0.036
Target genes							
ACADM	GCGAGTACCCTGTCCCATTA CCTCAGTCATTCTCCCCAAA	243	NM_001075235	-0.29	0.99	1.93	
ACOX1	CCATTGCCGTCCGATACAGT GTTTATATTGCTGGGTTTGATAATCCA	99	BC102761	-0.27	0.96	1.88	
ALDH9A1	CAGGATTCGGCAGAGAGAAC TGAGCCATGAAGAGCATCAC	229	NM_001046423	-0.28	0.99	1.90	
BBOX1	TCCAGCTGCCTACTCTGGAT AGCTGAACCTTACCCCAGGT	292	BC149884.1	-0.28	0.99	1.91	
CD36	GCATTCTGAAAGTGCGTTGA CGGGTCTGATGAAAGTGGTT	179	BC103112	-0.28	0.98	1.91	
CPT1A	CAAAACCATGTTGTACAGCTTCCA GCTTCCTTCATCAGAGGCTTCA	111	FJ415874	-0.32	0.99	2.09	
HMGCS2	GCCCAATATGTGGACCAAAC ATGGTCTCAGTGCCCACTTC	209	NM_001045883	-0.29	0.99	1.96	
PPARA	GGTGGAGAGTTTGGCAGAACCAGA TCCCACTGCCCAGCTCCGATC	168	BT020756.1	-0.23	0.99	1.70	
SLC22A5	CACAGTGGTCAGGAACATGG AATGGTGTCTGGGAGTGGAG	181	BC105377	-0.28	0.99	1.89	
SLC27A1	CTGAAGGAGACCTCCACAGC GTGGTACAGGGGCAGACAGT	208	NM_001033625.2	-0.30	0.99	1.99	
TMLHE	TGGCAGGACACTGCTAGTTG GACAGCCCGGTCATAGTTGT	222	NM_001076064.1	-0.31	0.99	2.05	

wk postpartum and was thereafter declining towards 14 wk postpartum (Table 3). Milk fat and milk protein contents were highest at 1 wk postpartum and were thereafter declining (Table 3).

Relative mRNA abundances of PPARA and genes involved in fatty acid uptake, fatty acid oxidation and ketogenesis in the liver of dairy cows in the transition period and at different stages of lactation

The relative mRNA abundance of PPARA in the liver was increased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$) and was thereafter declining, reaching values at 5 wk and 14 wk postpartum similar with that of 3 wk prepartum (Figure 1). In accordance with the expression pattern of PPARA, target genes involved in fatty acid uptake (SLC27A1, CD36), mitochondrial and peroxisomal β-oxidation (ACOX1, CPT1A, ACADM) and ketogenesis (HMGCS2) were rising from 3 wk prepartum to 1 wk postpartum ($P < 0.05$, Figure 1). From 1 to 5 and 14 wk postpartum, mRNA abundances of these genes, with the only exception of CD36, were declining ($P < 0.05$, Figure 1). mRNA abundances of ACOX1, SLC27A1 and HMGCS2 remained at a higher level at 5 and 14 wk postpartum than at 3 wk prepartum ($P <$

Table 3 Performance of dairy cows in the transition period and at different stages of lactation

Variable	3 wk prepartum	1 wk postpartum	5 wk postpartum	14 wk postpartum	wks 1 to 14 postpartum	*P*-value
Dry matter intake (kg/d)	13.4^{c} ± 0.33	13.4^{c} ± 0.48	18.4^{b} ± 0.62	20.4^{a} ± 0.53	18.5 ± 0.49	< 0.001
Milk yield (kg/d)	-	28.6^{c} ± 0.76	37.5^{a} ± 0.90	32.0^{b} ± 0.81	32.7 ± 0.67	< 0.001
FCM* (kg/d)	-	39.2^{a} ± 1.39	40.9^{a} ± 1.51	33.0^{b} ± 1.03	37.7 ± 0.87	< 0.001
Milk fat (%)	-	6.40^{a} ± 0.25	4.51^{b} ± 0.20	4.11^{b} ± 0.13	5.00 ± 0.17	< 0.001
Milk protein (%)	-	4.09^{a} ± 0.09	2.85^{c} ± 0.05	3.13^{b} ± 0.06	3.35 ± 0.08	< 0.001
Energy balance† (MJ NEL/d)	46.9^{a} ± 2.42	-64.9^{d} ± 5.15	-30.4^{c} ± 3.05	3.89^{b} ± 2.11	-12.5 ± 5.52	< 0.001
Energy intake† (% of requirement)	215.1 ± 7.92	58.9 ± 2.45	82.3 ± 2.22	102.1 ± 2.10	81.1 ± 2.63	< 0.001

Values are mean ± SE (n = 20)

$^{a, b, c, d}$ Means with different superscripts differ significantly ($P < 0.05$)

* Corrected for 4% milk fat content

† Calculated value

0.05, Figure 1). In contrast, mRNA abundances of CPT1A and ACADM, two enzymes of mitochondrial β-oxidation, returned to levels similar to those at 3 wk prepartum (Figure 1).

Relative mRNA abundances of genes involved in carnitine synthesis and carnitine uptake in the liver of dairy cows in the transition period and at different stages of lactation

mRNA abundances of the two genes involved in the formation of γ-butyrobetaine-the precursor of carnitine-TMLHE and ALDH9A1, were strongly (10-, and 6-fold, resp.) increased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$, Figure 2). mRNA abundance of BBOX1, the enzyme which converts γ-butyrobetaine into carnitine, was moderately (1.8-fold) increased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$, Figure 2). mRNA abundance of SLC22A5, the most important carnitine transporter, was strongly (13-fold) increased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$, Figure 2). With the exception of BBOX1, mRNA abundances of all these genes involved in carnitine synthesis pathway and carnitine uptake were declining from 1 wk to 5 and 14 wk postpartum. Nevertheless, mRNA abundances of these genes in 5 and 14 wk postpartum remained at levels higher than those at 3 wk prepartum ($P < 0.05$, Figure 2).

Concentrations of carnitine in liver, plasma and milk of dairy cows in the transition period and at different stages of lactation

In the liver, free carnitine was nearly the exclusive form of carnitine whereas concentrations of carnitine esters (< 1 nmol/g) were only slightly above the detection limit and are therefore not reported. Liver free carnitine concentration was rising from 3 wk prepartum to 1 wk postpartum ($P < 0.05$) and was thereafter falling to levels below those observed at 3 wk prepartum (Table 4). In plasma, the concentration of free carnitine was strongly decreasing from 3 wk prepartum to 1 wk postpartum and was thereafter rising to values which remained however below those observed 3 wk prepartum ($P < 0.05$, Table 4). In contrast, the concentration of carnitine esters in plasma was increasing from 3 wk prepartum to 1 wk postpartum ($P < 0.05$) and was thereafter decreasing; plasma concentration of carnitine esters at 14 wk postpartum was even lower than that 3 wk prepartum ($P < 0.05$, Table 4). Plasma concentration of total carnitine decreased from 3 wk prepartum to 1 wk postpartum ($P < 0.05$) and remained thereafter at a constant level (Table 4). Concentrations of free, esterified and total carnitine in milk were highest at 1 wk postpartum; concentrations were thereafter decreasing and were similar at 5 wk and 14 wk postpartum (Table 4).

Correlations between liver carnitine concentration and phenotypic measures (plasma NEFA and BHBA, hepatic concentration of TAG)

In order to assess whether plasma NEFA concentrations influence liver carnitine concentrations, we calculated a linear regression between plasma NEFA and liver carnitine concentrations. As expected, plasma NEFA concentrations were strongly increasing from 3 wk prepartum to 1 wk postpartum and were thereafter declining (Table 5). At all the three time points considered during lactation (1 wk, 5 wk, 14 wk postpartum), a significant positive correlation between plasma NEFA concentration and liver carnitine concentration was observed ($P < 0.05$, Table 6). In contrast, an inverse correlation between plasma NEFA concentration and liver carnitine concentration was observed at 3 wk prepartum. In order to elucidate whether liver carnitine status influences hepatic TAG accumulation or ketone body formation, we calculated linear regressions between liver carnitine concentrations and liver TAG concentrations or plasma BHBA concentrations. As expected, liver TAG

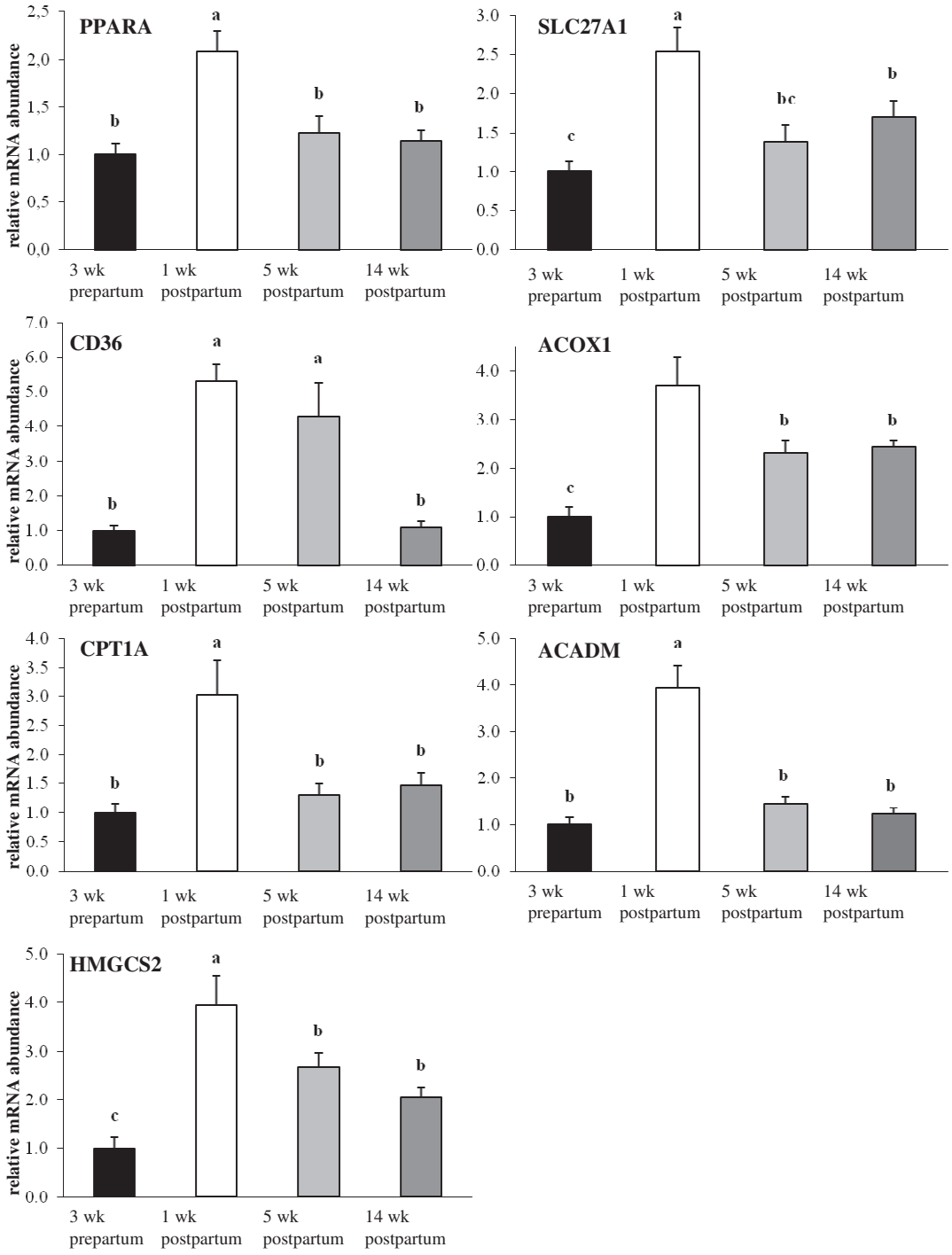

Figure 1

Figure 1 Relative mRNA abundances of PPARA and genes involved in fatty acid uptake, fatty acid oxidation and ketogenesis. Relative mRNA abundances of PPARA and genes involved in fatty acid uptake (SLC27A1, CD36), fatty acid oxidation (ACOX1, CPT1A, ACADM) and ketogenesis (HMGCS2) in the liver of dairy cows in the transition period and at different stages of lactation; bars represent means ± SE (n = 20) and are expressed relative to the mRNA abundance at 3 wk prepartum. [a, b, c] Bars with different superscripts differ significantly ($P < 0.05$).

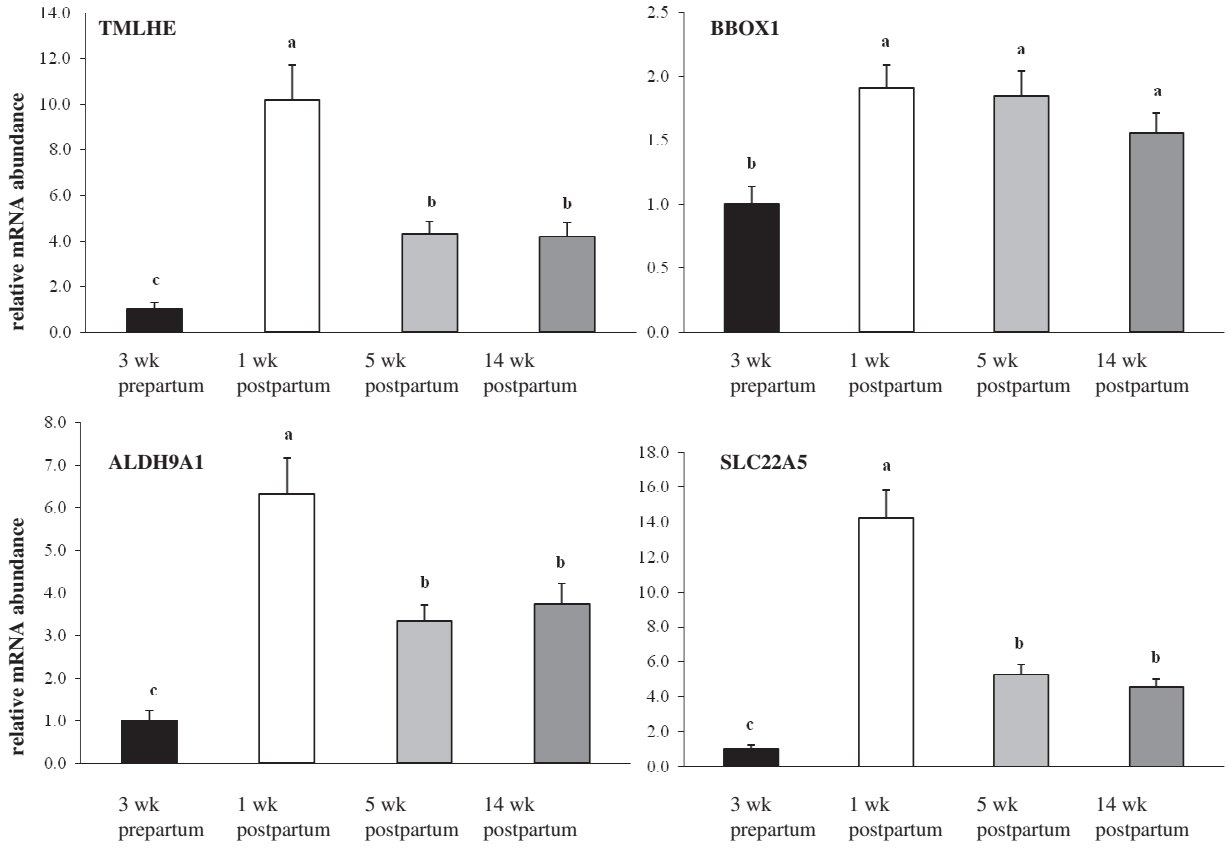

Figure 2

Figure 2 Relative mRNA abundances of genes involved in carnitine synthesis and carnitine uptake. Relative mRNA abundances of genes involved in carnitine synthesis (TMLHE, ALDH9A1, BBOX1) and carnitine uptake (SLC22A5) in the liver of dairy cows in the transition period and at different stages of lactation; bars represent means ± SE (n = 20) and are expressed relative to the mRNA abundance at 3 wk prepartum. [a, b, c] Bars with different superscripts differ significantly ($P < 0.05$).

Table 4 Concentrations of carnitine in liver biopsy, plasma and milk samples of dairy cows in the transition period and at different stages of lactation

	3 wk prepartum	1 wk postpartum	5 wk postpartum	14 wk postpartum	*P*-value
Liver tissue					
free carnitine, nmol/g wet weight	37.4^{b} ± 4.53	55.6^{a} ± 4.09	26.0^{c} ± 1.76	18.2^{c} ± 1.26	< 0.001
Plasma					
free carnitine, μmol/L	3.82^{a} ± 0.25	1.40^{c} ± 0.10	2.18^{b} ± 0.20	2.35^{b} ± 0.17	< 0.001
carnitine esters*, μmol/L	2.07^{b} ± 0.10	2.54^{a} ± 0.12	2.45^{ab} ± 0.18	1.64^{c} ± 0.11	< 0.001
total carnitine†, μmol/L	5.89^{a} ± 0.33	3.94^{b} ± 0.17	4.64^{b} ± 0.32	3.99^{b} ± 0.26	< 0.001
Milk					
free carnitine, μmol/L	-	84.5^{a} ± 5.92	50.3^{b} ± 4.34	60.2^{b} ± 2.65	< 0.001
carnitine esters*, μmol/L	-	131.1^{a} ± 12.1	65.9^{b} ± 6.23	41.3^{c} ± 2.60	< 0.001
total carnitine†, μmol/L	-	215.5^{a} ± 16.6	116.2^{b} ± 7.92	101.5^{b} ± 3.93	< 0.001

Values are mean ± SE (n = 20)

[a, b, c] Means with different superscripts differ significantly ($P < 0.05$)

*Sum of acetyl- and propionyl carnitine, †Sum of free carnitine and carnitine esters

concentration was highest at 5 wk postpartum and BHBA concentrations were highest at 1 wk and 5 wk postpartum (Table 5). However, no significant correlations between liver carnitine concentrations and liver TAG or plasma BHBA concentrations emerged, both pre- and postpartum (Table 6).

Discussion

This study was performed to investigate the hypothesis that the onset of lactation in dairy cows leads to an up-regulation of genes involved in hepatic carnitine synthesis and uptake of carnitine. As expected, the transition from late pregnancy to early lactation was associated with a strong negative energy balance resulting in increased concentrations of NEFA and BHBA in plasma and an increase in hepatic TAG concentration. Similar metabolic changes during the periparturient period in cows have been observed in many other studies [e.g. [16,18,29]]. In agreement with a recent study [16], we observed that the negative energy balance occurring at early lactation was associated with an increased expression of several PPARA target genes involved in fatty acid uptake, mitochondrial and peroxisomal fatty acid oxidation and ketogenesis. Although we were not able to give direct proof of PPARA activation due to small liver sample amount available, an up-regulation of various PPARA target genes is indicative of an activation of that transcription factor in the liver. While there is less research about activation of PPARA in cattle, studies in other species such as rodents or pigs have clearly shown that increased plasma NEFA concentrations, induced by energy deprivation, are leading to an activation of PPARA in liver and other tissues [12,13,30]. As long-chain fatty acids are also acting as agonists of PPARA in bovine cells [15], it seems justified to speculate that high plasma NEFA concentrations in dairy cows during early lactation were causing an activation of PPARA in the liver. It should be noted, however, that in opposite to our study and the study of Loor et al. [16], there are also studies which did not observe an up-regulation of PPARA and PPARA target genes in the liver of dairy cattle during early lactation, particularly when cows had prepartum a caloric intake in excess of 100% of their energy requirement [31,32]. The lack of up-regulation of PPARA during early lactation in these studies has been explained by a hepatic inflammatory response, induced by an excessive prepartum caloric intake, which decreased pre- and postpartum hepatic expression of expression of PPARA [32].

In accordance with the hypothesis of this study, we observed for the first time that the transition from late pregnancy to early lactation leads to an up-regulation of various genes involved in carnitine synthesis (ALDH9A1, TMLHE, BBOX1) and carnitine uptake (SLC22A5) in the liver of cows at 1 wk postpartum. As all these genes are PPARA target genes with functional PPREs identified in their promoters or first introns [32-35], we assume that the up-regulation of these genes in the liver of dairy cows at 1 wk postpartum was caused by a potential activation of PPARA. The finding of positive correlations between plasma concentrations of NEFA, which might be regarded as natural agonists, and hepatic carnitine concentrations during lactation supports a role of PPARA in the regulation of genes of carnitine synthesis and uptake.

The present study confirms previous studies in showing that liver carnitine concentration is increasing during the transition from late pregnancy to lactation and is thereafter continuously decreasing to values similar or even below those observed in pregnancy [18,19]. In the study of Carlson et al. [19], hepatic total carnitine concentrations were around 1.6-fold higher on 2 d of lactation compared to 3 wk prepartum, while values at d 28 were even lower than those 3 wk prepartum. In the study of Grum et al. [18], concentrations of acid-soluble carnitine (free carnitine plus short-chain acyl carnitine) sharply increased from 3 wk prepartum to 1 d postpartum and returned to prepartum values at d 21 postpartum. As carnitine is synthesized from trimethyllysine released primarily from turnover of skeletal muscle proteins, Grum et al. [18] suggested that an increased hepatic carnitine concentration at 1 d postpartum might be due to an enhanced catabolism of muscle protein in this early stage of lactation. Although we have not directly measured carnitine synthesis and uptake into cells, the findings of increased mRNA expression of genes of carnitine synthesis and uptake suggest that increased hepatic carnitine concentrations at early lactation could also

Table 5 Metabolic parameters in liver biopsy and plasma samples of dairy cows in the transition period and at different stages of lactation

	3 wk prepartum	1 wk postpartum	5 wk postpartum	14 wk postpartum	*P*-value
Plasma NEFA (μmol/L)	103^{d} ± 10	864^{a} ± 47	276^{b} ± 16	171^{c} ± 11	< 0.001
Liver triglyceride (mg/g wet weight)	4.56^{c} ± 0.45	23.5^{b} ± 2.6	64.3^{a} ± 10.3	3.18^{c} ± 0.28	< 0.001
Plasma BHBA (μmol/L)	484^{b} ± 23	707^{a} ± 42	724^{a} ± 46	521^{b} ± 24	< 0.001

Values are mean ± SE (n = 20).

[a, b, c, d] Means with different superscripts differ significantly ($P < 0.05$)

Table 6 Linear regression parameters for the relationship of concentration of free carnitine in liver biopsy samples (nmol/g wet weight) as predictor variable with different metabolic parameters of dairy cows in the transition period and at different stages of lactation as response variables

Response variable		3 wk prepartum	1 wk postpartum	5 wk postpartum	14 wk postpartum
Plasma NEFA (μmol/L)	Intercept	152.7	285.7	115.7	81.4
	Slope	-1.17	10.2	5.86	4.66
	R^2	0.29	0.64	0.38	0.26
	P-value	0.015	< 0.001	0.008	0.035
Liver triglyceride (mg/g wet weight)	Intercept	3.07	30.5	44.1	3.81
	Slope	0.04	-0.14	0.65	-0.05
	R^2	0.15	0.09	0.01	0.09
	P-value	0.112	0.291	0.673	0.305
Plasma BHBA (μmol/L)	Intercept	562.8	605.3	603.7	503.3
	Slope	-1.84	1.43	4.56	0.60
	R^2	0.13	0.02	0.03	0.00
	P-value	0.113	0.621	0.505	0.907

be due, at least in part, to an increased carnitine synthesis in the liver and an increased uptake of carnitine from blood into the liver. This suggestion is supported by studies in rodents and pigs which found that an up-regulation of enzymes of carnitine synthesis in the liver, caused either by treatment with PPARA agonists or by energy deprivation, leads to an increased hepatic carnitine concentration without changing the concentrations of BB or TML, the precursors of carnitine synthesis [7,8,36,37]. The finding that the concentration of free carnitine in plasma is strongly decreasing from 3 wk prepartum to 1 wk postpartum fits into this suggestion. Studies in rodents have shown that an up-regulation of SLC22A5 by treatment with PPARA agonists or by energy deprivation leads to a reduction of plasma carnitine concentration, due to an increased transport of carnitine from plasma into tissues [6,8,36]. The decrease in plasma carnitine concentrations during early lactation might be in part explained by the transfer of carnitine from plasma into the milk in mammary gland. Nevertheless, with respect to the findings in rodents, we assume that reduced plasma carnitine concentrations in early lactation could also be caused by an increased uptake of free carnitine from plasma into tissues, including the liver. In accordance with present study, Carlson et al. [19] also observed a strong reduction of plasma carnitine concentration from 21 d prepartum to 9 or 27 d postpartum. Those authors [19] also found that carnitine concentration in skeletal muscle is not changing significantly during transition from pregnancy into lactation. This finding also agrees with studies in rodents which show that energy deprivation does not influence muscle carnitine concentrations [7,36].

Furthermore, we observed that milk carnitine concentration is highest at 1 wk postpartum and is thereafter decreasing to 5 wk and 14 wk postpartum. This finding agrees with the study of Carlson et al. [19] which found a strong reduction of milk carnitine concentration from 2 wk to 6 wk postpartum. It is possible that the high carnitine concentration in milk at wk 1 postpartum is due to the strong negative energy balance of the cows. Carlson et al. [38] found that energy restriction of cows increased milk carnitine concentrations. In the mammary gland, carnitine is secreted into the milk by several transporters (SLC22A4, SLC22A5, OCTN3, SLC6A14, SLC6A10) [39]. Possibly, one or more of these transporters are up-regulated in a state of a negative energy balance. Studies in rats also found a reduction of milk carnitine concentration from early to later stage of lactation, due to a down-regulation of SLC22A5, OCTN3 and SLC6A14 [39].

Ketosis and fatty liver are two diseases in dairy cows during early lactation which are linked to hepatic fatty acid oxidation [40]. As carnitine is involved in β-oxidation due to its role in the transport of long chain fatty acids into the mitochondrion, it was interesting to explore whether plasma ketone body concentrations or hepatic TAG concentrations are correlated with hepatic carnitine concentrations. The observation that there were no correlations between hepatic carnitine concentration and both, plasma BHBA and hepatic TAG concentrations, at 1, 5 and 14 wk postpartum suggests that the availability of carnitine in the liver had no influence on ketogenesis and TAG accumulation in the liver. The observation that hepatic carnitine concentration does not correlate with BHBA concentration is in accordance with a study showing that carnitine supplementation does not influence plasma BHBA concentration in early lactating dairy cattle [41]. The finding that the activity of CPT1A does not play a primary role in the etiology of ketosis [42] is another indication that the availability of carnitine, which acts as a cofactor of that enzyme, is

not a key factor in the production of ketone bodies. Accumulation of TAG in the liver is explained by the observation that the capacity of bovine liver tissue to convert fatty acids to esterified products is strongly increased during the early postnatal period, whereas fatty acid oxidation is only slightly increased, meaning that NEFA from mobilization are directed towards conversion to TAG [43]. The finding that there was no correlation between hepatic carnitine concentration and hepatic TAG concentration thus indicates that a higher hepatic carnitine concentration did not stimulate hepatic fatty acid oxidation. This indication is, however, in contradiction to some in vitro and in vivo studies in dairy cows. In vitro studies using bovine liver slices have shown that addition of carnitine enhances the oxidation of palmitate [44,45]. Moreover, postruminal infusion of carnitine enhanced palmitate oxidation and decreased liver lipid accumulation in cows with experimentally induced negative energy balance [41]. These studies suggested that carnitine might be the rate-limiting factor of hepatic β-oxidation in dairy cows during the periparturient period and that carnitine supplementation might prevent the development of a fatty liver.

Conclusions

The present study shows for the first time that hepatic mRNA abundances of genes involved in carnitine synthesis and cellular uptake of carnitine in dairy cows are increased during the transition from late pregnancy to lactation. An up-regulation of genes involved in carnitine biosynthesis and uptake could contribute to elevated hepatic carnitine concentration in early lactation observed in this and previous studies.

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Author details

[1]Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany. [2]Institute of Agricultural and Nutritional Sciences, Martin-Luther-Universität Halle, Von-Danckelmann-Platz 2, D-06120 Halle, Saale, Germany. [3]Animal Nutrition, Technische Universität München, Liesel-Beckmann-Strasse 6, D-85354 Freising, Germany.

Authors' contributions

GS: conducted the animal experiment, performed the statistical analyses and wrote the manuscript. JK: performed the PCR analyses. FH and SG: performed the carnitine analyses and helped to draft the manuscript; FJS: participated in the design of the study and supervised the animal experiment; RR: supervised PCR analyses. GIS: supervised the carnitine analyses. KE: conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Expression of fibroblast growth factor 21 in the liver of dairy cows in the transition period and during lactation

G. Schlegel[1], R. Ringseis[1], J. Keller[1], F. J. Schwarz[2], W. Windisch[2] and K. Eder[1]

1 Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Gießen, Gießen, Germany, and
2 Chair of Animal Nutrition, Department of Animal Sciences, Center of Life and Food Sciences Weihenstephan, Technische Universität München, Freising-Weihenstephan, Germany

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Correspondence
K. Eder, Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Heinrich-Buff-Ring, 35390 Giessen, Germany. Tel: +49 641 9939230;
Fax: +49 641 9939239;
E-mail: klaus.eder@ernaehrung.uni-giessen.de

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Summary

Fibroblast growth factor 21 (FGF21) has been identified as a novel hormonal factor involved in the regulation of metabolic adaptations during energy deprivation. The present study aimed to investigate the expression of the *FGF21* gene in the liver of dairy cows during the transition from pregnancy to lactation. Therefore, the relative mRNA abundance of *FGF21* in liver biopsy samples of 20 dairy cows in late pregnancy (3 weeks pre-partum) and early lactation (1, 5, 14 weeks post-partum) was determined. It was observed that hepatic mRNA abundance of *FGF21* at 1 week post-partum was dramatically increased (110-fold) compared to 3 weeks pre-partum ($p < 0.001$). With progress of lactation, mRNA concentration of *FGF21* was declining; nevertheless, mRNA abundance at 5 and 14 weeks post-partum remained 25- and 10-fold increased compared to 3 weeks pre-partum ($p < 0.001$). Using a gene array technique, it was found that many genes involved in fatty acid oxidation, gluconeogenesis and ketogenesis were up-regulated during early lactation compared to late pregnancy. Moreover, there were positive linear correlations between hepatic mRNA concentration of *FGF21* and mRNA concentrations of genes involved in ketogenesis as well as carnitine synthesis and carnitine uptake at various time-points during lactation, indicating that FGF21 could play a role in ketogenesis and carnitine metabolism in the liver of dairy cows ($p < 0.05$). In overall, the present study shows that expression of the *FGF21* gene is strongly up-regulated during the transition period. It is assumed that the up-regulation of *FGF21* might play an important role in the adaptation of liver metabolism during early lactation in dairy cows such as in other species.

Introduction

In mammals, the liver plays an important role in maintaining systemic energy balance during energy deprivation by coordinate regulation of lipid and carbohydrate metabolism. During the early stage of energy deprivation, the liver mobilizes glucose from its glycogen stores. As fasting progresses and glycogen stores are depleted, β-oxidation of fatty acids deriving from adipose tissue is enhanced. Oxidation of fatty acids yields acetyl-CoA which is used for energy production via tricarboxylic acid cycle and oxidative phosphorylation and for the production of ketone bodies. These metabolic adaptations during energy deprivation are controlled mainly by hormones such as glucagon, catecholamines and glucocorticoids (Potthoff et al., 2009). Recently, fibroblast growth factor 21 (FGF21) has been detected and identified as an important hormonal factor involved in the regulation of metabolic adaptations during energy deprivation (Badman et al., 2007; Inagaki et al., 2007; Lundåsen et al., 2007). The *FGF21* gene

is expressed in liver, pancreas, white adipose tissue and skeletal muscle (Nishimura et al., 2000; Izumiya et al., 2008; Wang et al., 2008). Studies in rodents have shown that hepatic expression of *FGF21* is strongly up-regulated during fasting and is rapidly suppressed by refeeding, indicating a role of FGF21 in fasting-induced response (Badman et al., 2007; Inagaki et al., 2007; Lundåsen et al., 2007). Indeed, it was demonstrated that FGF21 stimulates hepatic lipid oxidation, ketogenesis and gluconeogenesis, pathways required to supply the body with sufficient energy fuels and glucose during fasting (Badman et al., 2007). It has been found that *FGF21* is up-regulated by peroxisome proliferator-activated receptor (PPAR)-α, a transcription factor which is activated during fasting by non-esterified fatty acids (NEFA) released from adipose tissue (Badman et al., 2007; Lundåsen et al., 2007; Oishi et al., 2008; Yu et al., 2012). The metabolic importance of FGF21 is evident from transgenic mice overexpressing *FGF21* and from FGF21 null mice. Mice overexpressing hepatic *FGF21* exhibit increased hepatic ketone body production and an enhanced expression of key genes involved in gluconeogenesis and fatty acid oxidation (Inagaki et al., 2007). In contrast, transgenic FGF21 null mice show reduced rates of gluconeogenesis, β-oxidation and ketogenesis during fasting (Potthoff et al., 2009). Hence, the induction of *FGF21* is required for the normal activation of hepatic lipid oxidation, gluconeogenesis and ketogenesis in response to nutritional challenges (Badman et al., 2007).

During early lactation, dairy cows are typically in a strong negative energy balance because of strong increase in the energy requirement for milk production, which cannot be met as the food intake capacity is limited. The negative energy balance is associated with complex metabolic changes in the liver which includes increased expression of genes involved in β-oxidation of fatty acids, ketogenesis and gluconeogenesis (Loor et al., 2005; Van Dorland et al., 2009; Graber et al., 2010, 2011). Moreover, it is expected that the negative energy balance during early lactation leads to an activation of PPARα in the liver, induced by an increased uptake of NEFA from the blood deriving from lipolysis in the adipose tissue. Indeed, there are few studies showing an up-regulation of *PPARα* and PPARα target genes in the liver of dairy cows during early lactation (Loor et al., 2005; Schlegel et al., 2012a). Owing to the fact that *FGF21* expression is regulated by PPARα, an activation of PPARα in early lactation is expected to cause an induction of *FGF21*. Indeed, Carriquiry et al. (2009) were the first who observed an up-regulation of *FGF21* in the liver of dairy cows during lactation. Interestingly, these authors did not observe an up-regulation of *PPARA* in the liver, which raises the possibility that *FGF21* was up-regulated during lactation in a PPARα-independent manner.

In contrast to the study of Carriquiry et al. (2009), we observed recently an increase of the mRNA level of *PPARA* and its target genes involved in fatty acid transport, fatty acid oxidation and carnitine synthesis, indicative of PPARα activation, in the liver of dairy cows during the transition from pregnancy to early lactation (Schlegel et al., 2012a). As activation of PPARα induces expression of *FGF21*, we hypothesized that expression of *FGF21* might be strongly increased in the liver of these cows during early lactation. To investigate this hypothesis, we determined mRNA abundance of *FGF21* in liver biopsy samples from a recently performed experiment with dairy cows (Schlegel et al., 2012a). To assess the expression of genes involved in hepatic β-oxidation, gluconeogenesis and ketogenesis – metabolic pathways regulated by FGF21 – we also determined relative mRNA concentrations of several genes involved in fatty acid metabolism, gluconeogenesis and ketogenesis in these samples using a gene array technique. To investigate the role of FGF21 in metabolic adaptations in hepatic lipid metabolism during lactation, we calculated correlations between hepatic mRNA concentration of *FGF21* and phenotypic variables of lipid metabolism as well as mRNA abundances of genes involved in hepatic lipid metabolism.

Materials and methods

The animal experiment was conducted at the Agricultural Experimental Station Hirschau of the Technical University of Munich, Germany. It was approved by the Bavarian state animal care and use committee (Az.: 55.2-1-54-2531-78-09).

Animals and feeding, sample collection

This study included 20 Holstein cows (four primi- and 16 multiparous, 2.7 ± 1.1 parities, mean ± SD) as experimental animals with an experimental period from 3 weeks pre-partum until 14 weeks post-partum. The animals were housed in a freestall barn. They received a partial mixed ration (PMR) for *ad libitum* intake of basic feed with separate and limited intake of concentrates [supplemental concentrate (SUPP), 0.63 kg DM/day for each cow; individual concentrate (CONC), individual access]. Partial mixed ration consisted [dry matter (DM), basis] of 33.7%

grass silage, 44.9% maize silage, 6.4% hay and 14.9% concentrate, while SUPP contained (DM basis) 24.4% soybean meal, 48.3% grain maize and 27.3% of a rumen-protected fat supplement. With an assumed dry matter intake of 16 kg of PMR/day and the allotted amount of SUPP, the calculated nutrient supply covered the energy and protein requirements for 23 kg of milk/day. CONC was individually allocated at four computer-operated feeding stations with an automatic feeding programme (DeLaval Alpro, Glinde, Germany). CONC was composed of 24.8% grain maize, 21.8% wheat, 20.1% soybean meal, 15.2% dried sugar beet pulp with molasses, 14.9% barley and 3.2% vitamin–mineral premix including limestone (DM basis). The allocation of CONC was increased from 1.2 to 8.0 kg of DM/day during the first 42 days of lactation, and thereafter, it was dependent on the milk performance of the individual cow. Daily intakes of PMR and CONC were recorded for each individual cow. Liver biopsies were taken from the right liver lobe (*Lobus hepatis dexter*) at 3 weeks pre-partum, and 1, 5 and 14 weeks post-partum before feeding between 07:00 and 09:00 hours. More details about feeding and collection of liver biopsies are given by Schlegel et al. (2012a).

RNA isolation and quality control

Total RNA was isolated from liver biopsies using Trizol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. RNA from 15 mg of each sample was isolated within 1 week after finishing the trial. Isolated RNA was preserved at −80 °C until use. RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively, using an Infinite 200M microplate reader and a NanoQuant Plate (both from Tecan, Männedorf, Switzerland). The A260/A280 ratios were 1.96 ± 0.05 (means ± SD). The integrity of the total RNA was checked by 1% agarose gel electrophoresis. RNA was judged as suitable for array hybridization only if the samples exhibited intact bands corresponding to the 18S and 28S ribosomal RNA subunits. Subsequently, five RNA pools, each for every time-point (3 weeks pre-partum, 1, 5 and 14 weeks post-partum), were prepared. Each RNA pool comprised RNA from four animals.

Microarray analysis

For microarray analyses, the RNA pools were sent to the Center of Excellence for Fluorescent Bioanalytics (KFB) at the University of Regensburg for hybridization to the Affymetrix GeneChip bovine genome arrays (Affymetrix, High Wycombe, UK). The Affymetrix GeneChip bovine genome array is based on content from UniGene and GenBank® mRNAs. It contains 24 027 probe sets representing more than 23 000 transcripts and includes approximately 19 000 UniGene clusters. In brief, total RNA was checked for quality and quantity using an Agilent Bioanalyzer 2100 machine, and total RNA was transcribed to first- and second-strand cDNAs. After purification and testing on an Agilent Bioanalyzer 2100 machine (Agilent Technologies, Santa Clara, CA, USA), the double-stranded cDNA served as a template for the *in vitro* transcription reaction for cRNA synthesis. The cRNA was labelled with biotin using the Affymetrix GeneChip labelling kit. After checking the quality and quantity of the labelled cRNA, cRNA was fractionated and hybridized with the Affymetrix GeneChips. GeneChips were washed and stained with the Affy-metrix GeneChip Fluidics station 450. The GeneChips were then scanned with an Affymetrix GeneChip scanner 3000. All procedures were performed according to Affymetrix protocols (GeneChip expression analysis, Technical manual from Affymetrix). The quality of hybridization was assessed in all samples following the manufacturer's recommendations. After scanning the arrays, cell intensity files containing a single intensity value for each probe cell were computed from the image data with the Affymetrix GeneChip Operating Software. Probe cell intensity data were further analysed in the Affymetrix Expression Console 1.1 software using the Affymetrix Microarray Suite Version 5.0 (MAS 5.0, Affymetrix, High Wycombe, UK) algorithm to create chip files. In the MAS 5.0 algorithm, a global scaling strategy is applied for normalization where the average signal intensity of all probe sets is scaled to a default target signal.

Quantitative real-time PCR

Relative mRNA concentration of *FGF21* was determined using quantitative real-time PCR (qPCR). For this end, cDNA was synthesized in less than a week after RNA extraction from 1.2 μg of total RNA from all individual samples (n = 20/group) contributing to the RNA pools for microarray analysis using 100 pmol dT18 primer (Eurofins MWG Operon, Ebersberg, Germany), 1.25 μl 10 mM dNTP mix (GeneCraft, Lüdinghausen, Germany), 5 μl buffer (Fermentas, St. Leon-Rot, Deutschland), and 60 units M-MuLV Reverse Transcriptase (MBI Fermentas, St. Leon-Rot, Germany) at 42 °C for 60 min, and a final inactivating step at 70 °C for

10 min in Biometra Thermal Cycler (Whatman Biometra®, Göttingen, Germany). Subsequently, cDNA was stored in aliquots at −20 °C. For the standard curve, a cDNA pool of all samples was made. qPCR was performed using 2 μl cDNA combined with 18 μl of a mixture composed of 10 μl KAPA SYBR FAST qPCR Universal Mastermix (Peqlab, Erlangen, Germany), 0.4 μl each of 10 μM forward and reverse primers and 7.2 μl DNase/RNase free water in 0.1 ml tubes (Ltf Labortechnik, Wasserburg, Germany). Gene-specific primer pairs obtained from Eurofins MWG Operon were designed using PRIMER3 and BLAST. Sequence of gene-specific primer pair for *FGF21* was as follows (forward, reverse; NCBI Genbank): 5′-GGCATCATCCGTGTAGAGGT-3′, 5′-TTCAAGCACTTGGGACTGTG-3′; XM_001789587.1). Expression value of *FGF21* was normalized using the GeNorm normalization factor. Procedure of normalization, the characteristics of gene-specific primers and the average expression stability ranking of the six potential reference genes in liver of cows were previously described in detail (Schlegel et al., 2012b). After normalization of gene expression data using the calculated GeNorm normalization factor, means and SD were calculated from normalized expression data for samples of the same treatment group. The mean of 3 weeks pre-partum was set to 1 and relative expression ratios of 1, 5 and 14 weeks post-partum are expressed as fold changes compared to 3 weeks pre-partum.

Determination of phenotypic variables and mRNA abundances of genes used for regression analysis

Variables considered for regression analysis [Plasma concentrations of NEFA and β-hydroxy butyrate (BHB), hepatic concentrations of triacylglycerols (TAG) and cholesterol, concentrations of total and free carnitine in plasma, milk and liver and hepatic mRNA abundances of genes involved in various pathways of lipid metabolism] were determined as described recently (Schlegel et al., 2012a,b).

Statistics

Data were statistically evaluated by ANOVA with time-point of sampling, parity of the cow (primi- vs. multiparous) and individual animal as factors, using the MINITAB Statistical Software Release 13.0 (Minitab, State College, PA, USA). Prior to statistical analysis, all data were checked for normality and outliers before statistical analysis. Means of mRNA concentrations of *FGF21* between the four time-points of sampling were compared by Fisher's multiple-range test. For estimation of correlations between hepatic *FGF21* mRNA abundance and metabolic variables and mRNA abundances of various hepatic genes, Pearson's correlation coefficients (r) were determined by linear regression analysis. Gene array data were evaluated by comparing the means of 1, 5 and 14 weeks post-partum with that of 3 weeks pre-partum using the Student's t-test.

Results

Expression of *FGF21* in the liver

Relative mRNA abundance of *FGF21*, determined by qPCR, was dramatically increasing (110-fold, $p < 0.001$) from 3 weeks pre-partum to 1 week post-partum and was thereafter declining (Fig. 1). Nevertheless, relative mRNA abundances of *FGF21* at 5 and 14 weeks post-partum were 25- and 10-fold, respectively, higher compared to the pre-partum level ($p < 0.001$, Fig. 1). There was no difference in relative hepatic mRNA abundance of *FGF21* between primi- and pluriparous cows ($p > 0.05$).

Expression of hepatic genes involved in hepatic β-oxidation

To assess the effect of lactation on hepatic mitochondrial β-oxidation, we considered the expression of 17 genes involved in that pathway (Table 1). In 1 week post-partum, all the genes considered, with the only

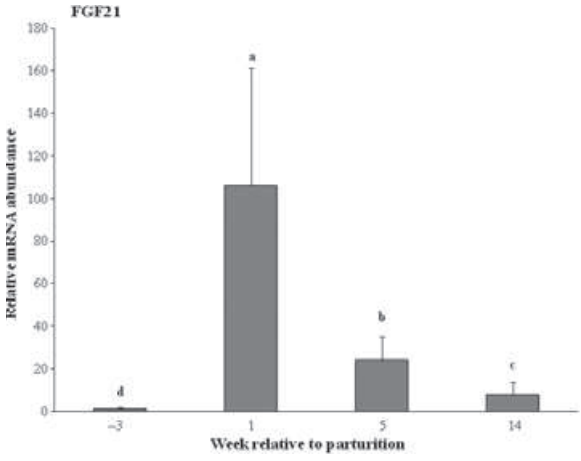

Fig. 1 Relative mRNA abundance of fibroblast growth factor 21 (*FGF21*) in the liver of dairy cows in the transition period and early stages of lactation; bars represent means ± SD ($n = 20$) and are expressed relative to the mRNA abundance at 3 weeks pre-partum (=1.00). Bars with different superscripts differ significantly by Fisher's multiple-range test ($p < 0.001$).

Table 1 Expression of genes involved in hepatic mitochondrial β-oxidation in Holstein cows at 1, 5 and 14 weeks post-partum (data are given as fold change compared to 3 weeks pre-partum)

Gene	Description	1 week post-partum	5 weeks post-partum	14 weeks post-partum
CPT1A	Carnitine palmitoyltransferase 1A	1.66*	1.11	1.12
CPT1B	Carnitine palmitoyltransferase 1B (muscle)	6.11*	2.03$^{\#}$	1.49^{+}
CPT1C	Carnitine palmitoyltransferase 1C	1.37*	1.31^{+}	1.08
CPT2	Carnitine palmitoyltransferase 2	2.35*	1.65$^{\#}$	1.35
SLC25A20	Solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	1.9^{+}	1.35^{+}	1.12
MLYCD	Malonyl-CoA decarboxylase	1.74*	1.24$^{\#}$	1.06
ACADS	Acyl-CoA dehydrogenase, C-2 to C-3 short chain	1.40*	1.37*	1.16^{+}
HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/ enoyl-CoA hydratase (trifunctional protein), alpha subunit	2.61*	1.61$^{\#}$	1.15
HADHB	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/ enoyl-CoA hydratase (trifunctional protein), beta subunit	2.40*	1.65$^{\#}$	1.25
ACADVL	Acyl-coenzyme A dehydrogenase, very long chain	2.14*	1.33^{+}	0.99
ACADM	Acyl-CoA dehydrogenase, C-4–C-12 straight chain	1.34*	1.17^{+}	1.18^{+}
ACADL	Acyl-CoA dehydrogenase, long chain	1.85$^{\#}$	1.31	1.13
ACADSB	Acyl-CoA dehydrogenase, short/branched chain	1.32^{+}	1.13	1.15
ECHS1	Enoyl-CoA hydratase, short chain, 1, mitochondrial	1.73^{+}	1.68^{+}	1.42
ACAA2	Acetyl-Coenzyme A acyltransferase 2	1.70$^{\#}$	1.42$^{\#}$	1.39^{+}
PCCA	Propionyl CoA carboxylase, alpha polypeptide	1.50^{+}	1.39	1.27
PCCB	Propionyl CoA carboxylase, beta polypeptide	1.36	1.52^{+}	1.26

Results are means of five RNA pools per time-point considered. Significance of differences compared to 3 weeks pre-partum: $^{+}p < 0.05$; $^{\#}p < 0.01$; $^{*}p < 0.001$.

exception of *PCCB* encoding propionyl CoA carboxylase, beta polypeptide were up-regulated (1.32–6.11-fold, $p < 0.05$) compared to 3 weeks pre-partum (Table 1). From 1 week post-partum to later lactation, expression of all the genes involved in mitochondrial β-oxidation decreased (Table 1). In 5 weeks post-partum, 13 of the genes considered remained increased in its expression in relation to 3 weeks pre-partum ($p < 0.05$), while in 14 weeks post-partum, only four of the genes considered remained up-regulated compared to 3 weeks pre-partum ($p < 0.05$, Table 1).

Expression of hepatic genes involved in hepatic gluconeogenesis

To assess the effect of lactation on hepatic gluconeogenesis, we considered the expression of 12 genes involved in that pathway (Table 2). In 1 week post-partum, the expression of all these 12 genes was up-regulated (1.48–4.52-fold, $p < 0.05$, Table 2). From 1 week post-partum to later lactation, expression of all the genes involved in hepatic gluconeogenesis decreased (Table 2). In 5 and 14 weeks post-partum, 8 and 4, respectively, of the 12 genes considered remained up-regulated compared to 3 weeks pre-partum ($p < 0.05$, Table 2).

Expression of hepatic genes involved in hepatic ketogenesis

To assess the effect of lactation on hepatic ketogenesis, we considered the expression of three genes involved in that pathway (Table 3). In 1 week post-partum, these three genes were 1.26–2.26-fold up-regulated compared to 3 weeks pre-partum ($p < 0.05$, Table 3). Although the expression of these genes was declining from 1 week post-partum to later lactation, the three genes involved in ketogenesis remained up-regulated in 5 weeks post-partum compared to 3 weeks pre-partum ($p < 0.05$, Table 3). In 14 weeks post-partum, expression of these genes was not different compared to 3 weeks pre-partum (Table 3).

Correlations between *FGF21* mRNA abundance and phenotypic variables or hepatic mRNA abundances of various genes

To assess the role of hepatic FGF21 in adaptation of hepatic lipid metabolism during lactation, linear regression analysis between hepatic *FGF21* mRNA concentration and phenotypic variables (plasma NEFA and BHB concentrations, hepatic TAG and cholesterol concentrations, concentrations of total

Table 2 Expression of genes involved in hepatic gluconeogenesis in Holstein cows at 1, 5 and 14 weeks post-partum (data are given as fold change compared to 3 weeks pre-partum)

Gene	Description	1 weeks post-partum	5 weeks post-partum	14 weeks post-partum
PC	Pyruvate carboxylase	4.38*	1.99*	1.35^{+}
PCK1	Phosphoenol pyruvate carboxykinase 1	1.53$^{\#}$	1.61$^{\#}$	1.40^{+}
PCK2	Phosphoenol pyruvate carboxykinase 2	1.60$^{\#}$	1.18	1.15
FBP1	Fructose-1,6-bisphosphatase 1	3.92*	1.85$^{\#}$	1.35
FBP2	Fructose-1,6-bisphosphatase 2	2.48	1.72	1.23
G6PC	Glucose-6-phosphatase	3.71*	2.01$^{\#}$	1.97$^{\#}$
GK	Glycerol kinase	4.52*	1.65	1.23
GPD1	Glycerol-3-phosphate dehydrogenase	1.82*	1.89*	1.52^{+}
LDHA	Lactate dehydrogenase A	3.66$^{\#}$	1.72	1.20
LDHB	Lactate dehydrogenase B	3.45*	2.24$^{\#}$	1.41
MDH1	Malate dehydrogenase 1 (soluble)	1.90$^{\#}$	1.64^{+}	1.39
MDH2	Malate dehydrogenase 2 (mitochondrial)	1.48$^{\#}$	1.34^{+}	1.20

Results are means of five RNA pools per time-point considered. Significance of differences compared to 3 weeks pre-partum: $^{+}p < 0.05$; $^{\#}p < 0.01$; $^{*}p < 0.001$.

Table 3 Expression of genes involved in hepatic ketogenesis in Holstein cows at 1, 5 and 14 weeks post-partum (data are given as fold change compared to 3 weeks pre-partum)

Gene	Description	1 week post-partum	5 weeks post-partum	14 weeks post-partum
HMGCL	3-hydroxymethyl-3-methylglutaryl-CoA lyase	1.26$^{\#}$	1.16$^{\#}$	1.07
HMGCS2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (mitochondrial)	2.26*	1.78$^{\#}$	1.38
ACAT1	Acetyl-CoA acetyltransferase 1	1.63*	1.43$^{\#}$	1.23

Results are means of five RNA pools per time-point considered. Significance of differences compared to 3 weeks pre-partum: $^{\#}p < 0.01$; $^{*}p < 0.001$.

and free carnitine in plasma, milk and liver) and mRNA abundances of genes involved in hepatic fatty acid uptake and oxidation [fatty acid transporter, member 1 (*SLC27A1*), CD36/fatty acid translocase (*CD36*), acyl-CoA oxidase 1, (*ACOX1*), carnitine palmitoyltransferase 1A (*CPT1A*), acyl-CoA dehydrogenase, C-4–C-12 straight chain (*ACADM*)], carnitine synthesis and uptake [butyrobetaine (gamma), 2-oxoglutarate dioxygenase (*BBOX1*), trimethyllysine hydroxylase, epsilon (*TMLHE*), aldehyde dehydrogenase 9 family, member A1 (*ALDH9A1*), organic cation/carnitine transporter 2 (*SLC22A5*)], fatty acid synthesis [sterol regulatory element-binding protein-1 (*SREBF1*), fatty acid synthase (*FASN*), acetyl-CoA carboxylase alpha (*ACACA*), stearoyl-CoA desaturase (*SCD*)], cholesterol synthesis [sterol regulatory element-binding protein-2 (*SREBF2*), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*)] and ketogenesis [3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2, mitochondrial (*HMGC2*)] was performed. In overall, there were only few significant correlations between hepatic *FGF21* mRNA abundance and other variables (Table 4). At 3 weeks ante-partum, there were positive linear correlations between hepatic *FGF21* mRNA abundance and mRNA abundances of *ACOX1* and genes involved in carnitine synthesis and uptake (*TMLHE*, *ALDH9A1*, *SLC22A5*) ($p < 0.05$, Table 4). At 1 week post-partum, positive linear correlations between relative hepatic *FGF21* mRNA abundance and hepatic TAG concentration as well as mRNA abundances of *SLC22A5*, *SREBF1*, *HMGCR* and *HMGCS2* were observed ($p < 0.05$, Table 4). At 5 weeks post-partum, there were positive linear correlations between relative hepatic *FGF21* mRNA abundance and mRNA abundances of *CD36*, *TMLHE* and *HMGCS2* ($p < 0.05$, Table 4). In 14 weeks post-partum, a positive linear correlation between hepatic *FGF21* mRNA abundance and concentration of free carnitine in milk was observed ($p < 0.05$, Table 4).

Discussion

In the present study, we used liver biopsy samples from dairy cows obtained at late pregnancy (3 weeks pre-partum) and early lactation (1, 5, 14 weeks

Table 4 Statistically significant linear positive correlations between relative hepatic mRNA abundance of *FGF21* and phenotypic parameters or mRNA abundances of hepatic genes in Holstein cows at 3 weeks ante-partum and 1, 5 and 14 weeks post-partum

Parameter	*r*	p
3 weeks ante-partum		
ACOX1	0.47	0.04
TMLHE	0.48	0.04
ALDH9A1	0.51	0.03
SLC22A5	0.33	0.05
1 week post-partum		
Hepatic TAG concentration	0.62	0.007
SLC22A5	0.45	0.03
SREBF1	0.54	0.02
HMGCR	0.92	<0.01
HMGCS2	0.55	<0.01
5 weeks post-partum		
Plasma NEFA concentration	0.45	<0.01
CD36	0.75	<0.01
TMLHE	0.54	<0.01
SLC22A5	0.31	0.04
HMGCS2	0.31	0.04
14 weeks post-partum		
Concentration of free carnitine in milk	0.47	0.01

Description of genes: *ACOX1*, acyl-CoA oxidase 1; *ALDH9A1*, aldehyde dehydrogenase 9 family, member A1; *CD36*, CD36/fatty acid translocase; *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *HMGCS2*, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2, mitochondrial; NEFA, non-esterified fatty acids; *SREBF1*, sterol regulatory element-binding protein-1; *SLC22A5*, organic cation/carnitine transporter 2; *TMLHE*, trimethyllysine hydroxylase, epsilon.

post-partum). The transition of the cows from late pregnancy to early lactation was associated with a strong negative energy balance (−65 MJ MJ NEL/day at 1 week post-partum), an increase of plasma concentrations of NEFA and BHB, and hepatic TAG concentration. Those cows, moreover, showed increased mRNA abundances of *PPARA*, and several of its target genes involved in fatty acid metabolism and carnitine synthesis, as determined by qPCR analyses, in 1 week post-partum. At 14 weeks post-partum, the cows of this study returned to a slight positive energy balance (data are shown in Schlegel et al., 2012a).

In the present study, we found that gene expression of *FGF21* was dramatically up-regulated during the transition from late pregnancy to early lactation. Noteworthy, an up-regulation of *FGF21* in the liver of early lactating dairy cows has already been observed by Carriquiry et al. (2009). In contrast to our study (Schlegel et al., 2012a) and the study of Loor et al. (2005), Carriquiry et al. (2009) – such as few other studies (Palin and Petit, 2004; Van Dorland et al., 2009; Graber et al., 2010) – did, however, not observe an up-regulation of *PPARA* during early lactation. Carriquiry et al. (2009) suggested that the lack of up-regulation of *PPARA* during early lactation might be explained by a hepatic inflammatory response, induced by an excessive pre-partum caloric intake in their cows, which decreased pre- and post-partum hepatic expression of *PPARA*.

Interestingly, the extent of up-regulation of hepatic *FGF21* mRNA concentration from late pregnancy (3 weeks pre-partum) to early lactation (1 week post-partum), being around 110-fold, was much greater than in study of Carriquiry et al. (2009) in which hepatic *FGF21* mRNA abundance increased around threefold from 12 day pre-partum to 10 days post-partum. Although results of mRNA measurements cannot be directly compared between different studies, the stronger up-regulation of *FGF21* in our study indeed could be due to the observed activation of PPARα in the cows used in this study (Schlegel et al., 2012a).

Very recently, a study of Schoenberg et al. (2011) was published which investigated plasma FGF21 concentration and expression of *FGF21* in various tissues of high-yielding dairy cows during early lactation. Those authors observed that plasma FGF21 concentrations are nearly undetectable at late pregnancy, sharply increase at 3 days pre-partum, peak at the day of parturition, and then stabilize at lower, chronical elevated concentrations during the energy deficit of early lactation. Their study also found that mRNA expression of *FGF21* is strongly increased from late pregnancy (4 weeks pre-partum) to early lactation (8 weeks post-partum) and that liver is the major source of plasma FGF21 with little or no contribution of white adipose tissue, skeletal muscle and mammary gland. In their studies, plasma FGF21 was also increased in non-lactating cattle when an energy deficit state was induced by feed restriction. Interestingly, the increase in *FGF21* mRNA abundance in that study (sevenfold from 4 weeks pre-partum to 1 week post-partum) was lower than that observed in our study. A drawback of our study is that we were, for technical reasons, not able to determine plasma concentrations of FGF21 and FGF21 protein concentrations in the liver of the cows. Nevertheless, our study confirms that study in showing that early lactation leads to a strong induction of the *FGF21* gene in the liver of dairy cows.

It has been shown that FGF21 stimulates β-oxidation of fatty acids, gluconeogenesis and ketogenesis in the liver (Badman et al., 2007). To assess the expression of hepatic genes involved in these metabolic processes, we performed a gene array analysis in the

liver samples. Although this technique generates rather semi-quantitative data, the use of a gene array has the great advantage that a great number of genes involved in several pathways can be detected simultaneously in a small sample amount, such as in liver biopsy samples. Consideration of the gene array data clearly indicates that hepatic fatty acid oxidation, gluconeogenesis and ketogenesis were up-regulated in 1 and 5 weeks post-partum, in comparison with pregnancy and later lactation. The finding that these pathways are stimulated in the liver of dairy cows with a negative energy balance agrees with several other studies, which considered the gene expression profile in the liver (Loor et al., 2005; van Dorland et al., 2009; Graber et al., 2010, 2011). It has been well established that genes involved in fatty acid oxidation, gluconeogenesis and ketogenesis are regulated by PPARα (Mandard et al., 2004). Therefore, it is likely that the up-regulation of these genes during early lactation was at least in part induced by PPARα. The finding that a knockdown of the *FGF21* gene leads to a reduction of the rates of fatty acid oxidation, gluconeogenesis and ketogenesis in mice during fasting clearly shows that FGF21 plays a crucial role in the up-regulation of these pathways independent of PPARα (Potthoff et al., 2009). However, the role of FGF21 in the metabolic adaptations in the liver of dairy cows during early lactation has not yet been investigated so far. In the present study, we observed a positive linear correlation between hepatic *FGF21* mRNA abundance and hepatic mRNA abundance of *HMGCS2*, the gene encoding the key enzyme of ketogenesis (at 1 and 5 weeks post-partum). This finding suggests that FGF21 could play a role in the regulation of ketogenesis in dairy cows during lactation, although there was no correlation between hepatic *FGF21* mRNA abundance and concentration of BHB in plasma. There were moreover positive correlations between *FGF21* mRNA abundance and mRNA abundances of genes involved in carnitine synthesis (*TMLHE*, *ALDH9A1*) and carnitine uptake (*SLC22A5*). Those correlations indicate that FGF21 could play a role in the regulation of hepatic carnitine synthesis and uptake. This indication is supported by the finding of a positive relationship between hepatic *FGF21* mRNA abundance and milk carnitine concentration at 14 weeks post-partum. However, with respect to the correlations observed, it should be noted that in rodents *FGF21* as well as genes involved in ketogenesis and carnitine metabolism (including *ALDH9A1* and *SLC22A5)* are directly regulated by PPARα (Rodríguez et al., 1994; van Vlies et al., 2007; Koch et al., 2008; Wen et al., 2010, 2012). The regulation of these genes in dairy cows has not yet been investigated. Nevertheless, based on the regulation of these genes in rodents, it cannot be excluded that the relationship between the abundances of *FGF21* mRNA and those of genes involved in ketogenesis and carnitine metabolism observed is not causative, but because of the common regulation of these genes by PPARα. Thus, further studies are warranted to investigate whether FGF21 directly regulates ketogenesis and carnitine metabolism in the liver of dairy cows. With respect to this, it is probably of interest to explore whether activation of FGF signalling, which is mediated at the cell surface by ligand binding to high affinity tyrosine kinase FGF receptors 1–4 (Johnson and Williams, 1993), leads to a PPARα-independent induction of genes regulating ketogenesis and carnitine metabolism.

Our regression analysis, moreover, showed correlations between hepatic *FGF21* mRNA abundance and plasma NEFA concentration (5 weeks post-partum) as well as hepatic TAG concentration (1 week post-partum). Relationships between hepatic *FGF21* mRNA concentration or plasma FGF21 levels and plasma NEFA concentration or hepatic TAG concentration, respectively, have already been reported (Carriquiry et al., 2009; Schoenberg et al., 2011). As the liver is not a site of lipogenesis in dairy cattle, hepatic TAG concentration reflects the uptake of NEFA into the liver (Schoenberg et al., 2011). Thus, the observed relationship between hepatic TAG and hepatic *FGF21* mRNA concentration also supports the view that expression of *FGF21* in the liver is induced by NEFA, mediated probably by activation of PPARα, such as observed in rodents (Badman et al., 2007; Lundåsen et al., 2007; Yu et al., 2012).

In rodents, it has been found that FGF21 has also important metabolic effects on tissues other than the liver. The most dramatic effects of FGF21 have been found in adipose tissue where this hormone stimulates glucose uptake by up-regulation of *SLC2A1* encoding the facilitated glucose transporter 1 and lipolysis by enhancing the activity of hormone-sensitive lipase (Inagaki et al., 2007; Coskun et al., 2008). Systemic administration of FGF21 in mice reduces plasma glucose and triglyceride, enhances insulin sensitivity, and lowers obesity and hepatosteatosis (Badman et al., 2007; Coskun et al., 2008; Xu et al., 2009). From these studies, it has been concluded that FGF21 might be a promising candidate for the treatment of insulin resistance and obesity (Kliewer and Mangelsdorf, 2010). On the basis of these recent findings, it is likely that the expression of *FGF21* during early lactation might be of great relevance for several

health-related aspects in dairy cows such as the development of ketosis or fatty liver, patho-physiological conditions which are closely related with lipolysis in adipose tissue, insulin sensitivity as well as lipid oxidation and ketogenesis in the liver. Thus, in our opinion, the expression of *FGF21* in dairy cows deserves further attention in the development of strategies to prevent metabolic diseases in dairy cows during early lactation.

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4. Diskussion

4.1 Wirkungen von pansengeschützter CLA auf Leistungsdaten und Fleischqualität beim weiblichen Jungrind

Zahlreiche Studien konnten an landwirtschaftlichen Nutztieren Effekte der Verabreichung von CLA auf wirtschaftlich bedeutende Faktoren wie Leistungsparameter, Schlachtkörperqualität und Fleischqualität nachweisen (Ostrowska et al., 1999; Thiel-Cooper et al., 2001; Gillis et al., 2004b; Suksombat et al., 2007). In *Studie 1* sollte der Einfluss der CLA-Supplementierung bei Mastrindern in der Anfangsmast, einer Phase des starken Wachstums, auf Leistungsdaten, Schlachtkörper- und Fleischqualitätsparameter untersucht werden. Mit einer CLA-Supplementierung bei Labortieren und wachsenden Schweinen konnten positive Auswirkungen auf Leistungsparameter wie Futterverwertung und tägliche Zunahmen nachgewiesen werden (Chin et al., 1994; Ostrowska et al., 1999; Eggert et al., 2001; Thiel-Cooper et al., 2001). In *Studie 1* konnte kein Einfluss der CLA-Supplementierung von wachsenden weiblichen Rindern auf deren mittlere tägliche Futteraufnahme, mittleren täglichen Zunahmen und Futterverwertung beobachtet werden, die Wirtschaftlichkeit der Mast konnte nicht verbessert werden. Diese Ergebnisse stimmen mit vorliegenden Studien an älteren Mastrindern, in denen keine Veränderung der Leistungsdaten durch die Verfütterung pansengeschützter CLA festgestellt werden konnte, überein (Gassman et al., 2000; Block et al., 2003; Gillis et al., 2004b). Bei laktierenden Schafen wurde die Futteraufnahme durch CLA-Fütterung ebenfalls nicht verändert (Lock et al., 2006; Sinclair et al., 2010). Deutliche Effekte der CLA-Supplementierung auf die Körperzusammensetzung konnten sowohl bei Labortieren als auch bei Schweinen und Geflügel beschrieben werden. Der Gesamtkörperfettanteil bei Mäusen und Ratten wurde durch Supplementierung mit dem *trans*-10,*cis*-12 CLA-Isomer reduziert (Park et al., 1997; Ryder et al., 2001; Yamasaki et al., 2003), wobei die Adipozytengröße und nicht die Anzahl der Zellen im Fettgewebe bei den supplementierten Tieren im Vergleich zu nicht-supplementierten Kontrolltieren verringert war (Azain et al., 2000). Damit einhergehend wurde der TAG-Gehalt im Fettgewebe verringert und der Magermasse- und Wasseranteil im Schlachtkörper erhöht (Park et al., 1997; Park et al., 1999). Der Magerfleisch- bzw. Muskelmasseanteil am Schweineschlachtkörper wurde in vielen Studien bei geringerem Rückenspeckanteil gesteigert (Dugan et al., 1997; Ostrowska et al., 1999; Thiel-Cooper et al., 2001; Wiegand et al., 2001; Tischendorf et al., 2002; Wiegand et al., 2002). Die Abnahme des Fettgehalts im Schlachtkörper oder in verschiedenen Teilstücken beim Hähnchen durch CLA-Fütterung wurde ebenso mehrfach beschrieben (Szymczyk et al., 2001; Du und Ahn, 2002; Suksombat et al., 2007). Die vorliegende Studie

konnte durch den Einsatz pansengeschützter CLA bei wachsenden weiblichen Jungrindern keine Unterschiede in der Körperzusammensetzung der Tiere feststellen. Beurteilt werden konnten der Magerfleischanteil und die Verfettung des Schlachtkörpers anhand der Einstufung in das EUROP-Klassifizierungssystem durch einen entsprechend geschulten Sachverständigen, die Gewichte verschiedener Fettgewebe und der intramuskuläre Fettgehalt. Diese Parameter waren bei mit pansengeschützter CLA supplementierten und nicht-supplementierten Rindern gleich. Die Supplementierung brachte somit hinsichtlich der Schlachterlöse keine Vorteile für den Fleischerzeuger. In früheren Studien wurde die Zusammensetzung des Rinder- sowie des Schafschlachtkörpers durch die CLA-Fütterung nicht beeinflusst (Block et al., 2003; Wynn et al., 2006; Sinclair et al., 2010). Die physikalischen und chemischen Fleischqualitätsparameter, die in *Studie 1* gemessen wurden, blieben durch die Supplementierung der wachsenden Färsen mit pansengeschützter CLA vollkommen unbeeinflusst. Diese Beobachtungen stehen im Gegensatz zu Studien an Schweinen und Geflügel, wo durchaus Veränderungen in der Fleischqualität bei der Supplementierung der Tiere mit CLA festzustellen waren (Wiegand et al., 2001; Parrish et al., 2003). Bezüglich der Beurteilung der Qualität des Fleisches von mit CLA supplementierten Rindern sind vorwiegend Daten zur sensorischen Qualität vorhanden. Gillis et al. (2007) berichten von verminderter Qualität von Hackfleisch, während andere Studien keine Beeinflussung von Zartheit, Saftigkeit, sensorischen Attributen oder der Fleischfarbe nachweisen konnten (Gillis et al., 2004b; Poulson et al., 2004; Flórez-Díaz et al., 2006). Hinsichtlich der Fleischfarbe, die 24 Stunden nach der Schlachtung in Proben aus dem LM gemessen wurde, wurden in Übereinstimmung mit Studien mit Schweinen keine Unterschiede durch die CLA-Supplementierung festgestellt (Wiegand et al., 2002; Dugan et al., 2003; Janz et al., 2008). Die positive Korrelation zwischen der Fleischfarbe und der Zartheit von Rindfleisch wurde belegt (Wulf et al., 1997). Dass in *Studie 1* die Fleischfarbe nicht beeinflusst wurde, lässt erwarten, dass die Zartheit des Rindfleisches durch die CLA-Supplementierung nicht verändert wurde. Eine direkte Beurteilung der Zartheit des Fleisches konnte in der vorliegenden Untersuchung jedoch nicht durchgeführt werden. Eine negative Korrelation besteht zwischen der Fleischfarbe und dem pH-Wert im Muskel 24 Stunden nach der Schlachtung (Eilers et al., 1996; Wulf et al., 1997; Page et al., 2001). Der pH-Wert des LM nach 24 Stunden in der Kontrollgruppe unterschied sich in vorliegender Studie numerisch gering, jedoch signifikant, von denen der CLA-Gruppen. Dieser Unterschied ist nicht erklärbar und die pH-Werte lagen in einem normalen Bereich. Dass die Fleischfarbe nicht beeinflusst war, unterstreicht, dass dieser Unterschied im pH-Wert keine Relevanz für die

Fleischqualität hat. In der Studie von Poulson et al. (2004) wurde vermutet, dass CLA-Fütterung die Stabilität der Rotfärbung von Ochsenfleisch negativ beeinflusst, indem die Oxidationsvorgänge im Fleisch beschleunigt werden. Indikativ für die Oxidationsanfälligkeit von Muskelgeweben ist dessen Gehalt an α-Tocopherol, dem wichtigsten Antioxidans im Organismus (Augustini et al., 1998; Schwarz et al., 1998). Es ist bekannt, dass erhöhte α-Tocopherolkonzentrationen im Rindfleisch die Farbstabilität verbessern (Liu et al., 1995). Im Gegensatz zu der Studie von Poulson et al. (2004) war in *Studie 1* bei den mit pansengeschützter CLA gefütterten Jungrindern der α-Tocopherolgehalt im LM im Vergleich zu den nicht-supplementierten Tieren erhöht. Bei Mäusen wurde bei CLA-Fütterung ein erhöhter α-Tocopherolgehalt in der Leber infolge des gesteigerten Gehalts am α-Tocopherol-Transferprotein nachgewiesen (Chao et al., 2010). Ob die Erhöhung des α-Tocopherols im Muskelgewebe der Jungrinder auf eine Stimulation des α-Tocopherol-Transferproteins zurückzuführen ist und möglicherweise eine bessere oxidative Stabilität des Fleisches nach sich zieht, muss in weiteren Untersuchungen geklärt werden. Deutliche Effekte der CLA-Supplementierung bei wachsenden Jungrindern konnten hinsichtlich der Fettsäurezusammensetzung des LM sowie des subkutanen Fettgewebes nachgewiesen werden. So kam es sowohl im Muskel- als auch im Fettgewebe zu einem signifikanten Anstieg des Gehaltes an *trans*-10,*cis*-12 CLA in Abhängigkeit von der Dosierung der CLA-Zulage, der bereits häufig bei Rindern (Gillis et al., 2004a; Poulson et al., 2004; Gillis et al., 2007), Geflügel (Szymczyk et al., 2001; Suksombat et al., 2007) und Schweinen (Kramer et al., 1998; Bee, 2000; Eggert et al., 2001; Thiel-Cooper et al., 2001; Wiegand et al., 2002; White et al., 2009) beschrieben wurde. Es ist folglich möglich, den CLA-Gehalt im Fleisch durch die Fütterung von pansengeschützter CLA auch beim Wiederkäuer zu erhöhen. Die Konzentration des *cis*-9,*trans*-11 CLA-Isomers blieb in *Studie 1* wie bei anderen allerdings unverändert (Gillis et al., 2004a; Poulson et al., 2004). Bei Fleischrindern stammen bis zu 90 % der gesamten CLA im Muskel aus der endogenen Synthese durch die Δ^9-Desaturierung von *trans*-11 Vaccensäure, die neben der Desaturierung von Stearin- zu Ölsäure durch das Enzym Δ^9-Desaturase katalysiert wird (Gillis et al., 2004a; Mosley et al., 2006). Dieses Enzym, das in vielen bovinen Geweben vorkommt wird beim Schwein durch CLA-Fütterung gehemmt (Smith et al., 2002). Bei Fleischrindern, die auf der Weide gehalten wurden, konnten deutliche Steigerungen des Gehalts an *cis*-9,*trans*-11 CLA im Muskelfleisch erzielt werden, während sich der Gehalt bei im Stall gefütterten, mit CLA supplementierten Rindern nicht steigern ließ (Poulson et al., 2004). Das ist dadurch zu erklären, dass durch die Weidefütterung große Mengen an Linol- und Linolensäure aufgenommen werden, bei deren

Abbau erhebliche Mengen an *trans*-11 Vaccensäure entstehen, die wiederum als Vorstufe für die CLA-Bildung durch die Δ^9-Desaturase dient (Harfoot, 1978; Mosley et al., 2006). Im Gegensatz dazu ist anzunehmen, dass in der Studie von Poulson et al. (2004) sowie in *Studie 1* die Δ^9-Desaturase durch die Supplementierung mit CLA gehemmt wurde, so dass bei den supplementierten Rindern die endogene Synthese der *cis*-9,*trans*-11 CLA eingeschränkt wurde. Schließlich muss die Aufnahme dieses CLA-Isomers über die Zulage den Gehalt in den Gewebelipiden so weit ausgeglichen haben, dass keine Unterschiede zwischen den Gruppen erkennbar waren. Als weiteres Indiz bezüglich der Hemmung dieses Enzyms war in der vorliegenden Studie der Δ^9-Desaturase-Index, repräsentiert durch das Verhältnis der Summe der gesättigten Fettsäuren zur Summe der einfach ungesättigten Fettsäuren, im Muskel- und Fettgewebe durch die CLA-Fütterung erhöht. Die Veränderungen im Fettsäuremuster des LM der mit CLA supplementierten Jungrinder reichen schließlich nicht aus, um die Eigenschaften hinsichtlich der Einordnung als „functional food“ zu verbessern. Die Erhöhung des CLA-Gehaltes rangiert in so geringem Umfang, dass sie ohne Auswirkungen auf die CLA-Aufnahme des Menschen über Rindfleisch bleiben würde.

Die Ergebnisse aus *Studie 1* zeigen auf, dass die Verfütterung pansengeschützter CLA an wachsende Jungrinder in der Anfangsmast keinen Einfluss auf Leistungsparameter, Schlachtkörperqualität oder Fleischqualitätsparameter hat. Die Fettsäurezusammensetzung von Muskel- und subkutanem Fettgewebe zeigt Änderungen dahingehend, dass der Gehalt an gesättigten Fettsäuren ansteigt und an einfach ungesättigten Fettsäuren abnimmt, was ernährungsphysiologisch unerwünscht ist. Aus wirtschaftlicher Sicht ist die CLA-Zulage an Mastrinder in der Anfangsmast somit nicht empfehlenswert.

4.2 Wirkungen von pansengeschützter CLA auf den hepatischen Lipidstoffwechsel bei der Milchkuh

In vielen Studien mit Labortieren wurden den CLA positive, z. B. antikanzerogene, antiatherogene, antidiabetogene, antiinflammatorische oder antiadipöse Effekte nachgewiesen (Ha et al., 1987; Lee et al., 1994; Park et al., 1997; Houseknecht et al., 1998; Yamasaki et al., 2003). Jedoch wurden besonders beim Einsatz von CLA bei Mäusen negative Nebenwirkungen wie eine massive Fettleber, einhergehend mit der enormen Vergrößerung der Leber und erhöhten hepatischen TAG- und Cholesterolgehalten, offenkundlich (Belury und Kempa-Steczko, 1997; Tsuboyama-Kasaoka et al., 2000; Clément et al., 2002; Degrace et al., 2003). Die Leberverfettung der Maus, die durch die Fütterung des *trans*-10,*cis*-12 CLA-

Isomers ausgelöst wird, ist vorwiegend das Resultat der verstärkten Aufnahme von NEFA aus dem Blut in die Leber und der gesteigerten hepatischen *de novo* Fettsäuresynthese (Clément et al., 2002). Mögliche negative Effekte der Supplementierung von Rindern mit CLA wurden bislang kaum untersucht, obwohl die CLA bereits in der praktischen Fütterung zum Einsatz kommt. Insbesondere wird CLA eingesetzt, um bei laktierenden Milchkühen eine Absenkung des Milchfettgehaltes herbeizuführen. Das Problem hochleistender Milchkühe zu Laktationsbeginn stellt das nahezu unvermeidliche Geraten in eine negative Energiebilanz dar, die es so gut wie möglich zu minimieren gilt (Drackley, 1999). Ein Ansatzpunkt dafür ist die Verringerung der Energieabgabe durch die Milch, wozu pansengeschützte CLA eingesetzt werden können. Aus diesem Grund wurde zu den Wirkungen der Supplementierung mit pansengeschützter CLA auf den hepatischen Lipidstoffwechsel eine Studie mit 40 hochleistenden, laktierenden Milchkühen (*Studie 2*) durchgeführt. Um Rückschlüsse auf den hepatischen Lipidstoffwechsel ziehen zu können, wurden der TAG- und Cholesterolgehalt im Lebergewebe der Tiere untersucht und Microarray-Analysen in den Leberbiopsieproben aus der fünften Laktationswoche durchgeführt. Zu diesem Zeitpunkt befanden sich die Tiere in der Spitze der Hochlaktation und wiesen den höchsten Lipidgehalt in der Leber auf. Eine Microarray-Analyse ermöglicht die gleichzeitige Messung der mRNA-Expressionen einer Vielzahl an Genen, in vorliegendem Falle mehr als 23000. Diese Messung ist eine Momentaufnahme des Expressionsstatus der Gene in einem Gewebe zu einem bestimmten Zeitpunkt und ermöglichte somit die Betrachtung der Auswirkungen der CLA-Supplementierung auf das Transkriptprofil der Leber in der Laktationsspitze. Die Vorteile dieser Microarray-Technologie überwiegen den Nachteil, dass eher semi-quantitative Daten geliefert werden. Aus diesem Grund wurden die Daten aus der Microarray-Analyse durch quantitative real-time PCR-Messungen der relativen mRNA-Konzentrationen mehrerer Schlüsselenzyme aus verschiedenen Stoffwechselwegen validiert. Die Milchkühe der CLA-Gruppe in *Studie 2* erhielten eine Menge von 3,8 g *trans*-10,*cis*-12 CLA je Tier und Tag. Zulagen in dieser Größenordnung sind ausreichend, um eine Milchfettdepression bei hochleistenden laktierenden Milchkühen auszulösen (de Veth et al., 2004; Liermann, 2008) und repräsentieren somit praxisnahe Einsatzmengen.

Baumgard et al. (2000) konnten erstmalig nachweisen, dass das *trans*-10,*cis*-12 CLA-Isomer die *de novo* Fettsäuresynthese in der Milchdrüse der laktierenden Kuh hemmt. Nachfolgende Studien beschrieben die Wirkungen der *trans*-10,*cis*-12 CLA auf die Expressionsraten verschiedener lipogener Enzyme in der Milchdrüse. Die Expression der Lipoproteinlipase

(LPL) und des Fettsäurebindungsproteins (*fatty acid binding protein*, FABP; Aufnahme und Transport von Fettsäuren), der Acetyl-CoA-Carboxylase (ACACA) und der Fettsäure-Synthase (*fatty acid synthase*, FASN; *de novo* Synthese von Fettsäuren), der Δ^9-Desaturase (Desaturierung von Fettsäuren) sowie der Glycerol-3-Phosphat-Acyltransferase (GPAT; TAG-Synthese) wird bei Zulage von *trans*-10,*cis*-12 CLA, nicht aber von *cis*-9,*trans*-11 CLA, im Eutergewebe bzw. in Euterepithelzellen reduziert (Baumgard et al., 2001; Baumgard et al., 2002b; Peterson et al., 2004; Kay et al., 2007). Die Regulation dieser lipogenen Schlüsselenzyme im Euter erfolgt vorwiegend durch den Transkriptionsfaktor SREBP-1 (Baumgard et al., 2002b). Die mRNA-Expression des *SREBF1* im Eutergewebe von laktierenden Milchkühen wurde bei *trans*-10,*cis*-12 CLA-Infusion vermindert (Harvatine und Bauman, 2006). Die Milchfettdepression, die durch *trans*-10,*cis*-12 CLA ausgelöst wird, resultiert somit aus der supprimierten Expression der durch SREBP-1 regulierten lipogenen Enzyme im Euter, in deren Folge die Synthese von Fettsäuren, die Aufnahme von Fettsäuren, die Desaturierung sowie die Veresterung von Fettsäuren zu TAG im Euter reduziert sind (Baumgard et al., 2002b; Bauman et al., 2008; Bauman et al., 2011). Interessanterweise führt die Verabreichung von *trans*-10,*cis*-12 CLA an Milchkühe in deren Fettgewebe zu erhöhten Expressionsraten der Gene, die für lipogene Enzyme codieren, wie FASN, LPL, SCD und FABP4, sowie des regulierenden Transkriptionsfaktors SREBP-1 (Harvatine et al., 2009). Es wird deutlich, dass die Wirkungen der CLA auf die Lipidsynthese in den verschiedenen Körpergeweben nicht identisch sind. Die Leber unterliegt als zentrales Stoffwechselorgan gerade zu Laktationsbeginn einer Vielzahl von Anpassungsreaktionen des Stoffwechsels und großen Belastungen (Drackley, 1999). Negative Auswirkungen von CLA auf den hepatischen Lipidstoffwechsel der Milchkuh, wie sie bei Mäusen beobachtet wurden, hätten in dieser Phase vermutlich besonders schwerwiegende Effekte auf die Gesundheit der Milchkuh. Deshalb sind die Auswirkungen einer CLA-Supplementierung außer im Euter- und im Fettgewebe besonders im Lebergewebe von großem Interesse. Verschiedene Autoren konnten bislang im Lebergewebe CLA-supplementierter Milchkühe keine Auswirkungen auf die Expressionsraten verschiedener Gene des Lipidstoffwechsels sowie der Glukoneogenese beobachten (Selberg et al., 2004; Selberg et al., 2005; Sigl et al., 2010).

Die Regulation der hepatischen Fettsäuresynthese erfolgt durch den Transkriptionsfaktor SREBP-1, während SREBP-2 die Cholesterolsynthese reguliert. Die Familie der SREBPs umfasst basic Helix-Loop-Helix-Leucin-Zipper (bHLH-Zip) Transkriptionsfaktoren, die als inaktive Vorstufen gebildet werden, die in einem Komplex mit dem Sterol-aktivierenden

Protein (Scap) an die Membran des endoplasmatischen Retikulums (ER) gebunden vorliegen. Durch Interaktion mit dem *Insulin induced gene 1* (Insig-1) bzw. *2* (Insig-2) wird die Aktivierung des SREBP bei hohen Cholesterolgehalten in der Zelle unterbunden. Scap dient als Cholesterolsensor, der bei geringem Cholesterolgehalt der Zelle die Auflösung der Interaktion mit Insig-1 bzw. Insig-2 sowie den Transport des Scap/SREBP-Komplexes zum Golgi-Apparat in Gang bringt (Chang et al., 2006). Zur Aktivierung der Zielgene muss dort die Amino-terminale Domäne, die die bHLH-Zip-Region enthält, durch proteolytische Spaltung freigesetzt werden, so dass sie in den Nukleus wandern und an Sterolregulationselemente (*sterol regulatory element*, SRE) in der Promoterregion der Zielgene binden kann, wodurch deren Transkription angeregt wird (Shimano et al., 1999; Horton et al., 2002; Eberlé et al., 2004; Desvergne et al., 2006). SREBPs liegen in drei Isoformen vor, SREBP-1a, SREBP-1c und SREBP-2, wobei SREBP-1a und SREBP-1c durch dasselbe Gen *SREBF1* codiert werden, das unterschiedlich transkribiert werden kann (Brown und Goldstein, 1997; Horton et al., 2002). In der Leber und den meisten Geweben werden überwiegend SREBP-1c, der im Speziellen die Fettsäuresynthese reguliert, und SREBP-2, der insbesondere für die Regulation der Cholesterolsynthese verantwortlich ist, exprimiert (Brown und Goldstein, 1997; Shimomura et al., 1997). Zu den Zielgenen des SREBP-1c gehören die Schlüsselenzyme der Fettsäure- und TAG-Synthese, wie die ATP-Citrat-Lyase (*ACLY*), *ACACA*, *FASN*, *SCD* oder *GPAT*. Zielgene des SREBP-2 umfassen die 3-Hydroxymethylglutaryl-CoA-Reduktase (*HMGCR)*, die 3-Hydroxymethylglutaryl-CoA-Synthase (*HMGCS*), die Farnesyl-Diphosphat-Synthase (*FDPS*) sowie die Squalen Synthase, die für Enzyme der Cholesterolsynthese codieren, und den LDL Rezeptor (*LDLR;* Horton et al., 2002). Es konnte bereits gezeigt werden, dass bei Mäusen, die CLA erhielten, der SREBP-1 in der Leber aktiviert wird und dadurch Enzyme der *de novo* Fettsäuresynthese und der TAG-Synthese hochreguliert werden (Clément et al., 2002; Takahashi et al., 2003). Diese durch den SREBP-1 vermittelte Steigerung der Fettsäure- und TAG-Synthese trägt möglicherweise zur Entstehung der Fettleber bei. Wie bereits erwähnt, wurden die Expressionsraten des *SREBF1* und der SREBP-1-Zielgene im Euter- bzw. Fettgewebe von Milchkühen bei Verabreichung von *trans*-10,*cis*-12 CLA beeinflusst (s. oben; Harvatine und Bauman, 2006; Harvatine et al., 2009). Hingegen wurde die hepatische mRNA-Expression des *SREBF1* und verschiedener SREBP-1-Zielgene in Studien, in denen wachsende Jungrinder, Ratten, Hamster oder Geflügel CLA-Zulagen erhielten, nicht verändert (Aletor et al., 2003; Giudetti et al., 2005; Miranda et al., 2009; Shibani et al., 2011). In Übereinstimmung damit verursachte die Verfütterung von *trans*-10,*cis*-12 CLA an laktierende

Milchkühe (*Studie 2*) weder in der hepatischen Expression verschiedener Zielgene des SREBP-1c und des SREBP-2 noch in der mRNA-Expression der Transkriptionsfaktoren selbst Unterschiede zwischen der Kontroll- und der Behandlungsgruppe. Es lag somit in *Studie 2* keine Aktivierung dieser wichtigen Regulatoren der Fettsäure- und Cholesterolsynthese in der Leber der Milchkühe durch die CLA vor, so dass davon auszugehen ist, dass die Fettsäuresynthese und die Cholesterolsynthese nicht gesteigert wurden.

Peroxisomenproliferator-aktivierte Rezeptoren sind ligandenaktivierbare Transkriptionsfaktoren und gehören zur Gruppe der nukleären Hormonrezeptoren. Der PPARα spielt eine bedeutende Rolle in der Kontrolle des Lipidstoffwechsels und der Energiehomöostase, besonders bei der Anpassung an einen Fastenzustand (Desvergne und Wahli, 1999; Kersten et al., 1999; Leone et al., 1999). Nach der Aktivierung durch die Bindung eines Liganden, bildet der PPARα ein Heterodimer mit dem Retinoid-X-Rezeptor (RXR), das schließlich im Zellkern an spezifische *peroxisome proliferator respone elements* (PPREs) in der Promoterregion oder an spezielle Sequenzen im Intronbereich der PPARα-Zielgene bindet. Über diesen Mechanismus erfolgt die Regulation der Transkription zahlreicher Gene wichtiger Stoffwechselwege des Lipidstoffwechsels, z. B. der zellulären Fettsäureaufnahme und –bindung, der mitochondrialen und peroxisomalen Fettsäureoxidation, der Ketogenese sowie der Carnitinsynthese und –aufnahme (Desvergne und Wahli, 1999; Kersten et al., 1999; Mandard et al., 2004). Konjugierte Linolsäuren, sowohl das *cis*-9,*trans*-11 als auch das *trans*-10,*cis*-12 Isomer, besitzen die Fähigkeit als Ligand an den PPARα zu binden und ihn zu aktivieren (Moya-Camarena et al., 1999a; 1999b). Besonders bei Labortieren wurde bereits mehrfach gezeigt, dass CLA durch diese Eigenschaft zur Stimulation verschiedener durch PPARα regulierter Stoffwechselwege, z. B. der mitochondrialen und peroxisomalen β-Oxidation, führen (Belury et al., 1996; Moya-Camarena et al., 1999b; Ringseis et al., 2004). Die relativen hepatischen mRNA-Konzentrationen des *PPARA* und verschiedener klassischer PPARα-Zielgene ließen in *Studie 2* keinen Einfluss der Fütterung pansengeschützter CLA an Milchkühe erkennen. Die CLA-Supplementierung führte somit nicht zur Aktivierung des PPARα in der Leber, so dass in der Folge keine Steigerung der durch PPARα regulierten Stoffwechselwege zu beobachten war. Diese Beobachtung konnte ebenso bei mit CLA supplementierten wachsenden Jungrindern gemacht werden (Shibani et al., 2011). NEFA, die im Energiemangel bei der Mobilisierung aus dem Fettgewebe freigesetzt werden, können den PPARα aktivieren

(Forman et al., 1997; Kliewer et al., 1997; Kersten et al., 1999; Mandard et al., 2004). Ein Einfluss von CLA auf den Lipidstoffwechsel wäre möglicherweise am NEFA-Gehalt im Blutplasma der Tiere erkennbar. Jedoch wurden die NEFA-Gehalte im Blutplasma in *Studie 2* durch die CLA-Supplementierung nicht beeinflusst, so dass es folglich nicht zu Unterschieden in der Aktivierung des PPARα durch Einflüsse auf den NEFA-Gehalt im Plasma kommen konnte. Weiterhin wurde der Gehalt an BHBA im Blutplasma der Milchkühe durch die CLA-Supplementierung nicht verändert. Damit übereinstimmend wurde in *Studie 2* die Expression der *HMGCS2*, des Schlüsselenzyms der Ketogenese, das durch PPARα reguliert wird, in der Leber nicht beeinflusst. Unveränderte NEFA- und BHBA-Gehalte im Blutplasma von Milchkühen, die mit CLA supplementiert wurden, konnten ebenso in anderen Studien beobachtet werden (Perfield et al., 2002; Bernal-Santos et al., 2003; Selberg et al., 2004; Pappritz et al., 2011). Die differierenden Ergebnisse bei Ratten hinsichtlich der Aktivierung des PPARα durch *trans*-10,*cis*-12 CLA könnten an der vergleichsweise niedrigen Dosierung der CLA bei den Rindern liegen – 0,02 % der TM-Aufnahme je Tier und Tag in *Studie 2* verglichen mit 0,5 bis 1,5 % bei Ratten (Moya-Camarena et al., 1999a; Ringseis et al., 2004) - oder an der speziesspezifischen Variabilität der Expression des *PPARA*, die bei proliferierenden Spezies wie Ratten sehr stark ist (Holden und Tugwood, 1999; Luci et al., 2007).

Wie bereits beschrieben, zeigte sich anhand der gemessenen mRNA-Konzentrationen in *Studie 2* kein Einfluss der CLA auf die relevanten durch SREBPs oder PPARα regulierten Stoffwechselwege, wie die Fettsäuresynthese, die Cholesterolsynthese, die Fettsäureaufnahme oder die Fettsäureoxidation. Die Genexpressionen der Enzyme des Lipoproteinmetabolismus blieben ebenso unverändert. Hierzu gehören beispielsweise VLDL Rezeptoren (VLDLR) und LDLR, die LPL oder das mikrosomale Triglycerid-Transferprotein (MTP) sowie verschiedene Apolipoproteine. Lipoproteine sehr geringer Dichte (*very low density lipoproteins*, VLDL) werden in der Leber für den Transport von TAG und Cholesterolestern (CE) zu extrahepatischen Geweben gebildet. Die TAG und CE befinden sich im Kern der VLDL und werden von einer Hülle aus Apolipoproteinen, Phospholipiden und Cholesterol eingeschlossen. Die Apolipoproteine (Apo), in VLDL v.a. ApoB100, ApoC-III, ApoE und ApoC-II, dienen u.a. als Erkennungssignal für die VLDLR. Die LPL spaltet Fettsäuren aus den VLDL ab, wodurch diese in Lipoproteine geringer Dichte (*low density lipoproteins*, LDL) umgewandelt werden, die schließlich für die Versorgung von extrahepatischen Geweben mit Cholesterol sorgen. Dazu erfolgt die Bindung der LDL über ApoB100 und ApoE an LDLR,

woraufhin sie aufgenommen und abgebaut werden (Koolman und Röhm, 1998; Katoh, 2002). Dass die in der Leber der Milchkühe in *Studie 2* in der fünften Laktationswoche gemessenen Expressionsdaten der entsprechenden Gene sich zwischen den Tieren der Kontrollgruppe und den Tieren der CLA-supplementierten Gruppe nicht unterschieden, erklärt den unveränderten TAG-Gehalt im Lebergewebe, der in *Studie 2* sowie in anderen Studien bereits beobachtet werden konnte (Bernal-Santos et al., 2003; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005; Shibani et al., 2011). Zusätzlich wurde die Cholesterolkonzentration in der Leber durch die CLA-Zulage nicht beeinflusst. Das steht in Übereinstimmung damit, dass die relativen mRNA-Konzentrationen der Gene der Cholesterolsynthese, reguliert durch SREBP-2, der Cholesterolaufnahme via LDL und der Cholesterolsekretion via VLDL keine Unterschiede zwischen den Gruppen aufwiesen. Die messbaren TAG- und Cholesterolkonzentrationen im Blutplasma der Rinder sind in großem Maße von der TAG-Synthese in der Leber und dem Lipoproteinmetabolismus abhängig. In *Studie 2* blieben sowohl die TAG- als auch die Cholesterolkonzentration im Blutplasma der mit CLA supplementierten Kühe unverändert. Dies spiegelt wider, dass der hepatische Lipidstoffwechsel, die Lipolyse im Fettgewebe, der Fettsäuretransport sowie der Lipoproteinmetabolismus wie bereits beschrieben keinen Veränderungen unterlagen.

Die Supplementierung von Milchkühen mit pansengeschützter CLA in *Studie 2* führte – zumindest bei einer Dosierung, die praxisnah und ausreichend ist, um eine Milchfettdepression auszulösen – zu keiner negativen Beeinflussung des hepatischen Lipidstoffwechsels oder der TAG- und Cholesterolkonzentrationen im Lebergewebe und im Blutplasma.

4.3 Veränderungen im hepatischen Lipidstoffwechsel von Milchkühen zu Laktationsbeginn

Anfang der Neunziger Jahre wurde der Begriff der „Transitkuh“ geprägt. Mit der Transitphase ist in den meisten Fällen der Zeitraum von 3 Wochen vor der Abkalbung bis etwa 3 Wochen nach der Abkalbung gemeint. In den letzten 20 Jahren wurde die Bedeutung des Managements der Transitkuh immer häufiger Gegenstand wissenschaftlicher Untersuchungen. Man hat erkannt, dass besonders die Gestaltung der Fütterung und die Energieversorgung in dieser kritischen Phase großen Einfluss auf die Gesundheit und die Leistung der Milchkuh in der gesamten Laktation nimmt (Donkin, 2012). Obwohl die Forschung hinsichtlich der Optimierung der Transitphase der Milchkuh in den letzten Jahren

stark zugenommen hat, werden für die detaillierte und umfassende Aufklärung der grundlegenden Mechanismen im Stoffwechsel der Kühe in der Transitphase noch zahlreiche Untersuchungen notwendig sein. Entscheidender Einfluss auf die Adaption an die Laktation kann dem Lipidstoffwechsel im Fettgewebe, in der Leber, im Darm und in der Milchdrüse zugemessen werden (Drackley, 1999). Um einen Beitrag hinsichtlich der Aufklärung des Lipidstoffwechsels in der Leber der Milchkuh in der Transitphase und in der Frühlaktation zu leisten, wurden die *Studien 3* und *4* durchgeführt.

Milchkühe reduzieren bereits einige Tage vor der Abkalbung die Futteraufnahme. In den letzten drei Wochen der Trächtigkeit beträgt die Reduktion typischerweise etwa 30 – 35 % (Grummer, 1995). Durch die eingeschränkte Futter- und damit Energieaufnahme um den Abkalbezeitpunkt kann die Kuh ihren Energiebedarf, der im Rahmen der massiven Anpassungsreaktionen und Stoffwechselbelastungen aufgrund der Abkalbung und des Laktationsbeginns stark ansteigt, nicht mehr decken. Nahezu jede hochleistende Milchkuh befindet sich in den ersten Laktationstagen und –wochen in einer mehr oder weniger ausgeprägten negativen Energiebilanz, die ihr Maximum in der ersten Laktationswoche erreicht (Grummer, 1995). Auch die Milchkühe in den vorliegenden Studien gerieten in der Frühlaktation in eine negative Energiebilanz. Die begrenzte Futteraufnahme von durchschnittlich 13,4 ± 0,48 kg Trockenmasse je Tier und Tag war in der ersten Laktationswoche nicht ausreichend, um den stark angestiegenen Bedarf für Erhaltung und Milchproduktion zu decken. Die Energiebilanz der Tiere lag drei Wochen vor der Abkalbung noch in einem positiven Bereich, während sie in der ersten Laktationswoche im Mittel einen extrem niedrigen Wert von -64,9 ± 5,15 MJ NEL/d erreichte. Sie verbesserte sich im Verlauf des ersten Laktationsdrittels, so dass in der 14. Laktationswoche eine ausgeglichene Energiebilanz vorlag. Um dem Energiemangel entgegenzuwirken, mobilisieren die Tiere Fettsäuren aus dem Körperfettgewebe. Sowohl endogene Einflüsse als auch der Umfang der Verringerung der Futteraufnahme beeinflussen die Körperfettmobilisierung und führen zu einem Anstieg der NEFA im Blutplasma (Bell, 1995; Grummer, 1995). In der Literatur werden besonders in der ersten Laktationswoche erhöhte Gehalte an NEFA im Blutplasma sowie erhöhte Gehalte an BHBA in den ersten Laktationswochen in vielen Studien beschrieben (u.a. Grum et al., 1996; Loor et al., 2005), die in den vorliegenden Studien ebenso beobachtet wurden. Da die bovine Leber Fettsäuren in großem Umfang aufnehmen und verestern kann, jedoch nur geringfügige Kapazitäten für den Export von Fettsäuren als VLDL aus der Leber hat, erhöht die Anflutung der NEFA aus dem Fettgewebe die Gefahr der

Leberverfettung und weiterer, oft sekundärer Erkrankungen, insbesondere der Ketose, in der Transitphase enorm (Bell, 1995; Grummer, 1995; Vernon, 2005). Als wichtige Regulatoren des Lipidstoffwechsels wurden verschiedene Transkriptionsfaktoren identifiziert, zu denen u.a. die PPARs, der FGF21, die SREBPs und der Liver-X-Rezeptor α (LXRα) gehören (Repa und Mangelsdorf, 1999; Horton et al., 2002; Mandard et al., 2004; Long und Kharitonenkov, 2011). Der nukleäre Hormonrezeptor PPARα spielt eine entscheidende Rolle in der Regulation des Lipidstoffwechsels und der Energiehomöostase (s. unter 4.2) und wird physiologischerweise während eines Fastenzustandes oder Energiemangels aktiviert. In Übereinstimmung mit Loor et al. (2005) stiegen die relativen mRNA-Konzentrationen mehrerer PPARα-Zielgene in der Leber der Milchkühe im Zeitraum der Körperfettmobilisierung während der negativen Energiebilanz in der Frühlaktation an. Hierzu zählen Gene der Fettsäureaufnahme, der Fettsäureoxidation und der Ketogenese. Die Hochregulierung dieser klassischen Zielgene des PPARα deutet stark auf seine tatsächliche Aktivierung hin. Man kann in den vorliegenden Studien folglich davon ausgehen, dass in der Frühlaktation die in der negativen Energiebilanz aus dem Fettgewebe verstärkt freigesetzten NEFA den PPARα als dessen Liganden in der Leber aktiviert haben, wie es bei anderen Spezies und bovinen Zellkulturen bereits nachgewiesen wurde (Forman et al., 1997; Kliewer et al., 1997; Kersten et al., 1999; Mandard et al., 2004; Loor et al., 2005; Bionaz et al., 2012).

4.3.1 Carnitinstoffwechsel der Milchkuh in Transitphase und Frühlaktation

Die oben beschriebene Aktivierung des PPARα in der negativen Energiebilanz führt zu der Hypothese, dass die Gene der Carnitinsynthese und des Carnitintransportes in dieser Phase des Laktationszyklus hochreguliert werden. Grundlage für diese Annahme ist, dass diese Gene funktionale PPREs in der Promoterregion oder den ersten Introns enthalten (Wen et al., 2010; 2011; 2012). Dadurch sind die Gene der 4-N-Trimethylaminobutyraldehyd-Dehydrogenase (*ALDH9A1*), der Trimethyllysin-Dioxygenase (*TMLHE*), der γ-Butyrobetain-Dioxygenase (*BBOX1*) und des OCTN2 (*SLC22A5*) als PPARα-Zielgene identifiziert worden. In *Studie 3* konnte nachgewiesen werden, dass die relativen mRNA-Konzentrationen dieser Gene der Carnitinsynthese und –aufnahme in der Leber hochleistender Milchkühe in der ersten Laktationswoche signifikant erhöht sind im Vergleich zum Beginn der Transitphase drei Wochen vor der Abkalbung bzw. zu späteren Zeitpunkten in der Laktation. Die gesteigerten Expressionsraten resultierten in *Studie 3* wie in früheren Studien an Schweinen und Nagetieren in einer Steigerung der Carnitinsynthese und des Carnitintransportes, die anhand erhöhter hepatischer Carnitinkonzentrationen und abnehmender Konzentrationen an

freiem Carnitin im Blutplasma in der ersten Laktationswoche im Vergleich zur Trächtigkeit nachvollzogen werden konnte (Luci et al., 2006; van Vlies et al., 2007; Koch et al., 2008; Luci et al., 2008; Ringseis et al., 2008). Frühere Studien beschrieben ebenso erhöhte Carnitinkonzentrationen in der Leber von Milchkühen in der Frühlaktation, während die Vorgänge auf Genexpressionsebene ungeklärt blieben (Grum et al., 1996; Carlson et al., 2007a). Positive Korrelationen zwischen dem Gehalt an NEFA im Blutplasma und der Carnitinkonzentration im Lebergewebe zu allen gemessenen Zeitpunkten im Laktationsverlauf unterstreichen außerdem den Zusammenhang der NEFA im Plasma mit der Aktivierung des PPARα und die Rolle des PPARα in der Regulation der Carnitinhomöostase. Ein gewisser Anteil des Carnitins wird aus dem Plasma über Carnitintransporter in die Milchdrüse aufgenommen und in die Milch transferiert. Die Carnitinkonzentration in der Milch von Kühen steigt infolge einer Energierestriktion an (Carlson et al., 2007b). Möglicherweise führt die stark negative Energiebilanz in der Frühlaktation durch die PPARα-Aktivierung neben der Steigerung der hepatischen Carnitinsynthese ebenso zur Hochregulierung der Carnitintransporter in der Milchdrüse. Diese würde das Maximum der Carnitinkonzentration in der Milch in der ersten Laktationswoche und die anschließend abnehmenden Gehalte in *Studie 3* und anderen Studien (Erfle et al., 1974; Carlson et al., 2007a) erklären. Ein solcher Mechanismus kann dazu dienen, die Versorgung des Kalbes in den ersten Lebenstagen mit Carnitin sicherzustellen. Die Aktivität des Schlüsselenzyms der Carnitinsynthese, BBD, variiert in der Leber bei Mensch, Ratte und Schwein altersabhängig und ist bei Jungtieren sehr gering (Olson und Rebouche, 1987; Galland et al., 1999; Fischer et al., 2009). Auch wenn die Aktivität der BBD bei Kälbern noch nicht untersucht worden ist, kann man annehmen, dass auch sie in den ersten Lebenstagen nur über eine begrenzte endogene Carnitinsynthese verfügen und auf die Zufuhr über die Milch angewiesen sind. Es gibt Studien, in denen bei Milchkühen durch postruminale Carnitininfusion die hepatische Fettsäureoxidation gesteigert und die Akkumulation von TAG gesenkt werden konnten (Drackley et al., 1991; Carlson et al., 2006), wodurch suggeriert wird, dass Carnitin der limitierende Faktor der hepatischen β-Oxidation sei und der Carnitinstatus Einfluss auf das Entstehen einer Leberverfettung habe. In *Studie 3* konnte hingegen kein Zusammenhang zwischen dem Ketonkörpergehalt im Plasma oder dem TAG-Gehalt in der Leber und der hepatischen Carnitinkonzentration nachgewiesen werden. In Übereinstimmung mit anderen Studien wurde bestätigt, dass die Carnitinverfügbarkeit die Entstehung von Ketose oder Leberverfettung nicht beeinflusst (Dann und Drackley, 2005; Carlson et al., 2006). In der

Konsequenz sollte hinterfragt werden, ob der Einsatz von Carnitinsupplementen in der praktischen Milchviehfütterung sinnvoll und wirtschaftlich ist.

4.3.2 Expression des FGF21 in der Transitphase

Einer der Regulatoren der Energiehomöostase im Fastenzustand ist der FGF21, der in verschiedenen Geweben wie der Leber, dem Pankreas, dem Fett- und dem Muskelgewebe exprimiert wird. Auf dem *FGF21*-Gen befindet sich in der Promoterregion ein PPRE, eine Erkennungssequenz für den PPARα, weshalb die Expression des *FGF21* durch PPARα beeinflusst wird. Die Aktivierung des PPARα, z. B. durch NEFA, führte bei Nagetieren in verschiedenen Studien zum Anstieg der *FGF21*-Expression in der Leber sowie des Plasmagehalts an FGF21 (Badman et al., 2007; Long und Kharitonenkov, 2011). FGF21 stimuliert die hepatische Lipidoxidation, die Ketogenese und die Glukoneogenese insbesondere im Zusammenhang mit einem Energiemangel (Badman et al., 2007). In *Studie 4* konnte dementsprechend sowohl ein massiver Anstieg der relativen mRNA-Konzentration von *FGF21* in der Leber der Milchkühe in der ersten Laktationswoche verglichen mit dem Beginn der Transitphase drei Wochen vor der Abkalbung als auch in Übereinstimmung mit anderen Studien die Hochregulation der Gene der Fettsäureoxidation, der Glukoneogenese und der Ketogenese in der Leber nachgewiesen werden (Loor et al., 2005; van Dorland et al., 2009; Graber et al., 2010; Graber et al., 2012). Neben der Regulation durch PPARα gibt es auch von PPARα unabhängige Einflüsse auf die Expression des *FGF21* (Carriquiry et al., 2009; Potthoff et al., 2009). Das besonders hohe Ausmaß der Hochregulation des FGF21 in der Frühlaktation in *Studie 4* entsteht möglicherweise aus dem Zusammenwirken der PPARα-Aktivierung und unabhängigen Regulationsmechanismen. Aus einer Vielzahl von Regressionsberechnungen in *Studie 4* konnten nur wenige deutliche Zusammenhänge zur Beschreibung der physiologischen Rolle des FGF21 in der Leber der Milchkuh bei der Adaption des Lipidstoffwechsels an die Laktation dargestellt werden. Positive Korrelationen der mRNA-Konzentration von *FGF21* in der Leber mit der NEFA-Konzentration im Plasma sowie der TAG-Konzentration in der Leber in *Studie 4* stimmen mit vorangegangenen Studien überein (Carriquiry et al., 2009; Schoenberg et al., 2011) und untermauern die Annahme, dass die Aktivierung des PPARα infolge des gesteigerten NEFA-Gehaltes im Plasma die Ursache für die Stimulation der Expression des *FGF21* ist (Badman et al., 2007; Lundåsen et al., 2007; Yu et al., 2012). Positive Korrelationen der hepatischen mRNA-Konzentration des *FGF21* mit der der *HMGCS2*, dem Schlüsselenzym der Ketogenese, in der ersten und fünften Laktationswoche deuten auf einen Einfluss des FGF21 auf die hepatische Ketogenese der

Milchkuh in der negativen Energiebilanz hin, allerdings wurde kein Zusammenhang des FGF21 mit dem Gehalt an BHBA im Plasma beobachtet. Weitere positive Korrelationen bestanden zu verschiedenen Zeitpunkten zwischen der mRNA-Konzentration des *FGF21* und denen der Gene der Carnitinsynthese und –aufnahme sowie der Carnitinkonzentration in der Milch. Dadurch kann man vermuten, dass der FGF21 an der Regulation der Carnitinsynthese und –aufnahme beteiligt ist. Sowohl die Ketogenese als auch die Carnitinsynthese und –aufnahme werden bei Nagetieren jedoch direkt durch den PPARα reguliert (Rodriguez et al., 1994; van Vlies et al., 2007; Koch et al., 2008; Wen et al., 2010; Wen et al., 2012). Obwohl die Regulation dieser Gene bei Milchkühen noch nicht vollständig aufgeklärt wurde, belegt *Studie 3* einen Zusammenhang zwischen der PPARα-Aktivierung und der Hochregulation der Gene der Carnitinsynthese und –aufnahme zu Laktationsbeginn. Somit muss man in Betracht ziehen, dass die beobachteten Zusammenhänge zwischen der Expression des *FGF21* und der Ketogenese bzw. dem Carnitinstoffwechsel durch die gemeinsame Regulation durch den PPARα entstanden sein könnten. Zur Aufklärung der Regulation der Ketogenese und des Carnitinstoffwechsels durch FGF21 in der Leber von Milchkühen müssen weitere Studien durchgeführt werden. Ebenso stellt die Expression des *FGF21* bei Milchkühen in der Frühlaktation im Zusammenhang mit der Entstehung von Stoffwechselkrankheiten wie Ketose und Lebersteatose ein neues zu untersuchendes Feld dar, da therapeutische Effekte von FGF21 bei Nagetieren beobachtet werden konnten (Badman et al., 2007; Coskun et al., 2008; Xu et al., 2009; Kliewer und Mangelsdorf, 2010).

Die Ergebnisse der *Studien 3* und *4* zeigen auf, dass in der Transitphase der Milchkuh bedeutende Veränderungen in der Expression zahlreicher Gene des Lipidstoffwechsels auftreten. Der Übergang von der Trächtigkeit zur Laktation ist mit entscheidenden physiologischen Anpassungsmechanismen der Lipidhomöostase verbunden, zu deren Aufklärung die vorliegenden Studien beitragen sollten und konnten.

5. Zusammenfassung

Konjugierte Linolsäuren (CLA) erweckten aufgrund ihrer an Labortieren nachgewiesenen positiven Eigenschaften großes Interesse. An landwirtschaftlichen Nutztieren wurden Effekte auf die Körperzusammensetzung, Leistungsparameter und Fleischqualitätskriterien beschrieben. Bei Mastrindern, die in der Endmastphase mit CLA supplementiert wurden, veränderte sich die Schlachtkörperzusammensetzung nicht, während Leistungs- und Fleischqualitätsparameter vereinzelt beeinflusst wurden. Da an Mastrindern bislang ausschließlich die CLA-Supplementierung in der Endmastphase mit der Schlachtung bei hohen Endlebendgewichten untersucht wurde, war die Supplementierung wachsender Jungrinder mit pansengeschützter CLA in unterschiedlichen Dosen in der Anfangsmast Gegenstand von ***Studie 1***. Die Ergebnisse der ***Studie 1*** belegen, dass die Verfütterung pansengeschützter CLA an junge Fleckviehrinder in der Anfangsmast keinen Einfluss auf Leistungsparameter wie Trockenmasseaufnahme, mittlere tägliche Zunahmen oder Futterverwertung ausübt. Sowohl die Schlachtkörperqualität als auch die physikalische und chemische Fleischqualität des *Musculus longissimus dorsi* blieben durch die Supplementierung mit pansengeschützter CLA unverändert. Die Veränderungen im Fettsäuremuster von Muskel- und Fettgeweben der supplementierten Jungrinder stimmen mit früheren Studien überein. Die Supplementierung wachsender Jungrinder mit pansengeschützter CLA bringt folglich weder wirtschaftliche Vorteile für den Tierhalter noch qualitative Vorteile für den Konsumenten. Bei frischlaktierenden Milchkühen werden pansengeschützte CLA zur Absenkung des Milchfettgehaltes eingesetzt, um den Energieoutput über die Milch zu verringern und das Ausmaß der negativen Energiebilanz, die in dieser Phase der Laktation typischerweise auftritt, zu mindern. Der milchfettsenkende Effekt des *trans*-10,*cis*-12 CLA-Isomers ist bei vielen Spezies nachgewiesen worden. Da die Verabreichung von *trans*-10,*cis*-12 CLA bei Labortieren jedoch zu schwerwiegenden Nebenwirkungen auf den hepatischen Lipidstoffwechsel führen kann, ist es von großem Interesse, ob es bei der Milchkuh zu negativen Begleiterscheinungen des CLA-Einsatzes kommt. Deshalb wurde ein Versuch mit 40 laktierenden Milchkühen über den Zeitraum von drei Wochen vor der Abkalbung (ante partum, a.p.) bis 14 Wochen nach der Abkalbung (post partum, p.p.) durchgeführt (***Studie 2***). Die Tiere der CLA-Gruppe nahmen täglich 3,8 g des *trans*-10,*cis*-12 Isomers auf. Diese Dosis war ausreichend, um eine Milchfettdepression auszulösen und die Energiebilanz der Tiere im ersten Laktationsdrittel zu verbessern. Die Ergebnisse der ***Studie 2*** belegen, dass die Triacylglycerid- und Cholesterolgehalte im Plasma und in der Leber der Milchkühe durch die Verfütterung pansengeschützter CLA nicht

verändert werden. Des Weiteren konnte kein Einfluss der CLA-Zulage auf die Expression verschiedener Gene des Fettsäurestoffwechsels (Fettsäureaufnahme, -oxidation, -synthese), des Carnitinstoffwechsels, des Cholesterolstoffwechsels (Cholesterolaufnahme, -synthese, -sekretion), des Lipoproteinstoffwechsels (Synthese, Sekretion) oder der Ketogenese in der bovinen Leber nachgewiesen werden. Die Verfütterung pansengeschützter CLA an Milchkühe in praxisnaher Dosierung hat folglich keine unerwünschten negativen Auswirkungen auf deren hepatischen Lipidstoffwechsel.

Der Übergang von der Trächtigkeit zur Laktation ist für Milchkühe mit zahlreichen Anpassungsreaktionen und Belastungen insbesondere des Leberstoffwechsels verbunden. Sehr häufig geraten frischlaktierende Milchkühe in ein Energiedefizit, da die begrenzte Futteraufnahme den hohen Energiebedarf nicht decken kann. Nicht-veresterte Fettsäuren werden aus dem Körperfettgewebe freigesetzt, die in der Leber oxidiert werden müssen. Der hepatische Lipidstoffwechsel spielt infolgedessen eine besonders herausragende und entscheidende Rolle in der Transitphase und Frühlaktation der Milchkuh. In den ***Studien 3*** und ***4*** wurden Untersuchungen zu den Veränderungen im hepatischen Lipidstoffwechsel hochleistender Milchkühe während der Transitphase und des ersten Laktationsdrittels durchgeführt. Unter anderem erfolgte die Analyse des hepatischen Transkriptprofils zu verschiedenen Zeitpunkten mithilfe der Microarray-Technologie sowie deren Validierung durch quantitative real-time PCR-Messungen ausgewählter Genexpressionen. In ***Studie 3*** sollte untersucht werden, ob die hepatischen Gene der Carnitinsynthese und der Carnitinaufnahme in der Frühlaktation im Vergleich zur Trächtigkeit hochreguliert werden. Die Regulation der Gene der Carnitinsynthese und –aufnahme erfolgt durch den Transkriptionsfaktor Peroxisomenproliferator-aktivierter Rezeptor (PPAR) α. Dieser spielt eine herausragende Rolle in der Adaption des Metabolismus an einen Fasten- bzw. Energiemangelzustand. Die wichtigste Aufgabe von Carnitin ist die Beteiligung am Transport aktivierter Fettsäuren in das Innere der Mitochondrien, wo der Ort der β-Oxidation liegt, wodurch Carnitin große Bedeutung im Lipidstoffwechsel der Milchkuh in der Frühlaktation hat. In ***Studie 3*** wurde nachgewiesen, dass die Expression verschiedener Zielgene des PPARα in der Leber der Milchkühe in der ersten Laktationswoche im Vergleich zur Trächtigkeit hochreguliert wird, so dass dessen Aktivierung anzunehmen ist. Zugleich war die hepatische Expression der Gene der Carnitinsynthese und –aufnahme in Woche 1 p.p. gegenüber Woche 3 a.p. signifikant gesteigert. Weiterhin konnten erhöhte Carnitinkonzentrationen im Lebergewebe und in der Milch in der ersten Laktationswoche nachgewiesen werden, die die

Steigerung der Carnitinsynthese und –aufnahme widerspiegelten. Gegenstand der ***Studie 4*** war die Untersuchung der hepatischen Expression des Fibroblasten-Wachstumsfaktors 21 (*fibroblast growth factor 21*, FGF21) bei Milchkühen zu Laktationsbeginn. Der FGF21 ist insbesondere im Fastenzustand ein wichtiger Regulator der Lipidhomöostase. Seine vermehrte Expression stimuliert die Ketogenese, die Fettsäureoxidation, den Citratzyklus sowie die Glukoneogenese in der Leber. Die Regulation des FGF21 erfolgt u.a. durch den PPARα. Die Ergebnisse der ***Studie 4*** belegen, dass die Expression des *FGF21* in der Leber hochleistender Milchkühe in Woche 1 p.p. gegenüber Woche 3 a.p. massiv hochreguliert wird. Sie sank im Verlauf des ersten Laktationsdrittels, blieb jedoch deutlich höher als in der Trächtigkeit. Anhand der Microarray-Analysen wurde zugleich die Hochregulation verschiedener Gene der Fettsäureoxidation, der Glukoneogenese und der Ketogenese nachgewiesen. Über die Berechnung von Korrelationen wurde des Weiteren ein möglicher Zusammenhang zwischen der *FGF21*-Expression und der Ketogenese sowie dem Carnitinmetabolismus in der Leber aufgedeckt. Insgesamt konnten in den ***Studien 3*** und ***4*** verschiedene Veränderungen im hepatischen Lipidstoffwechsel hochleistender Milchkühe während der Transitphase und des ersten Laktationsdrittels nachgewiesen werden. Somit tragen diese Untersuchungen schließlich zum besseren Verständnis des hepatischen Lipidstoffwechsels der Milchkuh während der Transitphase bei und können helfen, Strategien zur Vermeidung von Leberfunktionsstörungen bei Milchkühen zu entwickeln.

6. Summary

Conjugated linoleic acids (CLA) aroused interest because of the positive properties they exerted in experiments with laboratory animals. Effects on body composition, performance parameters and meat quality parameters were demonstrated in experiments with farm animals. Supplementation of beef cattle with CLA in the finishing period did not change body composition but influenced performance and meat quality parameters. ***Study 1*** aimed to investigate supplementation of young growing heifers with different doses of rumen-protected CLA in the early fattening period because to date there were only studies having focus on CLA supplementation of cattle in the finishing period. The results of ***study 1*** prove no influence of rumen-protected CLA fed to young Simmental heifers in the early fattening period on performance parameters such as dry matter intake, average daily gain and feed conversion. Both carcass quality and physical and chemical meat quality measured on *Musculus longissimus dorsi* were unaltered as a result of CLA supplementation. The fatty acid composition of muscle and adipose tissue changed in the same way as shown before. Consequently, feeding rumen-protected CLA to young growing cattle results neither in economical advantages for the farmer nor in qualitative advantages for the customer. Rumen-protected CLA are fed to fresh cows to lower milk fat content in order to reduce the energy output by the milk and improve the negative energy balance, which is typical for this stage in the lactation period. Milk fat depression caused by the *trans*-10,*cis*-12 CLA isomer was shown in many species. However, in laboratory animals, feeding of the *trans*-10,*cis*-12 CLA isomer can cause serious side effects on the hepatic lipid metabolism. Therefore, it is of great interest if dairy cows are also affected by negative effects as a consequence of CLA feeding. So, another experiment with 40 lactating dairy cows from 3 weeks prepartum to 14 weeks postpartum was conducted (***study 2***). The animals in the CLA group ingested 3.8 g of *trans*-10,*cis*-12 CLA per day. This dose was efficient to cause a milk fat depression and improve the animals' energy balance during the first third of lactation. The results of ***study 2*** prove that bovine plasma and liver concentrations of triglyceride and cholesterol do not change by feeding rumen-protected CLA. Furthermore, no changes in the expression of different genes of fatty acid metabolism (fatty acid intake, fatty acid oxidation, fatty acid synthesis), carnitine metabolism, cholesterol metabolism (cholesterol intake, cholesterol synthesis, cholesterol efflux), lipoprotein metabolism (synthesis, secretion) or ketogenesis in the bovine liver as a consequence of CLA feeding could be established in ***study 2***. In summary, feeding rumen-protected CLA in a practice-oriented dose to dairy cows exerts no negative side effects on hepatic lipid metabolism.

The transition of the dairy cow from gestation to lactation involves many metabolic adaptations and stress especially regarding the liver metabolism. Very frequently, fresh cows suffer an energy deficiency because feed intake is restricted and cannot meet the high energy requirements. Non-esterified fatty acids are released from adipose tissue and must be oxidized in the liver. Consequently, the hepatic lipid metabolism plays an outstanding and crucial role in the transition period and early lactation of dairy cows. ***Studies 3*** and ***4*** aimed to investigate changes in lipid metabolism of high-yielding dairy cows in the transition period and the first third of lactation. Among other things, the hepatic transcript profile of different dates in lactation was analyzed using microarray technology. Microarray data were validated based on quantitative real-time PCR analyses of selected genes. ***Study 3*** aimed to investigate if hepatic genes of carnitine synthesis and carnitine uptake are up-regulated in early lactation compared to gestation. Regulation of genes of carnitine synthesis and carnitine uptake is performed by the transcription factor peroxisome proliferator-activated receptor α (PPARα). PPARα plays a crucial role in the adaptation of the metabolism to fasting and energy deficiency, respectively. The most important function of carnitine is its contribution to the transport of activated fatty acids into the mitochondrial matrix, where β-oxidation takes place. Therefore, carnitine is essential for lipid metabolism of dairy cows in early lactation. In ***study 3***, up-regulation of the expression of several PPARα target genes in the liver of the dairy cows in early lactation compared to gestation was proven. Consequently, activation of PPARα can be assumed. Likewise, the hepatic expression of genes of carnitine synthesis and uptake in week 1 postpartum was significantly above that in week 3 prepartum. In addition, elevated carnitine concentrations in liver tissue and milk in the first weeks of lactation were detected reflecting increased carnitine synthesis and uptake. Object of ***study 4*** was the investigation of the bovine hepatic expression of fibroblast growth factor 21 (FGF21) at the onset of lactation. FGF21 regulates lipid homeostasis in fasting periods. Increased expression of FGF21 stimulates ketogenesis, fatty acid oxidation, the citric acid cycle and gluconeogenesis in the liver. FGF21 is regulated by PPARα. Results of ***study 4*** prove a strong up-regulation of the hepatic expression of *FGF21* in high-yielding dairy cows in week 1 postpartum compared to week 3 prepartum. Expression decreased during the first third of lactation but remained higher than in gestation. Furthermore, based on the microarray analyses the up-regulation of different genes involved in fatty acid oxidation, gluconeogenesis and ketogenesis was detected. Regression analysis revealed a possible relation of hepatic expression of *FGF21* and ketogenesis as well as carnitine metabolism. Altogether, ***studies 3*** and ***4*** detected various changes in hepatic lipid

metabolism of high-yielding dairy cows in the transition period and the first third of lactation. Finally, these investigations contribute to a better understanding of hepatic lipid metabolism during the transition period and help to develop strategies to avoid liver dysfunction in dairy cows.

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Erklärung

„Ich erkläre: Ich habe die vorgelegte Dissertation **„Untersuchungen zu Wirkungen von konjugierten Linolsäuren (CLA) auf Fleischqualitätsparameter und den hepatischen Lipidstoffwechsel beim Rind und zu Veränderungen im hepatischen Lipidstoffwechsel der Milchkuh beim Übergang von der Trächtigkeit in die Laktation“** selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Döhlau, den 08.04.2013 ……

Gloria Schlegel

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.

The curriculum vitae was removed from the electronic version of the paper.

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