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Effects of Essential Fatty Acids and Conjugated Linoleic Acid on Performance and Energy Metabolism in Dairy Cows from Late Gestation to Early Lactation

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“Don’t limit your challenges, challenge your limits.

Each day we must strive for constant and never ending improvement.”

Anthony Robbins

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ABBREVIATIONS

acetyl-CoA	acetyl-coenzyme A
ALA	α-linolenic acid
ANOVA	analysis of variance
AP	antepartum
ARA	arachidonic acid
BCS	body condition score
BFT	back fat thickness
BHB	β-hydroxybutyric acid
BW	body weight
cDNA	complementary deoxyribonucleic acid
CLA	conjugated linoleic acid
CTRL	control
CV	coefficient of variation
d	day
DM	dry matter
DMI	dry matter intake
DPA	docosapentaenoic acid
DHA	docosahexaenoic acid
EB	energy balance
ECM	energy corrected milk
EFA	essential fatty acids
ELISA	enzyme-linked immunosorbent assay
FA	fatty acid
FBPase	frucose-1,6-bisphosphatase (protein)
FE	feed efficiency
eGP	endogenous glucose production
GH	growth hormone
G6Pase	glucose-6-phosphatase (protein)
G6PC	glucose-6-phosphatase (gene)
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HPLC	high-performance liquid chromatography
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein

LA	linoleic acid
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LRP10	low-density lipoprotein receptor-related protein 10 (gene)
LSM	least squares means
MUFA	monounsaturated fatty acids
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NDF	neutral detergent fiber
NEFA	non-esterified fatty acid
NE_L	net energy for lactation
PPAR	peroxisome proliferator-activated receptor
PC	pyruvate carboxylase (protein)
PC	pyruvate carboxylase (gene)
PCC	mitochondrial propionyl-CoA carboxylase
PCCA	mitochondrial propionyl-CoA carboxylase alpha chain
PCK1	cytosolic phosphoenolpyruvate carboxykinase (gene)
PCK2	mitochondrial phosphoenolpyruvate carboxykinase (gene)
PCR	polymerase chain reaction
PEPCKc	cytosolic phosphoenolpyruvate carboxykinase (protein)
PEPCKm	mitochondrial phosphoenolpyruvate carboxykinase (protein)
PP	postpartum
POLR2A	RNA polymerase II (gene)
PUFA	polyunsaturated fatty acids
RIA	radioimmunoassay
RNB	ruminal nitrogen balance
SCC	somatic cell count
SCD	stearoyl-CoA desaturase
SE	standard error
TC	total cholesterol
TMR	total mixed ration
VLDL	very-low-density lipoprotein
wk	week

1. INTRODUCTION

In the last three decades, world milk production has increased by more than 59%, from 530 million tonnes in 1988 to 843 million tonnes in 2018 (FAO 2020). During this time in Germany, the total milk production, milk production per cow, and the average size of dairy farms increased, even though the number of farms decreased (BLE 2021). This improvement in dairy farming was only achieved by advancements in management and genetics. However, the increased milk production per cow led to higher energy requirements and caused a shift in the nutritional management in dairy cattle farming from pasture-based feeding to loose-housing systems. Therefore, in Germany, 83% of the cows are housed in free-stall barns and get total mixed rations (**TMR**) with mainly preserved components of high caloric content, like concentrates or corn silage, to meet their energy needs for milk production (Destatis 2021; Barkema et al. 2015). Seasonal variations in pasture availability, nutritive value, and the need to meet the nutritional requirements of high-producing cows are the main limitations of grazing systems (Khan et al., 2015). Cows on pasture take up high amounts of essential fatty acids (**EFA**), especially α-linolenic acid (**ALA**; Chilliard et al., 2001; Glasser et al. 2013; Khiaosa-ard et al., 2015), whereas corn silage is rich in linoleic acid (**LA**) but contains low levels of fat and ALA (Chilliard et al., 2001; Khan et al., 2015). The unsaturated fatty acids (**FA**), ALA and LA, are classified as EFA because of the inability of mammals, including ruminants, to synthesize them endogenously *de novo*, and should be supplied with the diet (Bézard et al. 1994; Palmquist 2010). Ingested FA are converted by rumen microbes through isomerization and biohydrogenation from unsaturated to saturated FA (Harvatine and Allen 2006b, Jenkins et al. 2008). Conjugated linoleic acid (**CLA**) is a bioactive compound formed either in the rumen, by biohydrogenation from EFA, or is synthesized in mammary gland tissue. Rumen CLA production depends on EFA intake and increases with pasture feeding (Kelly et al. 1998; Chilliard et al. 2001; Ferlay et al. 2006; Shingfield et al. 2010; Lahlou et al. 2014). Therefore, the forage type strongly affects the intake of EFA and the n-6/n-3 FA ratio in the diet as well as the CLA status of dairy cows (Chilliard et al. 2001; Shingfield et al. 2010; Khan et al. 2015). In the periparturient period of dairy cows, it was shown that EFA and CLA can change production performance and milk composition – affecting lipid and glucose metabolism by endocrine and metabolic changes. Different FA, e.g., ALA and CLA, demonstrate properties in affecting energy metabolism and reducing the incidence of negative energy balance (**EB**), and lowering non-esterified fatty acids (**NEFA**) and liver triglyceride (**TG**) accumulation (Petit et al. 2007; Schäfers et al. 2017). The milk fat depression in consequence of an increased CLA status can spare glucose with homeorhetic adaptation to partitioning the glucose to a greater synthesis of other milk components or reducing endogenous glucose production (**eGP**, Hötger et al.

2013). Modulating effects of EFA and CLA on insulin sensitivity and the somatotropic axis are particularly desirable in early lactation to decrease partitioning of energy stores in milk production and reduce the extent of body mass loss (Pires and Grummer 2008; Grossen-Rösti et al. 2018). Because of the key role of nutritive alterations during the transition period, a possible impact of a combined EFA and CLA supplementation, as this is the case when providing pasture or fresh grass, on metabolic and endocrine changes has to be studied in more detail to assess their benefits and disadvantages on performance and energy metabolism around calving.

Presently, there are no studies on the overlapping effects of EFA and CLA supplementation in dairy cows and if the distinct metabolic modulating effects of these FA on performance and metabolism might be independent or synergistic. Therefore, this study aims to conclude whether a FA supply high in ALA and CLA, as with pasture feeding, could be more effective in reducing the metabolic load and whether it could ensure a high production response in early lactation. The aim of this thesis is to characterize the impact of abomasal supplementation of EFA, e.g., ALA, together with CLA, on performance and energy metabolism in dairy cows from late gestation to early lactation. Another goal is to determine whether the effects of ALA and CLA supplementation could possibly lead to a stabilization of a cow's metabolism by compensating for an insufficient energy intake in early lactation, which could be utilized as a strategy to promote animal health and welfare.

In the present thesis, chapter 2 underlines the importance of EFA and CLA in the feeding regime of dairy cows and outlines the changes in dairy cows' metabolism. Furthermore, an overview is given of energy partitioning in early lactation as well as the current state of knowledge concerning the effect of EFA and CLA supplementation on production performance as well as metabolic- and endocrine-related changes during the transition period from late pregnancy up to early lactation in dairy cows. Chapter 3 deals with the effects of abomasal EFA and CLA supplementation on performance, milk and body composition, and plasma metabolites related to lipid metabolism in dairy cows during the time of calving. Chapter 4 focuses on glucose metabolism and the somatotropic axis in dairy cows after abomasal EFA and CLA infusion during the time of calving. In the final general discussion (chapter 5), the main findings of chapters 3 and 4 are critically reviewed and put into the context of the present literature.

2. LITERATURE OVERVIEW

2.1 The Role of Fatty Acids in the Nutrition Management of Dairy Cows

2.1.1 General Aspects of Transition Cow Feeding Management

In dairy cows, the time around calving is the most critical physiological stage of the lactation cycle because cows transit from late gestation and dry period to early lactation (Drackley 1999). Feeding management of a peripartum cow is of great significance for a successful transition, which would reduce the incidence of metabolic dysfunction and impaired performance (Overton and Waldron 2004; Ingvartsen 2006). The primary goal of the nutritional management of dairy cows during this period is to support metabolic adaptations in glucose, FA, and mineral metabolism for preparing the onset of lactation (Overton and Waldron 2004).

For dry cows, 2-group nutritional strategies are preferably used to minimize overfeeding of nutrients during the early dry period but increase nutrient supply to facilitate metabolic adaptation to lactation during the late dry period (Ingvartsen 2006). Rations during the last weeks (**wk**) antepartum (**AP**) with increased nutrient density (generally done by increasing the non-fibrous content) allow maintenance because pregnancy decreases intake capacity as energy requirements for fetal growth and mammary development increase (Ingvartsen 2006). It was estimated that for a Holstein cow producing 30 kg milk at day (**d**) 4 postpartum (**PP**), the mammary requirements for glucose, FA and amino acids are, respectively, 2.7, 4.5, and 2.0 times those of the gravid uterus during late pregnancy, and the estimated mammary requirement for energy is 3.0 times that of the uterus (Bell 1995). In early lactation, despite the role of the endocrine system in metabolic regulation and nutrient partitioning by mobilization of body stores of fat and protein to meet these demands, various nutritional strategies have been devised to maintain dry matter intake (**DMI**), prevent excessive mobilization of body reserves, and minimizing physiological imbalance (Roche et al. 2013). Increasing the amount of energy supplied through dietary carbohydrate (i.e., starch versus highly digestible non-detergent fiber), glucogenic precursors, dietary fat sources, or decreasing energy expenditure by supplying specific FA results in generally positive effects on metabolism and performance of transition cows (Overton and Waldron 2004; Ingvartsen 2006; Roche et al. 2013). The increasing energy requirements of high-yielding dairy cows have led to a steady rise in the use of fat supplements as an energy source in ruminant nutrition, but fat feeding is limited by the drawbacks of reduced feed intake, digestibility effects, and a negative impact on rumen fermentation (Palmquist and Jenkins 2017; Moallem 2018). The amount of fat in the diet ranges from less than 20 g/kg DM

to more than 80 g/kg DM if fat is supplemented (Jenkins 2020). In the diet or fat supplements it is necessary to consider the FA profile because, besides energy provision, specific FA can elicit markedly different production and metabolic responses in transition dairy cows (Overton and Waldron 2004; Roche et al. 2013; Palmquist and Jenkins 2017; Bionaz et al. 2015).

2.1.2 Classification and Characteristics of Fatty Acids in Dairy Cow Nutrition

In recent years, considerable research effort was directed toward the evaluation of production and metabolic responses, as well as health-promoting properties of dietary supplementation with individual FA. In general, ruminant rations include a mixture of FA from a variety of feedstuffs. The FA fed to dairy cows originate from the fat present in the feed ingredients or added as supplements. Among diet ingredients and fat additives, the FA composition differs from its major supplemented FA palmitic acid (C16), stearic acid (C18), oleic acid (C18:1), LA (C18:2), and ALA (C18:3; Palmquist and Jenkins 1980).

The FA are made up of carbon, hydrogen, and oxygen, with a methyl group at one end (ω/n end), as well as a carboxylic acid at the other end (α end) of the carbon chain. Fatty acids are classified in different ways, for example, according to chain length or the presence and number of double bonds in their carbon chain. Thus, FA can be subdivided into short-chain fatty acids (SCFA; C1-5), medium-chain fatty acids (MCFA; C6-12), long-chain fatty acids (LCFA; C13-21), and very long-chain fatty acids (VLCFA; C22 or more). The number of hydrogen atoms surrounding the carbon atoms determines whether the fatty acid is saturated or unsaturated. Saturated fatty acids (SFA) contain no double bonds, whereas the mono- (MUFA; one double bond) and polyunsaturated fatty acids (PUFA; more than one double bond) are more reactive. The position of the double bonds in a FA chain is always specified by giving the label of the carbon in relation to the carboxyl end (considered as carbon number 1). The double bond configuration is in one of two structural orientations: *cis* or *trans* (geometric isomerization; **Figure 2.1**). *Cis* indicates that the functional group (hydrogens) are on the same side while *trans* denotes that hydrogens are on opposing sides of the carbon chain.

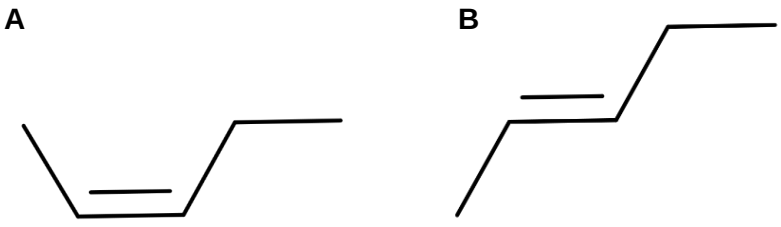

Figure 2.1 Structural orientation of double bond configuration in unsaturated fatty acids: *cis* (A) and *trans* (B)

2.1.3 Essential Fatty Acids

Long-chain PUFA can be divided into 4 main families, depending upon the site of the first double bond from the methyl side: n-3, n-6, n-7, and n-9 FA. The last two are the palmitoleic (n-7) and oleic (n-9) families that can be synthesized *de novo* in most cells, different from the linoleic (n-6) and linolenic (n-3) families. The LA and ALA are classified as EFA due to the inability of mammals, including ruminants, to synthesize them endogenously *de novo* (lack of Δ12 and Δ15 desaturase). As such, said acids have to be supplied with the diet (Bézard et al. 1994; Palmquist 2010). All the members of these two independent families n-6 and n-3 derive from their respective precursors, LA (C18:2 n-6) and ALA (C18:3 n-3), by alternate desaturation–elongation reactions (Bézard et al. 1994; **Figure 2.2**). Main metabolites of the n-6 family are arachidonic acid (**ARA**; C20:4 n-6) and docosapentaenoic acid (C22:5 n-6), and of the n-3 family the metabolites are eicosapentaenoic acid (**EPA**; 20:5 n-3), docosapentaenoic acid (**DPA**; C22:5 n-3) and docosahexaenoic acid (**DHA**; C22:6 n-3; James et al. 2000). The rate of endogenous biosynthesis of n-3 and n-6 PUFA is limited due to the competitive inhibition of LA and ALA for the initial Δ6 and Δ5 desaturation enzymes (Simopoulos 2016).

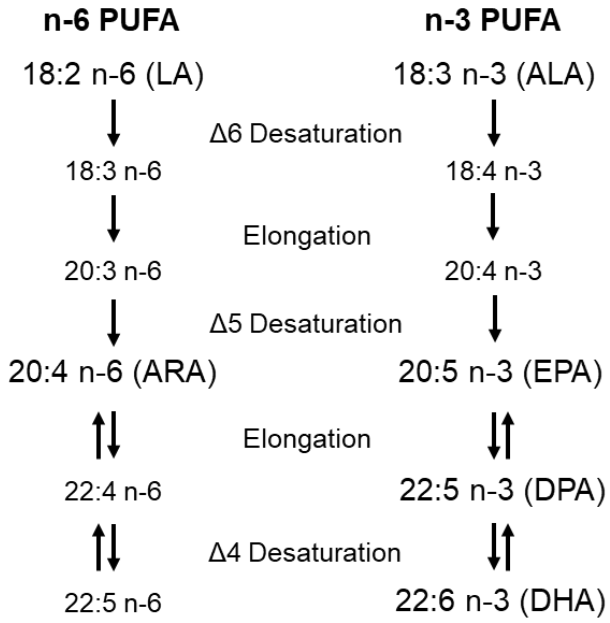

Figure 2.2 Pathways of n-6 and n-3 PUFA metabolism[1,2]

[1]Adapted from Bézard et al. (1994) and Simopoulos (2016)
[2]Abbreviations: ALA – α-linolenic acid; ARA – arachidonic acid; DHA – docosahexaenoic acid; DPA – docosapentaenoic acid; EPA – eicosapentaenoic acid; LA – linoleic acid; PUFA – poly unsaturated fatty acid

The EFA LA can be found in the seeds of most plants, such as corn, soybean, safflower, and sunflower (except for coconut, cocoa, and palm), whereas ALA appears mainly in chloroplasts of green vegetables, like grass or linseed (Glasser et al. 2008; Glasser et al. 2013; Simopoulos 2016). Grass, corn, and their preserved forms are the main EFA sources for cattle (Ferlay et al. 2017). Diets containing high proportions of corn silage provide a higher LA and energy intake, but low amounts of ALA as compared to pasture feeding or grass silage (Kliem et al. 2008; Khan et al. 2015; Khiaosa-Ard et al. 2015). Additional supplementation of oilseeds and their derived products (e.g., oil or meal) increase the intake of EFA. Linseed has a high oil level with 55% of ALA and fish oil is rich in FA from the n-3 family (Glasser et al. 2008, Petit 2010; Moallem 2018). Thus, the quality and quantity of EFA intake both depend on ration components, diet type (e.g., forage proportion, grazing vs. conserved forage), and FA supplementation (Khiaosa-Ard et al. 2015).

The n-3 and n-6 FA are involved in many biological processes, like cell proliferation and differentiation (Jump and Clarke 1999; Moallem 2018). They can affect cell membrane function (major components of phospholipids), enzyme activities, and can also be involved in the regulation of gene expression (Bézard et al. 1994; Jump and Clarke 1999; Palmquist 2010; Moallem 2018). Additionally, these FA have immunomodulatory and hemostatic effects, mostly mediated as precursors for eicosanoid synthesis, e.g., prostaglandins, leukotrienes, and thromboxane (Bézard et al. 1994; Palmquist 2010; Simopoulos 2016; Moallem 2018). Generally, FA of the n-3 series are known to be less inflammatory than n-6 FA (James et al. 2000; Moallem 2018). A variable combination of feed ingredients and additives modify and determine the final n-6/n-3 ratio in the diet and consequently in the metabolism (Moallem 2018). This can influence several metabolic processes and modify immune reactions, whereas a low n-6/n-3 ratio is preferred (Moallem 2018; Simopoulos 2016).

So far, the minimum intake of EFA in high-yielding dairy cows needed to maintain body functions and their requirements for milk performance is not well established (Palmquist 2010). In cows, ruminal anaerobic microbes split lipids of the diet by lipases and generated free FA are further processed by microbial hydrogenases (Ferlay et al. 2017). Ruminal microorganisms modify the dietary FA profile through isomerization (*trans*-FA intermediates) and biohydrogenation (reduction of double bonds) from unsaturated to SFA because rumen microbial growth is impaired by unsaturated FA (Harvatine and Allen 2006b, Maia et al. 2007; Jenkins et al. 2008). However, in cows, ruminal biohydrogenation eliminates large proportions of dietary LA and ALA with stearic acid as the final product of complete hydrogenation and reduces the amounts of EFA available for intestinal absorption (Shingfield et al. 2010). **Figure 2.3** shows major pathways of ruminal LA and ALA biohydrogenation, which varied from 70 to 95%,

and from 85 to 100%, respectively (Ferlay et al. 2017). These alterations in ruminal FA metabolism by ruminal bacteria are the main limitation for a conclusive investigation of an adequate EFA intake.

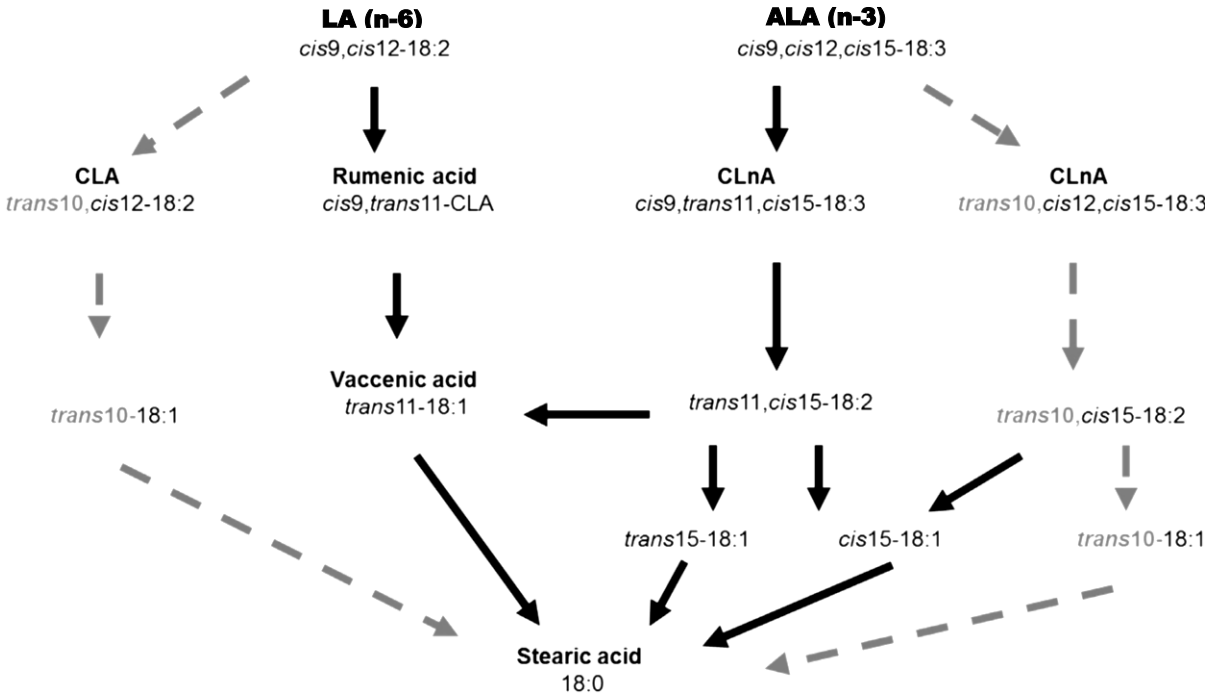

Figure 2.3 Major pathways of ruminal LA and ALA metabolism[1,2]

[1]Adapted from Ferlay et al. (2017)
[2]Meaning of the characters: → normal conditions, ⇢ reduced pH and/or diet rich in starch and supplemented with unsaturated fatty acids

2.1.4 Conjugated Linoleic Acid

The nomenclature CLA represents a variety of isomers of LA and is characterized by two conjugated double bonds (i.e., separated by one single bond, –C=C–C=C–). These double bonds can either be *cis* or *trans* (see **Figure 2.1**). The two most common CLA isomers are the *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA (**Figure 2.4**).

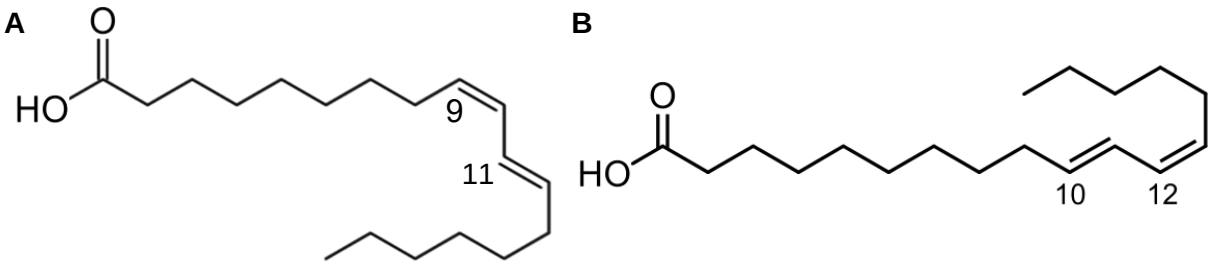

Figure 2.4 Chemical structure of the isomers *cis*-9,*trans*-11 CLA (A) and *trans*-10,*cis*-12 CLA (B)

The CLA are synthesized by rumen microbes as a result of incomplete ruminal biohydrogenation of dietary unsaturated FA to more saturated end products (Bauman et al. 2000). However, an incomplete hydrogenation results in the formation of intermediate products such as *cis* and *trans* isomers of C18 PUFA, like vaccenic acid, and variable CLA isomers (e.g., *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA). The second source of CLA synthesis is the production of CLA in the mammary gland or adipose tissue from vaccenic acid by Δ9 desaturase (Bauman et al. 2000).

The level of ruminal biohydrogenation and synthesis of CLA isomers varies and depends on diet composition (form and composition of supplemented FA, especially LA and ALA intake) and rumen environment (rumen pH) that alter rumen bacteria population (Bauman et al. 2000; Shingfield et al. 2010; Ferlay et al. 2017; Shokryzadan et al. 2017; Moallem 2018). Pasture feeding increases *cis*-9,*trans*-11 CLA production as opposed to feeding TMR with preserved forages (Ferlay et al. 2017). Forage maturity of the diet seems to be an important factor along with the increased formation of *cis*-9,*trans*-11 CLA in the early growth stage (Bauman et al. 2000; Ferlay et al. 2017). Regardless of the diet, the *cis*-9,*trans*-11 CLA isomer is the major isomer found in ruminant tissue and represents 80 to 90% of the total CLA in milk fat, but under certain dietary conditions the proportion and accumulation of *trans*-10,*cis*-12 CLA isomer increases (Bauman et al. 2000; Ferlay et al. 2017; Shokryzadan et al. 2017). A change of the dietary forage/concentrate ratio to low-fiber/high concentrate ratio (which result in decreased pH) or to diets rich in starch increases the formation of *trans*-10,*cis*-12 CLA and *trans*-10 18:1, instead of *cis*-9,*trans*-11 CLA and vaccenic acid, respectively (see **Figure 2.3**; Bauman et al. 2000; Ferlay et al. 2017).

The biological active CLA has been shown to have health-promoting effects both in humans and various other species. Isomer-specific studies revealed that the *trans*-10,*cis*-12 isomer was implicated in catabolic processes (lipolysis and fat oxidation), and the *cis*-9,*trans*-11 isomer was implicated in anabolic and anti-inflammatory effects (Ferlay et al. 2017). The *trans*-10,*cis*-12 CLA is the isomer that induces body composition changes (reduced body fat gain, enhanced lean body mass gain) and prevents cardiovascular diseases in several animal models as well as in humans (Pariza et al. 2000; Churruca et al. 2009; Kim et al. 2016; Shokryzadan et al. 2017). Both the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers have anticarcinogenic activity, antidiabetic properties (by improving insulin sensitivity), and have an immunological function in animal studies and humans (mostly cell lines), with predominated effects in *cis*-9,*trans*-11 CLA (Pariza et al. 2000; Churruca et al. 2009; Kim et al. 2016; Shokryzadan et al. 2017). The above are the reasons why distinct CLA isomers are desirable in human food products, and milk and meat from cattle comprise the most important natural source of CLA for human nutrition (Bauman et al. 2000; Ferlay et al. 2017; Simopoulos 2016; Shokryzadan et al. 2017)

2.2 Insight into Effects of Essential Fatty Acids and Conjugated Linoleic Acid on Production Performance and Metabolic and Endocrine Changes in the Transition Dairy Cow

Around calving, feed intake is not commensurate with the higher demand of energy and nutrients needed for the growth of the conceptus and lactation, which forces the transition cow to shift nutrients toward the mammary gland and use its own body reserves as an energy source (Bauman and Currie 1980; Wankhade et al. 2017). To accomplish the high energy needs for PP milk production, cows undergo challenging metabolic and endocrine changes (Drackley et al., 2001; Wankhade et al. 2017). The coordination of nutrient trafficking includes a plethora of hormones, with insulin and growth hormone (**GH**) as key regulators (Baumgard et al. 2017). The lactation performance of dairy cows depends to a large extent on their ability to cope with the metabolic demands of the periparturient period. In this respect, the research aims to prevent metabolic diseases and excessive body condition loss in early lactation by mitigating the natural homeorhetic partitioning of nutrients from body reserves to milk (Sundrum 2015; Habel and Sundrum 2020).

2.2.1 Energy and Nutrient Requirements for Milk Synthesis

According to the German Society of Nutrition Physiology (2001), the energy required for milk synthesis by a cow that has a performance of 11,000 kg milk/305 d amounts to more than 120 MJ NE_L/day. For milk production, the major macronutrient components are lactose (~5%), protein (2.5-4%), and fat (3-5%), all of which have to be synthesized (Osorio et al. 2016).

Milk lactose is the primary and milk-specific carbohydrate. It is a disaccharide composed of galactose and glucose subunits. As reviewed by Osorio et al. (2016), in the mammary gland, glucose transporters bring glucose out of the blood and into the cell. In the cytoplasm, glucose is converted to galactose, a process that requires energy input. Galactose is then actively transported by a specific transporter into the Golgi apparatus, where the complex of lactalbumin and β4-galactosyl transferase catalyzes the formation of the disaccharide lactose, which determines the volume of milk produced by maintaining the osmolarity of milk (Linzell 1972). In dairy cows, mammary tissue extracts ~20% of glucose from the blood for milk synthesis (Osorio et al. 2016).

The main proteins in milk are caseins and whey proteins. In the mammary gland, the synthesis of proteins requires the constituents of the protein synthesis machinery (including the large and small subunits of the ribosome: mRNA and tRNA), as well as the availability of amino acids combined with a large supply of energy. The lack of availability and faulty transport of amino

acids to the mammary gland are the two major limitations for milk protein synthesis (Osorio et al. 2016). Ideally, amino acids derived from the bloodstream can be used by the mammary gland for milk protein synthesis, but during nutrient deficiencies intramammary metabolism must be flexible enough in order to derive substrates for milk composition from supplied aminogenic, lipogenic, or glucogenic precursors. Protein balance calculations indicate that high-producing cows were in a slight deficit for the final 3 d of the prepartum period and that the most drastic imbalance occurred after calving (Grummer 1995). To support lactation contributions of amino acids and energy by body protein mobilization is a necessary homeorhetic adaptation (Bell et al. 2000). The mobilization of labile protein reserves is primarily regulated by hormonal changes and is less responsive to the moderate changes in metabolizable protein supply immediately PP (Lean et al. 2013).

Milk fat consists of more than 95% of triacylglycerol (Jensen 2002). A triacylglycerol molecule (also called triglyceride) consists of a glycerol backbone esterified with three FA. The glycerol-3-phosphate backbone for milk fat synthesis is synthesized in the mammary gland from glucose (glycolysis) or by phosphorylation of free glycerol taken up from blood during lipolysis. The linked FA can originate from 4 main sources: (1) from *de novo* synthesis in the mammary gland, (2) from feed, (3) from ruminal microbial metabolism (biohydrogenation and microbial lipids), and (4) from the mobilization of body reserves (Jensen 2002; Chilliard et al. 2007; Khiaosa-Ard et al. 2015). Short- and medium-chain FA (C4 to C14) and approximately half of C16 arise from *de novo* synthesis from acetate and β-hydroxybutyrate (**BHB**), whereas the remaining portion of C16 and all longer-chain FA (≥ C18) occur in the milk lipids as preformed FA and enter from circulation (Chilliard et al. 2007; **Figure 2.5**). The *de novo* FA synthesis in the bovine mammary gland needs reducing equivalents (nicotinamide adenine dinucleotide phosphate; NADPH) which are provided mostly via the citrate-isocitrate or pentose phosphate pathway (Linzell et al. 1976; Faulkner and Peaker 1982; Urrutia and Harvatine 2017). Thus, citrate and the pentose phosphate pathway, which branches from blood glucose, are indirectly associated with FA synthesis in the mammary gland of ruminants. Moreover, the elevated citrate concentration in the milk points at reduced *de novo* FA synthesis (Garnsworthy et al. 2006). Carbon atoms from glucose can also transfer via glycerol or via pyruvate and the acetyl-coenzyme A (**acetyl-CoA**) pathway into milk fat (Mellenberger et al. 1973).

In terms of energy expenditure, fat is the most expensive component of milk, so daily milk fat secretion in early lactating cows represents up to 35% of net energy intake (Bauman and Currie 1980). During early lactation, the high energy demands of milk fat secretion result in mobilization of reserves, mainly the body fat (Bauman and Currie 1980; Drackley 1999).

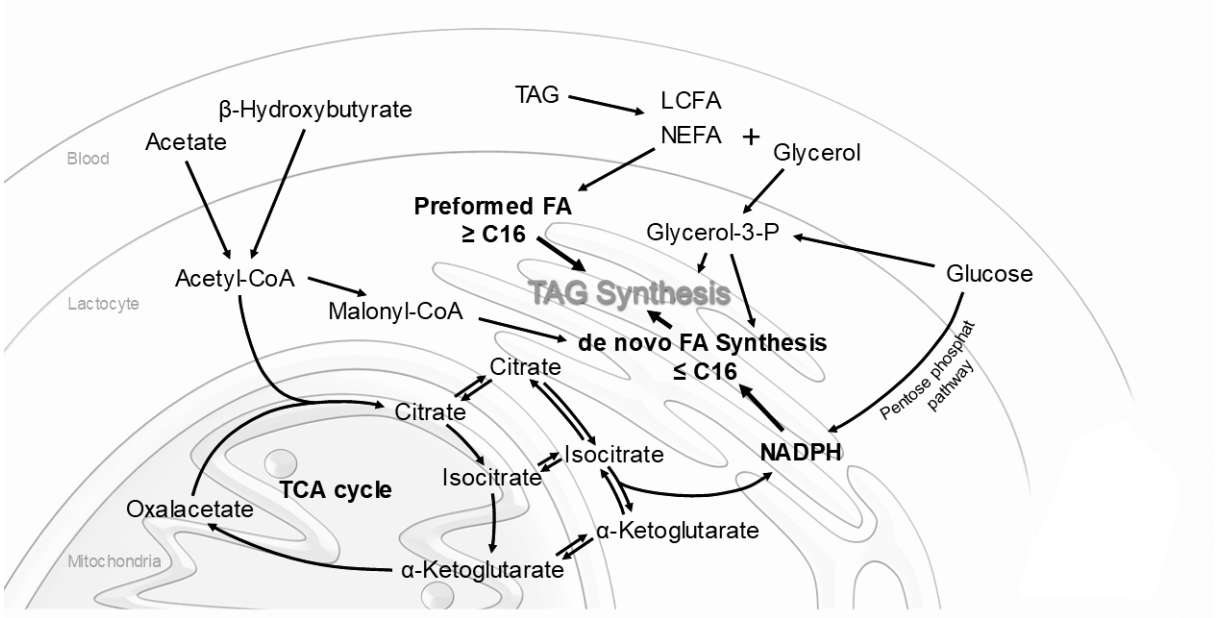

Figure 2.5 Milk fat synthesis in the mammary gland[1,2]

[1]Adapted from von Engelhardt et al. (2015)
[2]Abbreviations: LCFA – long-chain fatty acid; NADPH – nicotinamide adenine dinucleotide phosphate; TAG – triacylglycerol; NEFA – non-esterified fatty acid; TCA – tricarboxylic acid cycle

Essential Fatty Acids

Supplementation of EFA in the diet of dairy cows was shown to alter milk composition. The extent of the changes depends on (1) the amount and form of supplemented fat, (2) the degree of ruminal biohydrogenation; (3) the composition of the non-lipid component of the diet; (4) the influence of the lipid source on microbial FA synthesis; (5) the *de novo* synthesis of FA in the mammary gland; (6) the stage of lactation; and (7) the intestinal and mammary gland desaturase activity (Kennelly 1996).

As reviewed by Petit (2010) and Moallem (2018), the effects of feeding EFA as different forms of linseed on milk yield varied among studies. Said effects could be mediated by feed intake and seem to be neutral in the early stage of lactation. Abomasal LA or ALA supplementation (i.e., linseed oil) can increase milk fat percentage, whereas the inclusion of linseed products as EFA source in the diet of dairy cows has either no effect on milk fat concentration or leads to a slight decline of milk fat due to altered biohydrogenation and higher generation of CLA isomers (Benson et al. 2001; Petit et al. 2002; Gonthier et al. 2005; Petit et al. 2007; Carriquiry et al. 2009a, Kazama et al. 2010; Khas-Erdene et al. 2010; Petit 2010; Zachut et al. 2010; Côrtes et al. 2011; Moallem et al. 2012; Mach et al. 2013; Greco et al. 2015; Moallem 2018; Haubold et al. 2020a). The effect of feeding linseed on fat content in milk is highly related to the form of protection of FA, and therefore to the rate of exposure of unsaturated FA to biohydrogenation in the rumen. Supplemented EFA influences the FA profile in milk fat (Petit 2010; Moallem 2018). The shift to higher PUFA content in milk is compatible with a slight decline in

de novo FA synthesis (Khas-Erdene et al. 2010; Côrtes et al. 2011). In the end, the ability of the mammary gland to secrete EFA into milk is not a limiting factor in feeding strategies designed to alter milk composition, but the rumen conditions, rates of biohydrogenation, and protection against biohydrogenation by rumen microbes are the critical factors that influence the transfer of EFA from the diet into milk (Gonthier et al. 2005; Petit 2010).
Milk protein and lactose do not seem to be affected by the supplementation of a variety of EFA forms, as many reports have demonstrated either no effects or a slight decrease in milk protein (Petit 2010; Moallem 2018).

Conjugated Linoleic Acid

The CLA isomer *trans*-10,*cis*-12 has been shown to reduce milk fat concentration and yield by inhibiting the *de novo* FA synthesis (Baumgard et al. 2000; Bauman et al. 2008). Molecular mechanisms responsible for the reduction in lipid synthesis in the mammary gland involve a coordinated down-regulation of genes encoding *de novo* lipogenesis enzymes as well as uptake and transport of circulating FA and the transcription factor sterol response element-binding protein-1 (Baumgard et al. 2002b; Peterson et al. 2004; Harvatine and Bauman 2006; Bauman et al. 2008; Bauman et al. 2011). Response curves from a combined range of studies with different doses of *trans*-10,*cis*-12 CLA revealed a link between CLA dosage and the decrease of both milk fat yield and content as well as secretion of *trans*-10,*cis*-12 CLA (de Veth et al. 2004; Haubold et al. 2020a). The abomasal supplementation of 14 g/d *trans*-10,*cis*-12 CLA inhibits milk fat synthesis by 50% (Baumgard et al. 2001). The milk fat depressing effect of CLA supplementation seemed to be dependent on the supplemented formulation (protection against ruminal biohydrogenation) and lactation stage. In the established lactation, milk fat depression occurs shortly after starting the CLA supplementation, which differs from the delay in milk fat reduction that occurs in early lactation (Baumgard et al. 2000; Baumgard et al. 2001; Peterson et al. 2002; Bernal-Santos et al. 2003; Selberg et al. 2004; Castañeda-Gutiérrez et al. 2005; von Soosten et al. 2011; Hötger et al. 2013; Grossen-Rösti et al. 2018). The CLA-induced milk fat reduction is characterized by a more pronounced decrease in *de novo* synthesized FA, resulting in an altered milk FA pattern with a proportional shift to a greater percentage of longer-chain FA (Chouinard et al. 1999; Bauman and Griinari 2001; Baumgard et al. 2001; Perfield II et al. 2002; Mackle et al. 2003; Harvatine and Bauman 2011). Across studies, the transfer efficiency of abomasally infused *trans*-10,*cis*-12 CLA into milk fat was relatively constant at 22% (de Veth et al. 2004).
An increase in milk protein resulting from feeding CLA was mentioned by Bauman et al. (2008). However, the results of other studies revealed a milk protein reduction in early lactation (Moallem et al. 2010; Von Soosten et al. 2011). As mentioned by Bauman et al. (2008), during

times of inadequate nutrient intake, inducing a reduction in milk fat increases available energy that can be repartitioned toward an increased synthesis of milk and/or milk protein (Bernal-Santos et al. 2003; Mackle et al. 2003; Odens et al. 2007; Moallem et al. 2010; von Soosten et al. 2011; Hötger et al. 2013; Galamb et al. 2017; Chandler et al. 2017).

2.2.2 Energy Balance

The EB is the difference between energy consumed and energy used for maintenance and production (milk, meat, reproduction, etc.). The transition period is characterized by a negative EB caused by increased energy needs for fetal development and maintenance of conception, lactogenesis, and milk synthesis after calving (Bauman and Currie 1980; Roche et al. 2013; Baumgard et al. 2017). During the first one-third of the lactation period, micronutrient deficiencies lead to the use of body energy reserves stored in tissues to meet the needs (Bauman and Currie 1980; Drackley 1999; Roche et al. 2013). The high energy demands of milk production force a hypoglycemic status and causes mobilization of body fat and muscle tissue and consequently induces a loss of body condition (Drackley et al., 2001; van Dorland et al. 2009). During the transition period, to support lactation, a homeorhetic regulation of the energy metabolism orchestrates an adequate nutrient supply (carbohydrate, protein, fat, minerals, and vitamins) from body tissue to the mammary gland (Bauman and Currie 1980; Baumgard et al. 2017). The cow shifts its metabolism toward an increased hepatic glucose production, increased glucose use by the mammary gland, glucose sparing by non-mammary tissues, increased ketogenesis in the liver, use of ketone bodies in peripheral tissue such as muscle, decreased lipogenesis, increased lipolysis of body fat, and increased proteolysis in muscle tissue (Bauman and Currie 1980; Bell 1995; Drackley 1999; Roche et al. 2013). **Table 2.1** gives an overview of the most important metabolic adaptations after calving.

Essential Fatty Acids

Fatty acids may affect EB through changes in energy intake, nutrient digestibility, as well as milk and tissue synthesis via nutrient partitioning (Harvatine and Allen 2006a). Regarding the calculated EB by equation, a greater supply of n-3 FA had no effect on EB as long as performance (e.g., DMI, milk yield) and milk composition remain unaltered. Generally, the EB during the transition period is driven by a decrease in DMI by up to 30% (Wankhade et al. 2017). For EFA supplementation, the results regarding DMI are inconsistent. The effects on DMI may be attributed to many factors, such as the supplements' form, odor, and palatability; level and duration of supplementation; and stage of lactation and dietary composition, rather than to the FA composition of the supplements per se (Zachut et al. 2010; Moallem 2018). Generally, DMI

is not affected by postruminal n-3 supplementation from linseed nor by the feeding of n-3 supplements from linseed (Petit 2002; Petit et al. 2007; Carriquiry et al. 2009a; Khas-Erdene et al. 2010; Moallem et al. 2012; Mach et al. 2013; Moallem 2018). However, DMI decreased linearly with increasing amounts of supplemented n-3 FA in the face of a disturbed ruminal function (Maia et al. 2007; Kazama et al. 2010).

Table 2.1 Partitioning of nutrients as homeorhetic regulation to support lactation in dairy cows[1]

Tissue	Metabolic change[2]
Mammary tissue	▲ Nutrient use ▲ Blood supply
Liver	▲ Rates of gluconeogenesis ▲ Glycogen mobilization ▲ Fatty acid oxidation ▲ Ketogenesis ▲ Fatty acid esterification ▼ Lipoprotein metabolism ▲ Protein synthesis
Adipose tissue	▲ Lipolysis ▼ Preformed fatty acid uptake ▼ *De novo* fat synthesis ▼ Fatty acid re-esterification
Skeletal muscle	▼ Glucose utilization ▼ Protein synthesis ▲ Protein degradation
Bone	▲ Calcium and phosphor mobilization
Plasma hormones	▲ Growth hormone ▼ Insulin-like growth factor I ▼ Insulin ▲ Glucocorticoids

[1]Adapted from Baumgard et al. (2017) and Roche et al. (2013)
[2]Meaning of the characters: ▲ = increase; ▼ = decrease

Conjugated Linoleic Acid

The milk fat depressing effects of *trans*-10,*cis*-12 CLA on milk fat content and yield could be used as a management tool to temporarily reduce milk energy output, improve EB, and spare body reserves of dairy cows, especially in the transition period (Bauman et al. 2008; Trevisi et al. 2008; von Soosten et al. 2012). The CLA effect on EB during early lactation is inconsistent, several studies have provided evidence that decreases in milk fat content may improve EB or shorten the period where the EB is negative (Moore et al. 2004; Kay et al. 2006; Odens et al. 2007; Hutchinson et al. 2011). A missing effect of CLA supplementation on EB in some trials can be attributed to a lack of severe milk fat depression (dependency on supplementation period, formulation, or dose), enhanced milk energy output in the face of energy partitioning,

or reduced DMI (Bernal-Santos et al. 2003; Moallem et al. 2010; Sigl et al. 2010; Pappritz et al. 2011; von Soosten et al. 2011; Metzger-Petersen 2012; Hötger et al. 2013; Schäfers et al. 2017; Grossen-Rösti et al. 2018). Kay et al. (2006) reported a curvilinear relationship between the severity of milk fat depression and the shift of nutrient partitioning to a positive milk yield response during energy deficiency.

The effects of CLA on DMI as described in literature are dose- and time-depend (Schäfers et al. 2017). Higher doses and abomasal supplementation of *trans*-10,*cis*-12 CLA seemed to reduce DMI (Baumgard et al. 2000; Baumgard et al. 2001; Harvatine et al. 2009; Moallem et al. 2010; Pappritz et al. 2011; von Soosten et al. 2011; Hötger et al. 2013; Schäfers et al. 2017). A meta-analysis of Harvatine et al. (2009) indicated that the decrease in energy intake in the meta-analysis data set would account for 88% of the decrease in milk energy output during CLA-induced milk fat depression.

2.2.3 Lipid Metabolism

In early lactation, cows must cope with the genetically imposed burden of meeting the requirements for the metabolically prioritized mammary gland (Gross and Bruckmaier 2019). The energy stored in adipose tissue as lipids will be mobilized in early lactation (lipolysis), and the amount of NEFA in the blood will subsequently increase (Bauman and Currie 1980; Drackley et al. 2001).

The FA released from adipose stores are taken up by the liver and other tissues (e.g., skeletal muscle) and are utilized through the β-oxidation pathway in mitochondria to produce acetyl-CoA and enter the tricarboxylic acid cycle (complete oxidation; **Figure 2.6**). In periods of negative EB and high energy requirements (late pregnancy, early lactation) NEFA overflow in hepatocytes surpasses oxidation capacity and leads to incomplete oxidation of NEFA with an increase of intracellular acetyl-CoA levels and ketogenesis (Drackley 1999; Drackley et al., 2001). In the liver, acetyl-CoA is shunted off as the precursor for ketone body production, e.g., acetoacetate, BHB, and acetone, which can also be used as energy substrates in other tissues (Drackley et al., 2001). In addition, not-oxidized NEFA in the liver lead to increased TG synthesis and accumulation in periparturient dairy cows (Grummer 1995; Drackley 1999; Drackley et al. 2001). Because ruminants can not efficiently export FA as very-low-density lipoproteins (**VLDL**; low rates of hepatic VLDL synthesis and secretion), a significant amount of the NEFA taken up by the liver are re-esterified, stored, and accumulated as cytosolic lipid droplets in the liver (Grummer 1995; Bobe et al. 2004; Drackley et al., 2001). Increased lipogenesis and TG infiltration in the liver are associated with diminished metabolic capacity (Bobe et al. 2004). The packaging and transport of elevated re-esterified hepatic TG, cholesteryl esters, and cholesterol as VLDL via the bloodstream into the peripheral tissues is of secondary importance

compared to the low ability of the liver to secrete VLDL (Drackley 1999; Drackley et al. 2001). The VLDL are an endogenous source of FA that are used in the milk synthesis taking place in the mammary gland (Bell 1995). In peripheral circulation, VLDL are processed into intermediate-density lipoproteins and can be metabolized further to low-density lipoproteins (**LDL**). From extrahepatic tissues, cholesterol is returned to the liver in high-density lipoproteins (**HDL**).

Essential Fatty Acids

The ability to regulate the liver's transcriptional network in the peripartal cow via dietary LCFA, especially on transcription factors, was reviewed in detail by Loor (2010). The results provided evidence that LCFA, particularly PUFA, can activate the peroxisome proliferator-activated receptor α (PPARα) network of genes in ruminants which might, in turn, lead to a positive effect on liver function after calving, such as up-regulation of FA oxidation and less lipid accumulation via reduced TG synthesis and enhanced cholesterol synthesis for VLDL assembly/export (Jump and Clarke 1999; Loor 2010; Bionaz et al. 2015).

Such beneficial impact of lower plasma NEFA along with a greater clearance rate or lower liver TG accumulation with EFA supplementation was also demonstrated in cows (Mashek et al. 2005; Petit et al. 2007; Pires et al. 2008). However, during the transition period, reduced liver TG accumulation seemed to be more influenced by extrahepatic effects (e.g., reduced NEFA) than direct effects of ALA supplementation on the liver (Brickner et al. 2009; Loor 2010; Mach et al. 2013).

Conjugated Linoleic Acid

In cows, detecting the effect of CLA treatment on circulating plasma NEFA concentration is difficult because of the high variability in NEFA concentrations during the transition period. Inconsistent study results of CLA supplementation on plasma NEFA concentration indicate that CLA lowers circulating NEFA only during the challenging transition period after triggering a severe milk fat depression (Mackle et al. 2003; Castañeda-Gutiérrez et al. 2005; Kay et al. 2006; Odens et al. 2007; Hutchinson et al. 2011; Galamb et al. 2017). Several transition trials with CLA supplementation show no reduction in plasma NEFA and no effect on hepatic fat accumulation (Bernal-Santos et al. 2003; Moore et al. 2004; Selberg et al. 2004; Pappritz et al. 2011; von Soosten et al. 2011; Hötger et al. 2013; Schäfers et al. 2017; Grossen-Rösti et al. 2018). However, evidence exists for a reduced lipid mobilization in adipose tissue due to the gene expression changes and reduced lipolytic response (Baumgard et al. 2002b, Harvatine et al. 2009). Studies on liver lipid content indicated no effect of CLA supplementation on hepatic fat accumulation (Castañeda-Gutiérrez et al. 2005; Bernal-Santos et al. 2003; Selberg et al. 2004; Schäfers et al. 2017).

2.2.4 Glucose Metabolism

Glucose is a key nutrient used in metabolic processes of early lactating cows. Coordinated response of the tissues meets the need of up to 80% of the total glucose turnover for the secreting mammary gland; the rate of gluconeogenesis in the liver increases dramatically, and in addition, glycogen is mobilized (Bauman and Currie 1980).

The eGP, which is the sum of gluconeogenesis and glycogenolysis, provides most of the glucose for milk production because very little glucose originates from net portal absorption (Brockman 2005; Aschenbach et al. 2010; Hammon et al. 2016). Thus, in lactating dairy cows, glucose requirements need to be met mainly through the synthesis of glucose from non-carbohydrate sources, i.e., the main glucogenic precursor propionate from ruminal fermentation, followed by lactate, amino acids, and glycerol (Brockman 2005; Aschenbach et al. 2010; Hammon et al. 2016). Additionally, in early lactation, hepatic glycogenolysis can provide some immediate glucose by glycogen breakdown. The liver is the most important glucose-producing organ in ruminants, contributing up to 90% of whole-body glucose turnover (Brockman 2005). The pathways of precursor substrates for hepatic glucose production and key enzymes involved in hepatic gluconeogenesis are summarized in **Figure 2.6**. As reviewed by Hammon et al. (2016), pyruvate carboxylase (**PC,** *PC* [gene name in italic]) catalyzes the conversion of pyruvate to oxaloacetate and is allosterically activated by acetyl-CoA. Oxaloacetate is converted to phosphoenolpyruvate by two forms of the phosphoenolpyruvate carboxykinase (**PEPCKc**, *PCK1* – cytosolic form; **PEPCKm**, *PCK2* – mitochondrial form). Mitochondrial propionyl-CoA carboxylase (**PCC**, *PCCA*) is related to hepatic propionate entry into the gluconeogenic pathway. Glucose-6-phosphatase (**G6Pase**, *G6PC*) catalyzes the final step of the gluconeogenesis (conversion of glucose-6-phosphate to glucose) and is a prerequisite in tissues with endogenous glucose synthesis. During the transition period, the time pattern in mRNA expression for gluconeogenic enzymes is caused by a shift in gluconeogenic substrate availability after calving (Donkin 2016; Hammon et al. 2016). After calving, reduced propionate and increased lactate portions cause an immediate increase in mRNA abundance for *PC* and *PCK2*, but a delayed increase for *PCK1* and *PCCA*. The mRNA expression for *G6PC* is activated during elevated hepatic glucose production and less regulated on the transcriptional level (Hammon et al. 2016).

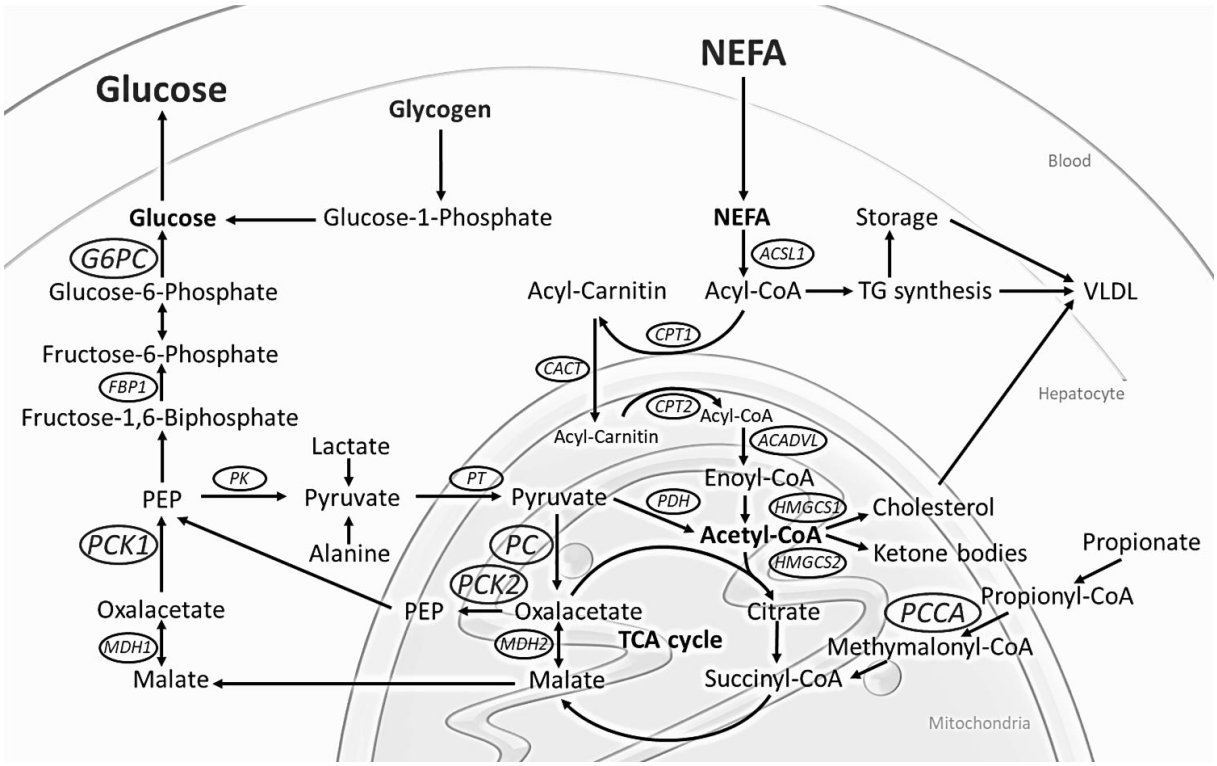

Figure 2.6 Simplified scheme of hepatic nutrient metabolism and related enzymes encoded by genes[1]

[1]Adapted from von Engelhardt et al. (2015)
[2]Abbreviations: *ACADVL* – acyl-CoA-dehydrogenase very long chain; *ACSL1* – acyl-CoA-synthetase long chain 1; *CACT* – carnitine-acylcarnitine translocase; *CPT1* – cytosolic carnitine-palmitoyl-transferase; *CPT2* – mitochondrial carnitine-palmitoyl-transferase; *FBP1* – fructose-1,6-biphosphatase; *G6PC* – glucose-6-phosphatase; *HMGCS1* – cytosolic hydroxyl-methyl-glutaryl-CoA-synthase; *HMGCS2* – mitochondrial hydroxyl-methyl-glutaryl-CoA-synthase; *MDH1* – cytosolic malate dehydrogenase; *MDH2* – mitochondrial malate dehydrogenase; *PC* – pyruvate carboxylase; *PCCA* – propionyl-CoA carboxylase alpha chain; *PCK1* – cytosolic phosphoenolpyruvate carboxykinase; *PCK2* – mitochondrial phosphoenolpyruvate carboxykinase; *PDH* – pyruvate dehydrogenase; PEP – phosphoenolpyruvate; *PK* – pyruvate kinase; *PT* – pyruvate translocase; TCA – tricarboxylic acid; TG – triglyceride; VLDL – very-low-density lipoprotein

Glucose metabolism in ruminants is regulated by the supply and removal of glucose and glucogenic precursors in the blood and is tightly controlled by altered concentrations of circulating hormones, i.e., insulin and glucagon (De Koster and Opsomer 2013). An exceptional feature is the insulin-independent glucose uptake by the mammary gland to support lactation (De Koster and Opsomer 2013). In early lactation, decreased glucose uptake and usage as an energy source by adipose tissue and skeletal muscle results in lower whole-body glucose oxidation (**GOx**) to ensure adequate glucose supply and allow partitioning of a greater percentage of glucose to the lactating mammary gland (Bauman and Currie 1980; Baumgard et al. 2017). A key aspect of this homeorhetic reaction is its mediation through altered responses to homeostatic effectors such as insulin and adrenergic agents (Bauman and Currie 1980). Cows enter an insulin-resistant state (decreased insulin responsiveness or sensitivity in peripheral tissues) when blood insulin concentration is at a low level in order to prevent the anabolic

processes and support lipolysis in adipose tissue and mobilization of amino acids from muscle tissue (Bauman and Currie 1980; De Koster and Opsomer 2013). Furthermore, glucocorticoids such as cortisol also cause peripheral insulin resistance and act as a gluconeogenic hormone in cattle (Brockman and Laarveld 1986; Kusenda et al. 2013). Elevated plasma cortisol reduces glucose tissue uptake in dairy cows, leading to increased plasma glucose concentrations, whereas hepatic glucose production is less affected (Kusenda et al. 2013). Thus, for dairy cows during the transition period, the most affected pathways during the insulin-resistant state are reduced glucose uptake by skeletal muscle and adipose tissue; reduced lipogenesis and increased lipolysis in adipose tissue; stimulation of the gluconeogenesis in the liver; suppressed protein synthesis and stimulated protein degradation of skeletal muscle to ensure a sufficient glucose supply for the gravid uterus and lactating mammary gland (De Koster and Opsomer 2013).

Essential Fatty Acids

Supplementation of ALA or an associated lower n-6/n-3 in the diet seemed to slightly affect plasma glucose in PP dairy cows (Carriquiry et al. 2009a, Zachut et al. 2010; Mach et al. 2013; Badiei et al. 2014; Greco et al. 2015; do Prado et al. 2016). In monolayer cultures of bovine hepatocytes, ALA supplementation elicited the highest rates of gluconeogenesis, (Mashek and Grummer 2003) whereas in cows ALA was shown to increase the concentration of glycogen in the liver before calving (Petit et al. 2007). The ability of different FA in blood plasma to mediate hepatic mRNA expression for gluconeogenic enzymes was theorized and initially examined in bovine cell cultures and seemed to be dependent on the concentration and profile of FA (White et al. 2011; 2012).

Dietary FA profile can modulate the response to insulin; particularly, n-3 PUFA may prevent the development of insulin resistance (Pires and Grummer 2008; Fortin et al. 2010). Additionally, greater insulin sensitivity was seen in pasture grazing cows as compared to exclusively TMR-fed cows in the PP period (Astessiano et al. 2015). However, there also exist results with no differences in plasma insulin response after supplementation of EFA or a modified n-6/n-3 ratio (Badiei et al. 2014; Greco et al. 2015).

Conjugated Linoleic Acid

Investigations on glucose metabolism with respect to CLA supplementation in dairy cows are performed to examine glucose concentrations in blood plasma, insulin responses, and changes in glucose turnover (Baumgard et al. 2002a, Moore et al. 2004; Odens et al. 2007; Bauman et al. 2008; Hötger et al. 2013; Urrutia and Harvatine 2017; Grossen-Rösti et al. 2018).

It was presumed that CLA supplementation can spare glucose, but study results demonstrate that the CLA induced reduction of milk fat synthesis occurs with little to no apparent alterations in glucose homeostasis or its regulating hormones, e.g. insulin and glucagon (Baumgard et al. 2002a, Bauman et al. 2008; Bauman et al. 2011; Grossen-Rösti et al. 2018). Only Hötger et al. (2013) showed that the *trans*-10,*cis*-12 CLA-induced milk fat depression results in a glucose-sparing effect with elevated plasma glucose concentration and reduced eGP to retain glucose homeostasis. Odens et al. (2007) attributed the increased glucose concentration to decreased whole-body insulin sensitivity. Additionally, CLA supplementation triggers glycogen storage, because early lactating cows exposed to an intramammary lipopolysaccharide challenge and supplemented with CLA provided more glucose and preferentially used BHB as an energy source during the immune response (Gross et al. 2018). However, in previous studies, CLA treatment had no effect on the hepatic glycogen concentration during the transition period, but EB was also not affected (Bernal-Santos et al. 2003; Hötger et al. 2013). Supplementation of CLA seemed to also have no effect on the hepatic mRNA expression of enzymes involved in gluconeogenesis (Selberg et al. 2004; Hötger et al. 2013).

An elevated insulin concentration in CLA-supplemented cows during the transition period has been detailed as well (Saremi et al. 2014; Grossen-Rösti et al. 2018). Findings of elevated insulin and less affected glucose concentration in CLA-supplemented cows were related to a decreased systemic insulin sensitivity or a CLA-induced stimulation of insulin secretion in pancreatic β cells (Saremi et al. 2014; Urrutia and Harvatine 2017; Grossen-Rösti et al. 2018).

2.2.5 Somatotropic Axis

Stimulation of hormones of the somatotropic axis exerts signaling functions in a series of metabolic processes and plays a key role in the control and regulation of the energy and nutrient distribution that support early lactation performance (Le Roith et al. 2001; Renaville et al. 2002).

Figure 2.7 shows the somatotropic axis consisting of stimulating and inhibiting factors of GH from the hypothalamus, GH, insulin-like growth factor-I (**IGF-I**), and their associated binding proteins and receptors, as well as their pathways and relationships. After endogenous pituitary GH release, GH binds to its receptor in the liver, mainly through GH receptor 1A (**GHR1A**), and stimulates the secretion of IGF-I (Le Roith et al. 2001; Renaville et al. 2002). IGF-I is secreted by peripheral tissues in addition to its primary release from the liver and exerts biological effects on most cell types (Thissen et al. 1994). Six different IGF binding proteins (**IGFBP**) act as carrier proteins by transporting IGF to the target tissues and prolonging the half-life of IGF by protecting them from proteolytic degradation (Le Roith et al. 2001). Nutrient-induced changes in the concentrations of the IGFBP could alter the clearance of circulating

IGF-I (Thissen et al. 1994). Nutritional restriction and PP negative EB decrease serum IGFBP-3 and increase serum IGFBP-1 and IGFBP-2 concentrations in order to lower the bio-availability of IGF-I to peripheral tissues (Renaville et al. 2002). The IGF-I and GH are regulated in a negative feedback loop, so that, as IGF-I decreases, the negative feedback on GH is reduced, resulting in an increase in plasma GH (Le Roith et al. 2001; Butler et al. 2003; Lucy 2004).

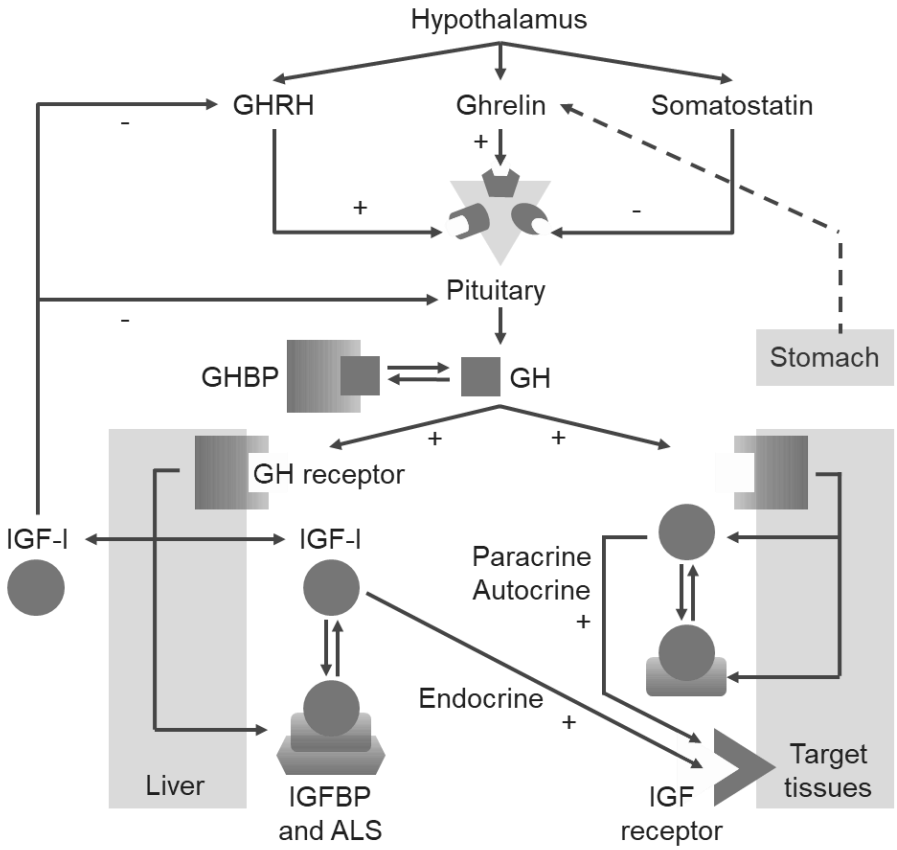

Figure 2.7 Pathways and relationships of the somatotropic axis[1,2]

[1]Adapted from Holt (2002)
[2]Abbreviations: GH – growth hormone; GHRH – GH releasing hormone; GHBP – GH binding protein; IGF – insulin-like growth factor; IGFBP – IGF binding protein; ALS – acid-labile subunit

A series of endocrine mechanisms involving GH, GHR, IGF-I, as well as insulin coordinate metabolic events and ensure nutrient partitioning to milk production and performance in early lactation (Lucy 2004). In dairy cows after calving, the somatotropic axis becomes uncoupled through downregulation of GHR1A in the liver (decreased *GHR1A* mRNA expression immediately before parturition), which leads to a reduced binding of GHR1A, loss of GH action, and low IGF-I concentrations (reduced *IGF1* mRNA expression, IGF-I synthesis and secretion shortly after parturition), a reduced negative feedback on GH, and an increase of plasma GH concentration (Thissen et al. 1994; Butler et al. 2003; Radcliff et al. 2003a, b; Lucy 2004). The

recoupling of the somatotropic axis during early lactation has been linked to PP nutrition as well as EB and is dependent on the stimulatory effect of insulin on liver GHR1A expression (Radcliff et al. 2006; Butler et al. 2003; Lucy 2004). It was shown that insulin causes opposite effects on GHR in adipose tissue (Butler et al. 2003). Thus, during times of low insulin, plasma GH concentration (via low GHR1A expression and less IGF-I negative feedback), adipose GHR concentration, and lipid mobilization all increase (Lucy 2004). The GH antagonizes anabolic effects of insulin and IGF-I, which has a nutrient partitioning effect and promotes lipolysis and eGP to meet the requirement for mammary milk synthesis in early lactation (Etherton and Bauman 1998; Lucy 2004).

Essential Fatty Acids

Study results show that the effects of different FA profiles in blood on the somatotropic axis are not well established. As such, said results are classified as heterogeneous. This could potentially be because of the changes in blood hormone concentrations and related alterations in hepatic mRNA that caused the uncoupling of the somatotropic axis in dairy cows during the transition period (Kim 2014). Altering the dietary n-6/n-3 ratio seemed to have no effect on hormones of the somatotropic axis or related expression of hepatic genes, suggesting that an improved ALA status in periparturient cows did not distinctly influence the somatotropic axis or key metabolic genes in the hepatic tissue (Carriquiry et al. 2009b, Greco et al. 2015). Despite all that, it was demonstrated that supplementation of n-3 FA either altered hepatic expression of genes related to the somatotropic axis or increased plasma concentration of IGF-I, glucose, and insulin (Carriquiry et al. 2009a; Dirandeh et al. 2016).

Conjugated Linoleic Acid

The somatotropic axis plays a key role in the co-ordination and regulation of intermediary metabolism to partition energy substrates (Breier et al. 1999). Some studies observed effects of CLA on the somatotropic axis, describing the interactions of GH, IGF-I, and their binding proteins. It was shown that a CLA supplement containing both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers can increase blood GH leading to an increased plasma glucose concentration, whereas other regulatory hormones remain unchanged (Qin et al. 2018). Moreover, in PP dairy cows, supplementation mixtures of both CLA isomers increased IGF-I blood concentration, suggesting an earlier recoupling of the GH–IGF-I axis, possibly due to a higher plasma insulin response (Castañeda-Gutiérrez et al. 2007; Csillik et al. 2017). These studies indicate a stimulatory effect of CLA on the somatotropic axis and a direct relationship between the amount of *trans*-10,*cis*-12 CLA supplementation and an elevated plasma IGF-I concentration. However, evaluation of the effects of specific CLA isomers show no effect of *trans*-10,*cis*-12 CLA but a

slightly greater plasma concentration of IGF-I after abomasal *cis*-9,*trans*-11 CLA infusion (Baumgard et al. 2000). Because of the inverse relationship between EB and plasma IGFBP-2 concentration in dairy cows, CLA treatment appeared to be responsible for the decrease in plasma IGFBP-2 due to the improvement in the energy status of cows in established lactation (Haubold et al. 2020a, b). Beneficial effects of an improved energy status in CLA-treated cows refer to the stimulation of the somatotropic axis, changes in the respective hormones, and related hepatic gene expression (Castañeda-Gutiérrez et al. 2007; Csillik et al. 2017; Qin et al. 2018; Haubold et al. 2020b).

2.3 Scope of the Thesis

The requirement of further studies concerning the impact of effects of EFA and CLA supplementation on performance and energy metabolism in dairy cows during the transition period was demonstrated by the aforementioned heterogeneous results of previous studies, which differed in their experimental designs, application forms, and duration of supplementation. As has been shown, LA and ALA are the main precursors for CLA formation and must be provided by feed, particularly in the form of fresh grass. Therefore, feeding regime and forage type strongly affects the intake of EFA and n-6/n-3 FA ratio as well as the CLA status of dairy cows as it changed from pasture-based feeding to barn systems with incorporation of preserved feed with corn silage as the main component in the diet of common dairy cow nutrition. The EFA and CLA isomers might have distinct metabolic modulating characteristics and functions in dairy cows and CLA effects can be partly independent from or synergistic to EFA's effects. Considering the mitigation of the metabolic load during the transition period, EFA and CLA supplementation may help to avoid production decreases in dairy cows and may also contribute to increasing well-being of dairy cows, as this is most likely the case in pasture-fed cows. Because of the key role of nutritive alterations during the transition period, a possible impact of EFA and CLA supplementation on the metabolic and endocrine changes has to be studied in more detail to assess their benefits as well as disadvantages on performance and energy metabolism around calving in dairy cows.

Therefore, a study with high-yielding dairy cows was conducted that aimed to balance and characterize the importance of EFA and CLA supplementation on performance and energy metabolism during the transition period and assess whether insufficient supply could possibly lead to impairment of metabolic functions. To investigate EFA and CLA supply for physiological functions, their impact on metabolic and endocrine changes had to be investigated in transition cows with a reduced EFA and CLA status. Therefore, cows were fed with a corn-silage based TMR to provide low amounts of EFA, especially ALA and CLA. Cows were assigned to one of 4 treatment groups: control (CTRL; coconut oil, 76 g/d), EFA (linseed and safflower oil, 78 and 4 g/d), CLA (*cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA in equal amounts, 10 g/d), and EFA+CLA. The FA treatments and dosages used in the following experiment were chosen after considering their effects based on pre-study results (Weber et al. 2016; Haubold 2020a, b). In the described study, postruminal infusion was used to avoid direct effects of supplemented FA on ruminal microbes or microbial hydrogenation of PUFA, and to make sure that investigated effects are directly linked to the supplemented FA.

Manuscript 1 focused on the impact of long-term supplementation of EFA, mainly ALA, and CLA on performance, milk, and body composition when fed with a diet consisting of low fat and a low n-3 FA contents, resulting in an increased n-6/n-3 FA supply.
Manuscript 2 focused on the glucose metabolism and endocrine changes in dairy cows after abomasal infusion of EFA together with CLA during late gestation and early lactation, and estimated their contributions to overcoming post-calving metabolic stress.

The outcome of this study provides new information on the importance of EFA and CLA supply in promoting the performance and energy metabolism in dairy cows and could help answer the scientific question whether EFA supply together with CLA is a significant factor in stabilizing metabolic functions, independently or in combination with each other, especially during the transition period.

The following hypotheses were established in the course of the described experiment:

- A combined EFA and CLA supplementation changes milk composition and reflects the FA pattern in milk of pasture-based dairy nutrition.
- A combined EFA and CLA supplementation affects performance and energy utilization during late gestation and early lactation.
- A combined EFA and CLA supplementation has an impact on lipid metabolism in the transition dairy cow.
- A combined EFA and CLA supplementation alters glucose metabolism in the transition dairy cow.
- A combined EFA and CLA supplementation affects the endocrine regulation of nutrient partitioning regarding the somatotropic axis.

2.4 References

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3. MANUSCRIPT 1

Effects of abomasal infusion of essential fatty acids together with conjugated linoleic acid in late and early lactation on performance, milk and body composition, and plasma metabolites in dairy cows

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3.1 Abstract

Rations including high amounts of corn silage are currently very common in dairy production. Diets with corn silage as forage source result in a low supply of EFA, such as ALA, and may lead to low CLA production. The present study investigated the effects of abomasal infusion of essential FA, especially ALA, and CLA in dairy cows fed a corn silage–based diet on performance, milk composition, including FA pattern, and lipid metabolism from late to early lactation. Rumen-cannulated Holstein cows (n = 40) were studied from wk 9 AP to wk 9 PP and dried off 6 wk before calving. The cows were assigned to 1 of 4 treatment groups. Cows were abomasally supplemented with coconut oil (CTRL, 76 g/d), linseed and safflower oil (EFA, 78 and 4 g/d; linseed/safflower oil ratio = 19.5:1; n-6/n-3 FA ratio = 1:3), Lutalin (CLA, 38 g/d; BASF SE, Ludwigshafen, Germany; isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 each 10 g/d) or EFA+CLA. Milk composition was analyzed weekly, and blood samples were taken several times before and after parturition to determine plasma concentrations of metabolites related to lipid metabolism. Liver samples were obtained by biopsy on d 63 and 21 AP and on d 1, 28, and 63 PP to measure TG concentration. Body composition was determined after slaughter. Supplementation of CLA reduced milk fat concentration, increased body fat mass, and improved EB in late and early lactation, but EB was lowest during late lactation in the EFA group. Cows with CLA treatment alone showed an elevated milk citrate concentration in early lactation, whereas EFA+CLA did not reveal higher milk citrate but did have increased acetone. Milk protein was increased in late lactation but was decreased in wk 1 PP in CLA and EFA+CLA. Milk urea was reduced by CLA treatment during the whole period. After calving, the increase of NEFA in plasma was less in CLA groups; liver TG were raised lowest at d 28 in CLA groups. Our data confirm an improved metabolic status with CLA but not with exclusive EFA supplementation during early lactation. Increased milk citrate concentration in CLA cows points to reduced *de novo* FA synthesis in the mammary gland, but milk citrate was less affected in EFA+CLA cows, indicating that EFA supplementation may influence changes in mammary gland FA metabolism achieved by CLA.

Key words: dairy cow, α-linolenic acid, conjugated linoleic acid, milk components, energy balance

3.2 Introduction

Essential fatty acids, particularly ALA and linoleic acid LA and their metabolites EPA, DPA, and docosahexaenoic acid, as well as ARA, are important for several biological functions, such as immune functions, blood coagulation, vascular resistance, enzyme activities, cell proliferation and differentiation, and receptor expression (Moallem 2018). Mammals, including ruminants, are not able to synthesize EFA; therefore, they must be obtained from food (Palmquist 2010; Spector and Kim 2015). In addition, the isomer-specific, health-promoting effects of CLA in humans are well known (Nagao and Yanagita 2005; Shokryazdan et al. 2017), and ruminant products are a natural source for CLA isomers (Bauman et al. 2000). Conjugated linoleic acids are produced in the rumen by EFA transformation, and therefore rumen CLA production depends on EFA intake (Chilliard et al. 2001; Shingfield et al. 2010). Some CLA isomers reveal metabolic effects in dairy cows, such as milk fat reduction and glucose-sparing effect (Bauman et al. 2000; Hötger et al. 2013). These effects are able to improve the energy status of dairy cows, especially in the transition period (Trevisi et al. 2008; von Soosten et al. 2012). In recent studies, lower milk protein and urea levels, which are possibly related to higher body protein accretion and nitrogen retention, were found following CLA supplementation (von Soosten et al. 2012; Haubold et al. 2020).

Over the last few decades, diets for dairy cows have changed dramatically. With increasing milk yield, it has become a common practice to feed TMR containing high amounts of corn silage rather than pasture-based feeding systems (Barkema et al. 2015). Therefore, the FA supply and the intake of EFA and level of rumen and tissue CLA production have also changed. (Chilliard et al. 2001; Shingfield et al. 2010). Cows on pasture take up high amounts of EFA, especially ALA (Chilliard et al. 2001; Khiaosa-ard et al. 2015), and CLA production in the rumen and mammary gland tissue increases with pasture feeding (Kelly et al. 1998; Ferlay et al. 2006; Lahlou et al. 2014). The importance of n-3 FA in dairy production has already been reviewed (Palmquist 2010; Moallem 2018). Corn silage is rich in LA but contains low levels of fat and ALA (Chilliard et al. 2001; Khan et al. 2015). In high-yielding herds, fat supplementation is a common feeding strategy to improve energy intake. However, commercial ruminal inert fats containing palmitic acid are usually used (Palmquist 2010), and low levels of n-3 FA are available to cows (Chilliard et al. 2001; Khan et al. 2015). Therefore, the forage type strongly affects the intake of EFA and the n-6/n-3 FA ratio in the diet, as well as the CLA status of dairy cows (Chilliard et al. 2001; Shingfield et al. 2010; Khan et al. 2015).

It is not known how administration of combined EFA and CLA administration affects performance and lipid metabolism in high-yielding dairy cows around calving, when cows are sub-

jected to significant metabolic stress. In addition, most studies on either EFA or CLA supplementation are short-term, and no long-term studies are available with a combined EFA and CLA supplementation from late gestation through subsequent lactation. It is obvious that the nutrient supply during late gestation affects early lactation performance and metabolism (Drackley 1999). Therefore, the aim of the present study was to investigate the long-term effects of a combined EFA and CLA supplementation on lactation performance, milk and body composition, and lipid metabolism in dairy cows when starting the supplementation late in the previous lactation. The cows received a corn silage–based TMR with low fat and especially low n-3 FA intake. The treatments focused on the supply of FA that provide EFA (mainly ALA), CLA, or the combination of both. Such a treatment model refers to the supply of EFA and related rumen and tissue CLA production in dairy cows receiving fresh grass or on pasture (Kelly et al. 1998; Ferlay et al. 2006; Lahlou et al. 2014). To avoid rumen degradation of the supplemented FA, all FA were infused into the abomasum. We hypothesized that an elevated combined intake of EFA and CLA would change performance, milk composition including FA pattern, and lipid metabolism of dairy cows during the transition from late pregnancy to early lactation. Doses for the supplied EFA (linseed and safflower oil in a ratio of 19.5:1; providing an n-6/n-3 FA ratio of 1:3 in the supplement mixture) and CLA were recently evaluated in a companion dose-response study in mid-lactating dairy cows (Haubold et al. 2020).

3.3 Materials and Methods

3.3.1 Animals, Husbandry, and Fatty Acid Supplementation

All experimental procedures were carried out in accordance with the German Animal Welfare Act and were approved by the relevant Department for Animal Welfare Affairs of the state of Mecklenburg-West Pomerania (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern, Germany; LALLF M-V/TSD/7221.3–1-038/15).

Forty Holstein cows were purchased in blocks of 8 cows from a local farm in approximately wk 18 of gestation in their second lactation. The cows were kept in a freestall barn at the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. During the preparation time of the study, cows were adapted to the new environmental conditions and diet and were surgically fitted with rumen cannulas (#2C or #1C 4-inch, Bar Diamond Inc., Parma, ID) 9 to 8 wk before beginning of the experiments and abomasal infusion lines [Teflon tube (inner diameter 6 mm) with 2 perforated Teflon flanges (outer diameter 120 mm)] 3 to 2 wk before beginning of the experiments, as previously described (Haubold et al. 2020). Two cows per block were assigned to 1 of 4 treatment groups with comparable projected milk production (11,101 ± 1,118 kg milk/305 d in second lactation, mean ± SD), BW (662 ± 56 kg, mean ± SD),

and predicted calving interval (395 ± 39 d, mean ± SD). Two cows calved prematurely and had to be excluded from the study. Cows were daily abomasally supplemented from d 63 AP until d 63 PP with coconut oil, providing no ALA or CLA (CTRL, n = 9; Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard KG, Bad Ditzenbach, Germany); a combination of linseed oil (DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany) and safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany) to provide an n-6/n-3 FA ratio of 1:3 in the supplement mixture (**EFA**, n = 9); Lutalin (**CLA** treatment, n = 10; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, 10 g/d each; BASF SE, Ludwigshafen, Germany); or a combination of EFA and CLA (**EFA+CLA**, n = 10). The amounts of daily infused supplements are given in **Table 3.1**. Treatments were infused using 60-mL catheter-tip syringes twice a day (2 equal portions) at 0700 and 1630 h. All supplements were liquified by heating to 38°C to allow infusion. The FA compositions of the added lipids are shown in **Table 3.7**. During the dry period, each dose was halved. Sampling started at wk 10 AP and was terminated at wk 9 PP.

Table 3.1 Amounts of daily abomasally infused supplements[1]

	Treatments						
	CTRL[2]	EFA		CLA[2]	EFA+CLA		
Supplementation	**Coconut oil[3]**	**Linseed oil[4]**	**Safflower oil[5]**	**Lutalin®[6]**	**Linseed oil[4]**	**Safflower oil[5]**	**Lutalin®[6]**
Daily infused oils (g/d)							
Lactation dosage	76	78	4	38	78	4	38
Dry period dosage	38	39	2	19	39	2	19
Daily infused fatty acids (g/d) at the lactation dosage[7]							
18:3 *cis*-9,*cis*-12,*cis*-15	0.00	39.9	0.01	0.00	39.9	0.01	0.00
18:2 *cis*-9,*cis*-12	1.39	12.4	2.48	1.34	12.4	2.48	1.34
18:2 *cis*-9,*trans*-11	0.00	0.00	0.01	10.3	0.00	0.01	10.3
18:2 *trans*-10,*cis*-12	0.00	0.02	0.01	10.2	0.02	0.01	10.2

[1]Cows were supplemented daily with coconut oil (CTRL), linseed and safflower oil (EFA), Lutalin® (CLA, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany), or EFA+CLA.
[2]Addition of vitamin E (0.06 g/d), Covitol 1360 (BASF SE), to compensate for the vitamin E in linseed oil (0.07%) and safflower oil (0.035%).
[3]Sanct Bernhard, Bad Ditzenbach, Germany.
[4]DERBY, Derby Spezialfutter GmbH, Münster, Germany.
[5]GEFRO, Memmingen/Allgäu, Germany.
[6]BASF SE.
[7]The lactation dosage was halved during the dry period.

3.3.2 Feeding, Feed Samples and Analyses, and Body Condition

Cows were fed with corn silage–based TMR during lactation (wk −22 to −7 AP and wk 1 to 9 PP) and during the dry period (wk −6 to −1 AP). Diets were fed ad libitum at 0600 h, and the cows had free access to water as well as trace-mineralized salt blocks. After calving, a calcium bolus (Rumin Ca^{DL}; Wirtschaftsgenossenschaft Deutscher Tierärzte eG, Garbsen, Germany) as well as 300 mL/d of 1,2-propanediol (Propylenglykol USP; Dr. Pieper Technologie- und Produktentwicklung GmbH, Wuthenow, Germany) were administered intraruminally on 3 consecutive days. Feed samples of TMR and corn silage were taken weekly, and samples from concentrates and straw were taken every 2 mo for the determination of DM content. Additional samples of single components were stored at −20°C, and nutrient compositions were determined at the Agricultural Analysis and Research Institute (LUFA), Rostock, Germany. Based on analysis of the individual TMR components, the compositions of the lactation and dry period diets were formulated and calculated according to the feeding standards of the German Society of Nutrition Physiology (Gesellschaft für Ernährungsphysiologie 2001; 2008; 2009) and the German Agricultural Society (Deutsche Landwirtschaftliche Gesellschaft; DLG 2013). The ingredients and chemical compositions of the diets with a planned low fat content are shown in **Table 3.2**. The FA compositions of the diets were determined via GC and are shown in **Table 3.3**. For extraction and direct FA methylation of diets, a modified method from Sukhija and Palmquist (1988) using 5% methanolic HCl and 6% K_2CO_3 solution was applied. The FA analysis of the FAME was performed using capillary GC with a CP-Sil 88 CB column (100 m × 0.25 mm; Agilent, Santa Clara, CA; Kalbe et al. 2019). Individual daily feed intake was recorded as disappearance of feed from troughs connected to an electronic scale to which access was controlled by an individual transponder (Institute for Agricultural Engineering and Animal Husbandry ILT, Bavarian State Research Center for Agriculture LfL, Freising, Germany).

Table 3.2 Ingredients and chemical compositions of the diets

	Diet	
Item (g/kg of DM)	**Lactation**	**Dry period[1]**
Ingredients		
Corn silage	457	421
Straw	97	223
Compound feed DEFA[2] (granulated)	446	–
Dried sugar beet pulp	–	163
Extracted soybean meal	–	99
Grain of rye	–	75
Mineral-vitamin mixture[3]	–	10
Urea[4]	–	9
Chemical composition		
NE_L (MJ/kg DM)[5]	7.1	6.5
Crude fat	23	21
Crude fiber	173	219
Crude protein	146	141
Utilizable protein[5]	143	141
NFC	432	379
NDF	346	423
ADF	197	249
RNB[5, 6]	0.5	0.0

[1]The dry period diet was fed from wk 6 to wk 1 before calving.
[2]Ceravis AG, Malchin, Germany. Ingredients: 46.5% dried sugar beet pulp, 25.3% extracted soybean meal, 23.8% grain of rye, 1.4% urea, 1.1% premix cow, 1.00% calcium, 0.37% phosphorus, 0.42% sodium, vitamins A, D3, E, copper, ferric, zinc, manganese, cobalt, iodine, selenium. Chemical composition: 44.4% NFC, 24.1% crude protein, 21.6% NDF, 12.4% ADF, 9.3% crude fiber, 8.2% crude ash, 1.8% crude fat, 7.9 MJ NE_L/kg DM.
[3]KULMIN®MFV Plus (Bergophor Futtermittelfabrik Dr. Berger GmbH & Co. KG, Kulmbach, Germany): 8.5% magnesium, 7.5% phosphorus, 6.5% sodium, 3.5% HCl-insoluble ash, 1.5% calcium; additives and trace minerals per kg: 1,000,000 I.E. vitamin A, 200,000 I.E. vitamin D_3, 10,000 mg vitamin E, 180 mg vitamin B_1, 90 mg vitamin B_2, 90 mg vitamin B_6, 200 mg vitamin B_5, 2500 mg vitamin B_3, 675 mg vitamin B_{12}, 12 mg vitamin B_9, 100 mg vitamin H, 2500 mg zinc, 3500 mg manganese, 500 mg copper, 20 mg cobalt, 75 mg iodine, 30 mg selenium as sodium selenite, 15 mg *Saccharomyces cerevisiae.*
[4]Piarumin® (SKW Stickstoffwerke Piesteritz GmbH, Lutherstadt Wittenberg, Germany): 99% urea, 46.5% total nitrogen.
[5]German Society of Nutrition Physiology (2001; 2008; 2009) and DLG (2013).
[6]RNB = ruminal nitrogen balance.

Table 3.3 Fatty acid composition of the experimental diets

	Diet	
Fatty acid (g/kg of DM)	**Lactation**	**Dry period[1]**
10:0	0.01	0.01
12:0	0.04	0.03
14:0	0.12	0.18
15:0	0.04	0.04
16:0	4.73	4.53
16:1, *cis*-9	0.06	0.05
17:0	0.09	0.08
17:1, *cis*-9	0.01	0.01
18:0	0.63	0.60
18:1, *cis*-9	4.82	3.84
18:1, *cis*-11	0.28	0.21
18:2, *cis*-9,*cis*-12	9.63	9.32
18:3, *cis*-9,*cis*-12,*cis*-15	1.35	1.37
18:4, *cis*-6,*cis*-9,*cis*-12,*cis*-15	0.04	0.02
20:0	0.15	0.16
20:1, *cis*-11	0.08	0.06
20:2, *cis*-11,*cis*-14	0.05	0.02
21:0	0.01	0.02
22:0	0.18	0.25
22:1, *cis*-13	0.01	–
22:2, *cis*-13,*cis*-16	0.01	0.04
23:0	0.05	0.02
24:0	0.23	0.29
SFA[2]	6.27	6.21
MUFA[3]	5.27	4.17
PUFA[4]	11.08	10.77
Sum of n-3 fatty acids[5]	1.39	1.39
Sum of n-6 fatty acids[6]	9.69	9.38
Ratio of n-6/n-3	7.00	6.76

[1]The dry period diet was fed from wk 6 to 0 before calving.
[2]Sum of 10:0; 12:0; 14:0; 15:0; 16:0; 17:0; 18:0; 20:0; 21:0; 22:0; 23:0 and 24:0.
[3]Sum of 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 20:1 *cis*-11 and 22:1 *cis*-13.
[4]Sum of 18:2 *cis*-9,*cis*-12; 18:3 *cis*-9,*cis*-12,*cis*-15; 18:4 *cis*-6,*cis*-9,*cis*-12,*cis*-15; 20:2 *cis*-11,*cis*-14 and 22:2 *cis*-13,*cis*-16.
[5]Sum of 18:3 *cis*-9, *cis*-12, *cis*-15 and 18:4 *cis*-6, *cis*-9, *cis*-12, *cis*-15.
[6]Sum of 18:2 *cis*-9, *cis*-12; 20:2, *cis*-11, *cis*-14 and 22:2 *cis*-13, *cis*-16.

The feed efficiency for milk production (**FE_{MY}**) was calculated as kilograms of milk per kilograms of DMI and feed efficiency for ECM production (**FE_{ECM}**) as kilograms of ECM per kilograms of DMI (Moallem 2016). According to the German Society of Nutrition Physiology (2001), the following formula was used to calculate the EB:

$$\text{EB MJ NE}_L\text{/d} = \text{NE}_L \text{ intake} - \text{NE}_L \text{ maintenance} - \text{NE}_L \text{ gestation} - \text{NE}_L \text{ milk production},$$

$$\text{NE}_L \text{ intake (MJ of NE}_L\text{/d)} = \text{kg of DMI} \times \text{MJ of NE}_L\text{/kg of DM} + \text{energy content provided by the supplements},$$

$$\text{NE}_L \text{ maintenance (MJ of NE}_L\text{/d)} = 0.293 \text{ MJ of NE}_L \times \text{kg of BW}^{0.75},$$

$$\text{NE}_L \text{ gestation (MJ of NE}_L\text{/d)} = 0.044 \times e^{0.0165 \times t} \text{ MJ of NE}_L, \text{ where t is the day of gestation, and}$$

$$\text{NE}_L \text{ milk production (MJ of NE}_L\text{/d)} = \text{kg of ECM} \times 3.14 \text{ MJ of NEL}.$$

Body weight, BCS, and back fat thickness (**BFT**) were measured after the morning milking once per week. The BCS was scored based on a 5-point scale according to Edmonson et al. (1989), and BFT was determined via ultrasonic measurements (SonoSite Titan; Fujifilm SonoSite Inc., Bothell, WA) described by Schröder and Staufenbiel (2006).

3.3.3 Milk Sampling and Analyses

Cows were milked twice daily at 0630 and 1800 h, and milk yield was recorded electronically after each milking. Colostrum samples from the first milking and pooled milk samples from one evening and the successive morning milking were taken weekly during late and early lactation and analyzed by the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg-Vorpommern e.V. (Güstrow, Germany). Determination of milk protein, milk fat, and milk lactose was performed using an infrared spectrophotometric method (MilkoScan FT6000, Foss GmbH, Hamburg, Germany) and SCC by a fluorescence-optical counting system (Fossomatic FC, Foss GmbH). According to Reist et al. (2003), the following formula was used to calculate the ECM:

$$\text{ECM (kg)} = (0.038 \times \text{g of crude fat} + 0.024 \times \text{g of CP} + 0.017 \times \text{g of lactose}) \times \text{kg of milk}/3.14.$$

Colostrum and milk were centrifuged at 50,000 × *g* (4°C, 20 min), and milk fat was removed. In colostrum samples protein was precipitated with 1.5 *M* perchloric acid and 2 *M* calcium carbonate and centrifuged at 13,000 × *g* (4°C, 10 min). The whey was stored at −20°C until the milk urea content was determined weekly during lactation by photometric measurements (ABX Pentra 400; Horiba ABX SAS, Montpellier, France) using the kit #LT-UR 0010 from Labor+Technik, Eberhard Lehmann GmbH (Berlin, Germany). The citrate concentration in milk samples was determined at wk −10 and −7 AP and weekly PP at the Institut für Analytik, Hygiene und Produktqualität (MQD, Güstrow, Germany) using a commercial enzymatic kit (#10139076035) from R-Biopharm AG (Darmstadt, Germany). Milk acetone was determined

weekly PP at MQD by the Skalar method using a continuous flow analyzer (SAN++, Skalar Analytic GmbH, Erkelenz, Germany), following the procedure described by de Roos et al. (2007). The FA composition of milk fat was determined in milk samples from wk −10 and −7 AP, as well as in first colostrum milking after calving and in milk samples from wk 4 and 8 PP at the Bavarian Center for Biomolecular Mass Spectrometry (BayBioMS), Technical University of Munich (Freising, Germany). Single FA in milk fat were determined via lipid extraction and gas chromatography (Agilent CP7420, select FAME 100 × 0.25-mm × 0.25-µm column) with flame ionization detection, as described by Firl et al. (2014). Methylation was performed by the trimethylsulfonium hydroxide method. The apparent transfer efficiencies of ALA and *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA were estimated by dividing the amount of FA in milk fat (minus the CTRL yields) by the amounts of the infused FA (Moallem et al. 2012). Fatty acids representing the product and substrate for Δ^9-desaturase were used to calculate the desaturase indexes (Castañeda-Gutiérrez et al. 2005).

3.3.4 Blood and Liver Sampling and Analyses

Blood samples were taken on d 63, 42, 35, 28, 21, and 10 before expected calving, on d 1 PP, and then once weekly up to d 56 immediately after morning milking before feeding, via jugular vein puncture using the Vacuette system (Greiner Bio-One International GmbH, Kremsmünster, Austria). Samples were immediately placed on ice and centrifuged within 30 min (at 1,565 × *g* for 20 min at 4°C), and the harvested plasma was stored at −20°C until analysis. Evacuated tubes containing sodium fluoride in combination with potassium oxalate as an anticoagulant were used to measure the plasma concentrations of NEFA, TG, low-density lipoprotein cholesterol (**LDL-C**), high-density lipoprotein cholesterol (**HDL-C**), and total cholesterol (**TC**). Plasma metabolites were analyzed using an automatic spectrophotometer (ABX Pentra 400; Horiba) and respective kits: NEFA, #434 91795 (acyl-CoA synthetase−acyl-CoA oxidase method) from Wako Chemicals GmbH (Neuss, Germany); TG, #A11A01640 (lipoprotein lipase–glycerin kinase−glycerin-phosphate-oxidase method), LDL-C, #A11A01638 (direct measurement of cholesterol in LDL by the cholesterol esterase and cholesterol oxidase and LDL cleavage), and HDL-C, #A11A01636 (direct measurement of cholesterol in HDL by accelerator selective detergent method with cholesterol esterase) from Horiba; and TC, #553-126 (cholesterol oxidase method) from mti Diagnostics GmbH (Idstein, Germany).

Liver tissue samples were obtained by needle biopsy on d −63 and −21 AP, on d 1 and 28 PP, and during slaughter on d 63 PP, as previously described (Weber et al. 2013), to measure the TG concentration. Liver TG concentration was determined using a Triglyceride Quantification Fluorometric Kit (#MAK266, Merck, Darmstadt, Germany).

3.3.5 Slaughtering and Body Composition

The cows were slaughtered on d 63 PP in the experimental abattoir of the FBN, which was approved by the EU and the German quality management system QS (QS Qualität und Sicherheit GmbH, Bonn, Germany). After morning milking, cows were weighed, transported to the slaughter facilities, stunned with a bolt gun, and exsanguinated. The head, mammary gland, feet, and skin with the tail were detached first. Thereafter, the mammary gland was weighed. The full gastrointestinal tract was removed, and the liver, kidneys, spleen, pancreas, and retroperitoneal adipose depot were dissected and weighed. Adherent mesenteric fat at the intestine and the omental adipose depot were cut off and weighed. Subcutaneous adipose tissue depots (from the sternum and perineal fat) were manually dissected and weighed. The hot and cold carcass weights were measured as described by Pfuhl et al. (2007). Total fat was calculated as the sum of the omental, mesenteric, retroperitoneal, and subcutaneous fat.

3.3.6 Statistical Analyses

Statistical analyses were performed using SAS for Windows, release 9.4 (SAS Institute Inc. Cary, NC). Performance data and plasma concentrations of metabolites were analyzed using the MIXED procedure by repeated-measures ANOVA containing EFA (level: yes, no), CLA (level: yes, no), time (levels: day or week relative to calving), block (levels: 1 to 5), and the respective interactions (EFA × CLA; EFA × time; CLA × time; EFA × CLA × time) as fixed effects. The calving interval and projected milk yield during the second lactation were used as covariates. Repeated measures on each cow were considered by using the repeated statement of the MIXED procedure with compound symmetry (timeline d or wk) covariance structure. The levels of the repeated variable time for performance data were late lactation (wk −10 to −7 AP), dry period (wk −6 to −1 AP), transition period (wk −3 AP to 4 PP), postpartum or early lactation (wk 1 to 8 PP), and entire period (wk −10 AP to 8 PP). Alternatively, for the metabolites, the AP period (d −63 to −1 AP) was evaluated. Data were analyzed for each observation period separately. The least squares means (**LSM**) and their standard errors (**SE**) were computed for each fixed effect in the ANOVA model to display the results. Additionally, group differences in these LSM were tested using the Tukey-Kramer procedure. The SLICE statement of the MIXED procedure was used to assess partitioned analyses of the LSM for interactions. All differences with $P < 0.05$ were considered significant.

3.4 Results

3.4.1 Animal Performance

In late lactation, DMI declined ($P < 0.05$) in CLA-treated cows from wk −10 to wk −8 AP by 7% and tended to be lower ($P < 0.1$) in wk −8 in CLA- than non-CLA-treated cows. (CLA × time interaction: $P = 0.06$; **Figure 3.1A**). After drying off, DMI decreased ($P < 0.001$) and after calving increased ($P < 0.001$) in all groups. At wk 7 and 8 PP, DMI was lower ($P < 0.05$) in CLA than in non-CLA-treated groups. The NE_L intake (**Table 3.4**) showed a similar time pattern to that of DMI, and PP NE_L intake was lower ($P < 0.05$) in CLA than that in EFA (wk 7) and in CTRL (wk 8). In late lactation, milk yield (**Figure 3.1B**) declined ($P < 0.001$) in all groups but not in EFA, and after parturition, milk yield increased ($P < 0.001$) without significant group differences. In late lactation, EB was higher ($P < 0.05$) in the EFA+CLA cows (wk −8 AP) and CLA cows (wk −7 AP; CLA effect, $P < 0.05$) than that in EFA cows. During early lactation, EB increased ($P < 0.001$) in all groups and was less negative ($P < 0.01$) in both CLA-treated groups than in CTRL and EFA (**Figure 3.1C**). Energy-corrected milk decreased during late lactation ($P < 0.05$) in CLA and EFA+CLA cows, was affected by CLA treatment ($P < 0.05$), and was lower in wk −8 AP in EFA+CLA than in EFA and CTRL cows and, in wk −7 AP, was lower in CLA and EFA+CLA cows than in EFA cows (**Figure 3.1D**). In early lactation, ECM increased ($P < 0.05$) from wk 1 to wk 2 in all groups and was reduced ($P \leq 0.01$) in CLA and EFA+CLA compared with the CTRL and EFA groups. The FE_{MY} showed a similar time pattern to that of milk yield and FE_{ECM} as ECM (**Table 3.4**). In late lactation, FE_{MY} declined more (CLA × time interaction $P < 0.001$) in CLA-treated groups compared with non-CLA-treated groups, and FE_{ECM} was higher in EFA ($P < 0.05$) compared with EFA+CLA (wk −8 and −7 AP) and CLA (wk −7 AP). In early lactation, FE_{ECM} was lower ($P < 0.001$) in CLA-treated than in non-CLA-treated groups.

During the last 10 wk of gestation, all groups gained similar BW (time: $P < 0.001$), BFT (time: $P < 0.001$), and BCS (time: $P < 0.05$; **Table 3.4**), reaching the same levels at calving. After calving, BCS and BFT decreased ($P < 0.001$) continuously, but BW rapidly decreased ($P < 0.001$) in all groups up to wk 3 and declined to a lesser extent until the end of the study. In wk 6 PP, we detected a CLA effect on BW reduction, and the decline of BW relative to wk −1 AP was less ($P < 0.05$) in the CLA group compared with CTRL. In wk 7 and 8 PP, BFT was higher ($P < 0.05$) in CLA- than in non-CLA-treated groups.

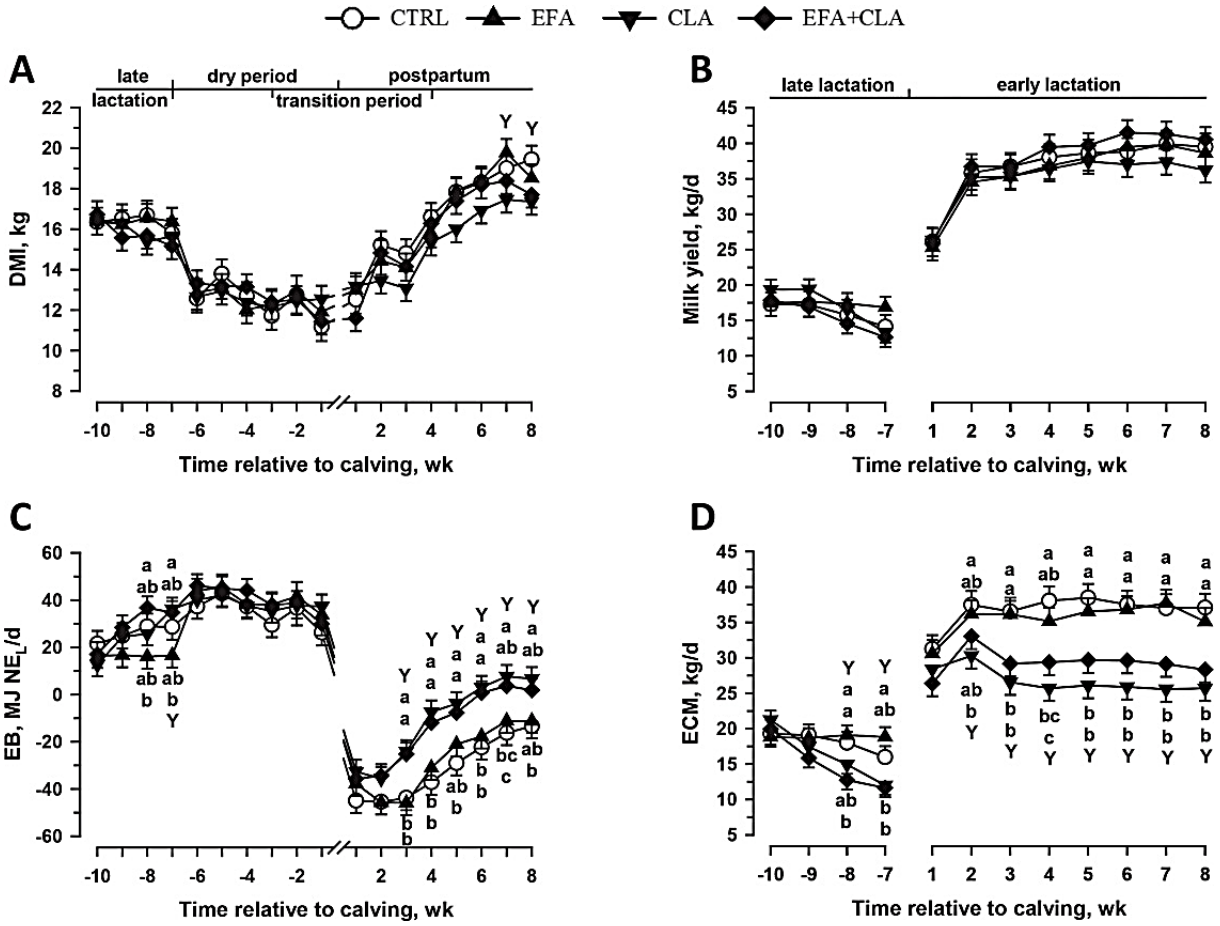

Figure 3.1 DMI (A), milk yield (B), energy balance (EB; C), and ECM yield (D) in cows supplemented abomasally daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; n = 10), or EFA+CLA (◆; n = 10) from wk 9 antepartum until wk 8 postpartum.
Data are presented as LSM ± SE; LSM with different lower-case letters (a–c) differ ($P < 0.05$) at the respective time point. Y = CLA effect at respective time point. Significant ($P < 0.05$) effects for DMI during late lactation (time), dry period (time), transition (time; EFA × CLA × time interaction), postpartum (time; CLA), and during the entire study (time). Significant ($P < 0.05$) effects for milk yield during late lactation (time; CLA × time interaction) and early lactation (time). Significant ($P < 0.05$) effects for energy balance during late lactation (time; CLA × time interaction), dry period (time), transition (time; CLA; CLA × time interaction), postpartum (time; CLA), and during the entire study (time; CLA; CLA × time interaction). Significant ($P < 0.05$) effects for ECM during late lactation (time; CLA; EFA × time interaction; CLA × time interaction) and early lactation (time; CLA; CLA × time interaction).

Table 3.4 Performance data during late lactation, dry and transition periods, postpartum or early lactation, and over the entire study of cows supplemented abomasally daily with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10), or the combination (EFA+CLA; n = 10) from wk 9 antepartum until wk 8 postpartum

		Treatment				Fixed effect, *P*-value					
Variable[2]	Time	CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA × CLA	Time	EFA × time	CLA × time
NE_L intake,	Late lactation	120.2 ± 4.6	116.7 ± 4.2	114.2 ± 3.9	113.8 ± 3.9	0.7	0.3	0.7	0.12	0.6	0.05
MJ NE_L/d	Dry period	80.6 ± 3.1	81.9 ± 1.9	81.4 ± 2.8	84.1 ± 2.8	0.5	0.6	0.8	0.001	0.7	0.5
	Transition period	93.9 ± 3.3	93.6 ± 3.1	91.2 ± 2.9	93.4 ± 2.9	0.8	0.6	0.7	0.001	0.9	0.6
	Postpartum	120.8 ± 3.8	119.4 ± 3.7	109.8 ± 3.5	115.2 ± 3.5	0.6	0.04	0.3	0.001	0.5	0.4
	Entire Study	106.6 ± 3.3	106.4 ± 3.1	101.2 ± 3.0	104.6 ± 2.9	0.6	0.2	0.6	0.001	0.9	0.05
FE_{MY},	Late lactation	0.96 ± 0.11	1.10 ± 0.10	1.11 ± 0.09	0.98 ± 0.09	0.9	0.9	0.19	0.001	0.06	0.01
kg milk/kg DMI	Early lactation	2.25 ± 0.10	2.25 ± 0.10	2.37 ± 0.09	2.43 ± 0.09	0.7	0.13	0.7	0.001	0.5	0.9
FE_{ECM},	Late lactation	1.08 ± 0.10	1.19 ± 0.09	1.05 ± 0.08	0.95 ± 0.09	0.9	0.13	0.2	0.001	0.04	0.001
kg ECM/kg DMI	Early lactation	2.31 ± 0.11[a]	2.28 ± 0.10[a]	1.84 ± 0.10[b]	1.95 ± 0.10[ab]	0.7	0.001	0.5	0.001	1.0	0.6
BW, kg	Late lactation	701 ± 21	666 ± 20	676 ± 19	670 ± 19	0.3	0.6	0.5	0.001	0.9	0.12
	Dry period	742 ± 22	700 ± 21	710 ± 20	718 ± 20	0.4	0.7	0.2	0.001	0.3	0.4
	Transition period	690 ± 20	654 ± 19	664 ± 18	672 ± 18	0.5	0.8	0.3	0.001	0.7	0.4
	Postpartum	634 ± 18	604 ± 18	622 ± 17	621 ± 17	0.4	0.9	0.4	0.001	0.8	0.16
	Entire Study	685 ± 20	649 ± 19	663 ± 18	665 ± 18	0.4	0.9	0.3	0.001	0.8	0.04
BCS	Late lactation	3.62 ± 0.11	3.50 ± 0.11	3.48 ± 0.10	3.29 ± 0.10	0.16	0.10	0.7	0.001	0.4	0.9
	Dry period	3.72 ± 0.12	3.73 ± 0.12	3.62 ± 0.11	3.62 ± 0.11	1.0	0.4	0.9	0.001	0.1	0.02
	Transition period	3.54 ± 0.12	3.55 ± 0.11	3.50 ± 0.11	3.50 ± 0.11	1.0	0.7	1.0	0.001	0.7	0.8
	Postpartum	3.12 ± 0.11	3.13 ± 0.11	3.15 ± 0.10	3.10 ± 0.10	0.8	1.0	0.8	0.001	0.7	0.19
	Entire Study	3.43 ± 0.11	3.41 ± 0.10	3.38 ± 0.10	3.31 ± 0.10	0.7	0.5	0.8	0.001	0.11	0.02

Table 3.4 Continuation

Variable[2]	Time	Treatment				Fixed effect, *P*-value					
		CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA × CLA	Time	EFA × time	CLA × time
BFT, mm	Late lactation	13.4 ± 1.0	12.2 ± 0.9	12.0 ± 0.9	11.3 ± 0.9	0.3	0.2	0.8	0.001	0.18	0.6
	Dry period	15.3 ± 1.1	14.3 ± 1.0	15.8 ± 1.0	14.6 ± 1.0	0.3	0.7	0.9	0.001	0.5	0.4
	Transition period	14.7 ± 1.1	14.2 ± 1.0	15.5 ± 1.0	14.5 ± 1.0	0.5	0.6	0.8	0.001	0.9	0.5
	Postpartum	12.1 ± 1.0	11.7 ± 0.9	13.5 ± 0.9	12.6 ± 0.9	0.5	0.2	0.8	0.001	0.9	0.001
	Entire Study	13.5 ± 1.0	12.7 ± 0.9	14.0 ± 0.9	13.0 ± 0.9	0.3	0.7	0.9	0.001	0.8	0.001

[a,b]Means within a row with different lowercase superscripts differ ($P < 0.05$).
[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE. FE_{MY} = feed efficiency for milk production; FE_{ECM} = feed efficiency for ECM production; BFT = back fat thickness.

3.4.2 Milk Composition

Cows receiving CLA showed reduced milk fat concentration ($P < 0.001$; **Figure 3.2A**) compared with the concentration in the CTRL and EFA groups, by an average reduction of 40% AP and 50% PP. After calving, a decrease in milk fat concentration was found until wk 2 in all groups ($P < 0.001$), and the milk fat concentration continued to decrease in both CLA-treated groups until wk 4 PP and remained at that low concentration until the end of the study. Milk fat yield declined ($P < 0.001$) in the CLA groups and was reduced ($P < 0.001$) in both CLA groups in late lactation (wk −9 to −7 AP) by more than 50% and in early lactation on average by 50% compared with the CTRL and EFA groups (**Table 3.5**). The milk citrate concentration increased ($P < 0.05$) during late lactation in CLA groups and showed higher concentration in CLA than non-CLA-treated groups ($P < 0.05$; **Figure 3.2B**). During early lactation, milk citrate decreased ($P < 0.05$) in cows not treated with CLA. Milk citrate was higher ($P < 0.05$) in CLA than in non-CLA-treated groups during the whole PP period, was higher ($P < 0.05$) in CLA-treated than in EFA and CTRL cows in wk 2, 5, 7, and 8 PP, and was highest in CLA cows in wk 6 PP. Milk acetone indicated a CLA effect ($P < 0.05$) and increased the highest ($P < 0.001$) in EFA+CLA at 2 wk PP (**Table 3.5**). Milk protein concentration during late lactation increased in both CLA groups more than in CTRL and EFA (CLA × time, $P < 0.05$), and in wk −7 AP the milk protein concentration was higher ($P < 0.05$) in EFA+CLA and CLA cows than in EFA cows (**Figure 3.2C**). After calving we detected a CLA effect ($P < 0.05$) for the whole period, and in wk 1 protein concentration was lower ($P < 0.05$) in both CLA groups than in EFA. In addition, we detected a CLA effect with lower milk protein concentration in CLA- than in non-CLA-treated groups in wk 7 PP. The milk urea concentration peaked before drying off ($P < 0.001$) in all groups (**Figure 3.2D**). During early lactation, urea in milk decreased ($P < 0.05$) in all groups but not in EFA cows. Milk urea was reduced ($P < 0.05$) by CLA treatment ($P < 0.05$) during the whole period and was affected by EFA × time interaction ($P < 0.05$). Milk urea concentration in EFA was higher than in both CLA groups in wk 4 PP, was higher than in CLA in wk 7 PP, and was higher than in CLA and CTRL in wk 8 PP. Lactose concentration and yield (kg/d) decreased in late lactation ($P < 0.01$) and increased after the onset of lactation until wk 3 PP (**Table 3.5**). In late lactation, lactose yield declined more distinctly (CLA × time interaction: $P < 0.001$) in CLA than in non-CLA-treated groups, and lactose concentration in wk −7 AP was lower ($P < 0.05$) in CLA- than in non-CLA-treated groups. During early lactation, the lactose concentration in wk 8 was higher ($P < 0.05$) in EFA- than in non-EFA-treated groups. The SCC increased ($P < 0.05$) in the CLA-treated groups before drying off, was higher ($P < 0.05$) in CLA-treated than in non-CLA-treated groups at wk −7, and remained unchanged in early lactation in all groups (**Table 3.5**).

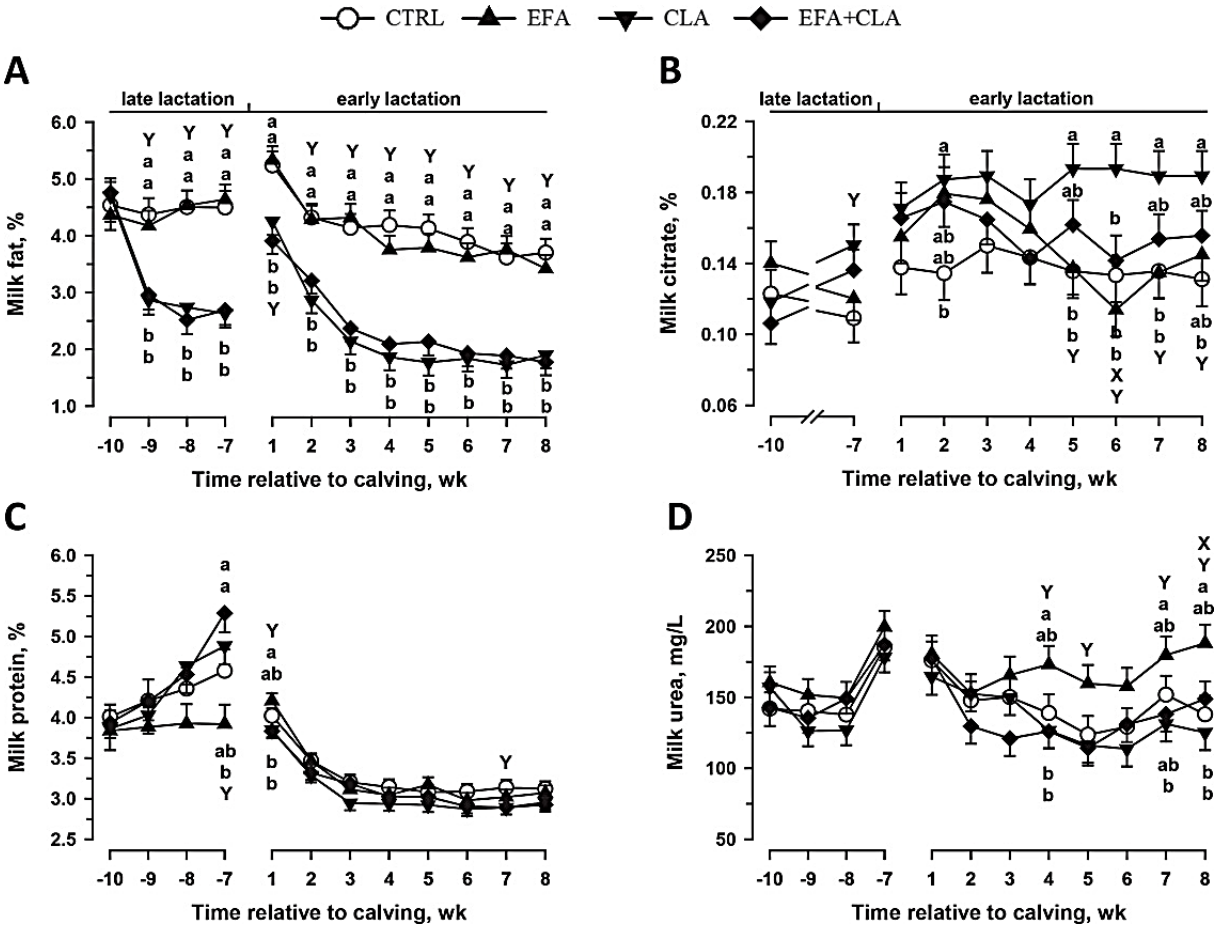

Figure 3.2 Milk fat concentration (A), milk citrate concentration (B), milk protein concentration (C), and milk urea concentration (D) in cows supplemented abomasally daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; n = 10), or EFA+CLA (◆; n = 10) from wk 9 antepartum until wk 8 postpartum. Data are presented as LSM ± SE; LSM with different lowercase letters (a, b) differ ($P < 0.05$) at the respective time point. X = EFA effect at respective time point. Y = CLA effect at respective time point. Significant ($P < 0.05$) effects for milk fat concentration during late lactation (time; CLA; CLA × time interaction) and early lactation (time; CLA; CLA × time interaction). Significant ($P < 0.05$) effects for milk citrate concentration during late lactation (CLA × time interaction) and early lactation (time; CLA; EFA × time interaction). Significant ($P < 0.05$) effects for milk protein concentration during late lactation (time; CLA × time interaction) and early lactation (time; CLA). Significant ($P < 0.05$) effects for milk urea concentration during late lactation (time) and early lactation (time; CLA; EFA × time interaction).

Table 3.5 Milk components during late and early lactation of cows supplemented abomasally daily with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10), or the combination (EFA+CLA; n = 10) from wk 9 antepartum until wk 8 postpartum

		Treatment				Fixed effect, *P*-value					
Variable[1]	Time	CTRL	EFA	CLA	EFA+ CLA	EFA	CLA	EFA × CLA	Time	EFA × time	CLA × time
Milk fat, kg/d	Late lactation	0.72 ± 0.06^{ab}	0.78 ± 0.06^{a}	0.54 ± 0.05^{b}	0.51 ± 0.05^{b}	0.8	0.001	0.5	0.001	0.4	0.001
	Early lactation	1.48 ± 0.08^{a}	1.41 ± 0.08^{ab}	0.78 ± 0.07^{c}	0.86 ± 0.07^{c}	1.0	0.001	03	0.001	0.5	0.001
Milk acetone, mmol/L	Early lactation	0.05 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.13 ± 0.02	0.18	0.04	0.2	0.11	0.6	0.2
Milk protein, kg/d	Late lactation	0.67 ± 0.05	0.64 ± 0.05	0.72 ± 0.05	0.64 ± 0.05	0.3	0.7	0.6	0.001	0.13	0.18
	Early lactation	1.17 ± 0.05	1.15 ± 0.05	1.07 ± 0.04	1.17 ± 0.04	0.4	0.3	0.19	0.001	0.9	0.9
Milk lactose, %	Late lactation	4.71 ± 0.11	4.61 ± 0.10	4.51 ± 0.09	4.45 ± 0.09	0.4	0.06	0.8	0.002	0.5	0.3
	Early Study	4.78 ± 0.04	4.85 ± 0.04	4.73 ± 0.04	4.79 ± 0.04	0.1	0.2	0.8	0.001	0.7	0.3
Milk lactose, kg/d	Late lactation	0.75 ± 0.08	0.81 ± 0.07	0.79 ± 0.07	0.71 ± 0.07	0.9	0.6	0.4	0.001	0.05	0.001
	Early Study	1.76 ± 0.09	1.75 ± 0.08	1.67 ± 0.08	1.81 ± 0.08	0.4	0.9	0.4	0.001	0.06	0.8
SCC × 1000/mL	Late lactation	248 ± 193	400 ± 172	502 ± 163	454 ± 166	0.8	0.4	0.6	0.002	0.7	0.18
	Early Study	167 ± 82	224 ± 79	222 ± 75	203 ± 74	0.8	0.8	0.6	0.6	0.4	0.6

$^{a–c}$Means within a row with different lowercase superscripts differ ($P < 0.05$).
[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as the LSM ± SE

3.4.3 Milk Fatty Acid Pattern

The concentration of ALA in milk fat increased 5-fold in EFA and 12-fold in EFA+CLA after beginning of supplementation and was higher in both EFA groups than in CTRL and CLA ($P < 0.001$) before drying off and in early lactation. Enrichment of ALA was higher in EFA+CLA than in EFA at wk −7 AP and at wk 4 and 8 PP ($P < 0.001$; **Figure 3.3A**). During the whole supplementation period, the EPA and DPA concentrations in milk fat were higher ($P < 0.05$) in EFA and EFA+CLA than in CTRL and CLA (**Figure 3.3B** and **3.3C**). The EPA concentration was higher ($P < 0.05$) in wk −7 AP and wk 4 and 8 PP in EFA than in EFA+CLA; the DPA concentration was higher ($P < 0.05$) in wk −7 AP but lower in wk 1 ($P < 0.05$) in EFA than in EFA+CLA. The LA concentration in milk fat increased the most in EFA+CLA ($P < 0.001$; 1.9-fold from wk −10 to wk −7 AP) and was lowest in CTRL ($P < 0.01$) in late and early lactation (**Figure 3.3D**). The concentrations of ARA but not of dihomo-γ-linolenic acid (**DGLA**) in milk fat were higher ($P < 0.001$) before drying off in the CTRL and EFA groups than those in the CLA and EFA+CLA groups (**Figure 3.3E** and **3.3F**). The highest concentration of ARA was reached in the colostrum sample ($P < 0.001$), and the concentrations of ARA and DGLA were higher in CTRL and CLA (not significant for DGLA) than those in EFA and EFA+CLA (not significant for ARA) in colostrum ($P < 0.05$). In addition, DGLA concentrations were lower ($P < 0.05$) in EFA- than in non-EFA-treated groups in wk −7 AP and wk 4 PP, and ARA concentrations were lower in CLA- than in non-CLA-treated groups in wk 8 PP. Milk fat concentrations of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA increased ($P < 0.001$) in late lactation (2.1-fold for *cis*-9,*trans*-11 and 3.4-fold for *trans*-10,*cis*-12) and after calving (3.4-fold for *cis*-9,*trans*-11 and 2.8-fold for *trans*-10,*cis*-12) in both CLA-treated groups, and concentrations were higher in CLA-treated than in non-CLA-treated groups ($P < 0.001$; **Figure 3.3G** and **3.3H**). The FA composition in milk fat (%) and milk FA yield (g/kg of milk) for all analyzed FA are presented in **Table 3.8** and **Table 3.9**.

The apparent transfer efficiencies of ALA and *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA changed with time ($P < 0.001$), and the lowest enrichment of these FA was detected in wk 1 PP ($P < 0.001$; ALA 26 ± 5.6% in EFA+CLA and 15 ± 5.6% in EFA cows; *trans*-10,*cis*-12 CLA 4.3 ± 4.1% in CLA cows and 4.3 ± 3.5% EFA+CLA cows; *cis*-9,*trans*-11 CLA was close to 0 in both CLA groups). In wk −7 AP, the apparent transfer efficiency of ALA was lower ($P < 0.01$) in EFA+CLA (30 ± 5.6%) than in EFA (58 ± 5.9%), whereas in early lactation, the efficiencies were quite similar for EFA (wk 4: 66 ± 5.9%; wk 8: 60 ± 5.9%) and for EFA+CLA (wk 4: 65 ± 5.6%; wk 8: 51 ± 5.6%). The apparent transfer efficiency of *trans*-10,*cis*-12 CLA in the EFA+CLA and CLA groups was lower ($P < 0.05$) in wk −7 AP (12.9 ± 3.5 and 9.4 ± 3.5%; $P < 0.05$) compared with that in early lactation (wk 4: 33 ± 3.5 and 25 ± 3.5%; wk 8: 27 ± 3.5 and 22 ± 3.5%, respectively). The apparent transfer efficiency of *cis*-9,*trans*-11 in EFA+CLA

and CLA groups did not differ between wk −7 AP and wk 4 and 8 PP (17.3 ± 5.7% for EFA+CLA and 10.6 ± 5.7% for CLA).

The n-6/n-3 FA ratio in milk fat decreased with the start of supplementation in EFA and EFA+CLA ($P < 0.001$) and was lower in both EFA groups than in CTRL and CLA at wk 7 AP ($P < 0.001$). In early lactation, the ratio changed from 8.3 in CTRL to 1.9 in EFA and 1.4 in EFA+CLA ($P < 0.001$; **Table 3.7**). The concentration of FA synthesized *de novo* (<16 carbons) decreased ($P < 0.001$), and in accordance, the content of preformed FA (>16 carbons) increased ($P < 0.001$) in milk fat of cows supplemented with CLA (**Table 3.7**). In late lactation, the desaturase indexes of 14:1, 16:1, and 18:1 were higher ($P < 0.05$) in CLA than in EFA, whereas the same desaturase indexes were reduced in CLA and EFA+CLA ($P < 0.05$) compared with those in the CTRL group in early lactation (**Table 3.7**). The 18:2 *cis*-9,*trans*-11 CLA index was higher in the CLA and EFA+CLA groups ($P < 0.05$) than in CTRL and EFA in both lactation periods.

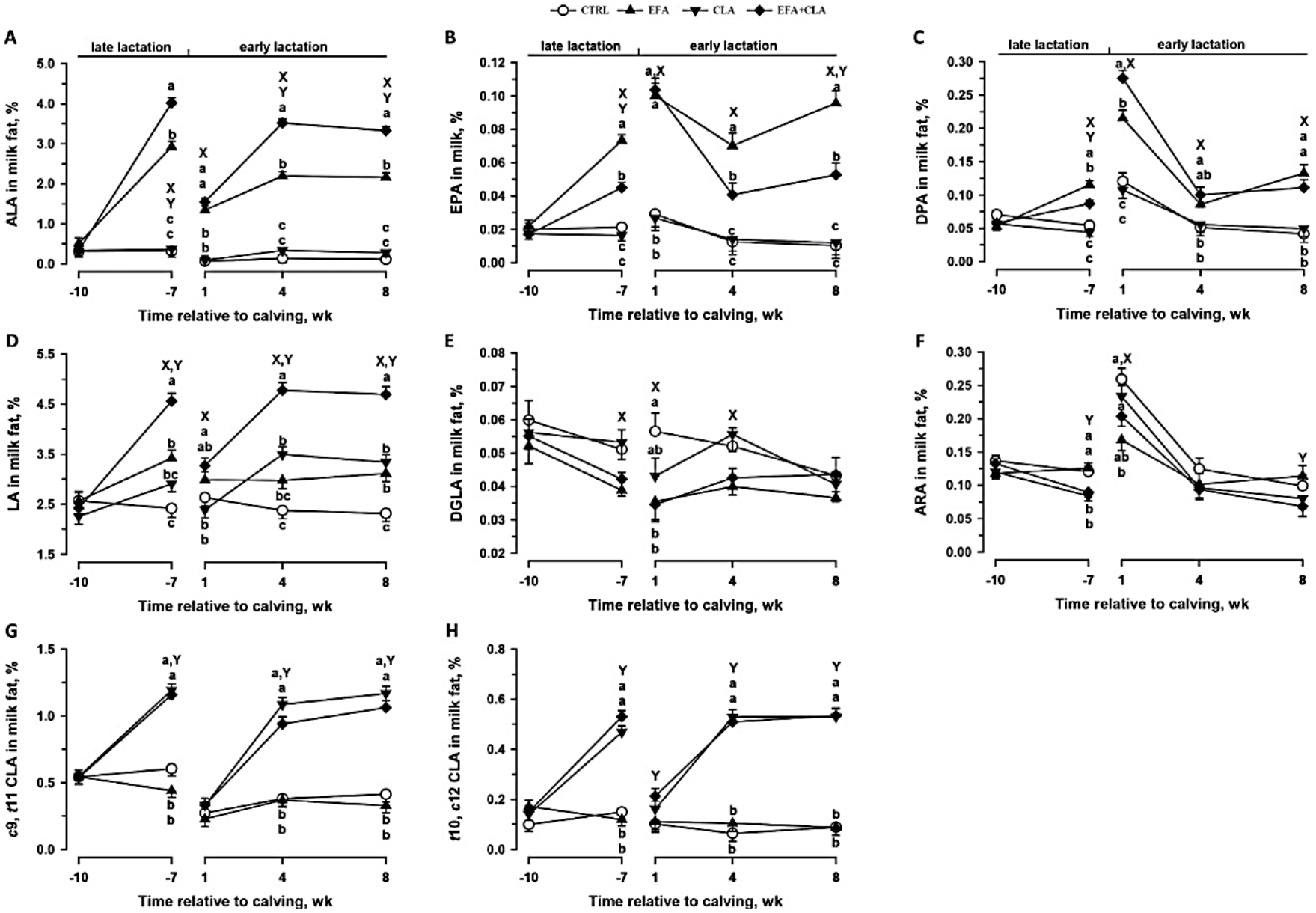
CTRL
EFA
CLA
EFA+CLA
A
ALA in milk fat, %
B
EPA in milk, %
C
DPA in milk fat, %
D
LA in milk fat, %
E
DGLA in milk fat, %
F
ARA in milk fat, %
G
c9, t11 CLA in milk fat, %
H
t10, c12 CLA in milk fat, %
late lactation
early lactation
Time relative to calving, wk

Figure 3.3 Milk fat concentrations of α-linolenic acid (ALA; A), eicosapentaenoic acid (EPA; B), docosapentaenoic acid (DPA; C), linoleic acid (LA; D), dihomo-γ-linolenic acid (DGLA; E), arachidonic acid (ARA; F), *cis*-9,*trans*-11 CLA (G), and *trans*-10,*cis*-12 CLA (H) in cows supplemented abomasally daily with either coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA *cis*-9,*trans*-11 and *trans*-10, *cis*-12; BASF SE, Ludwigshafen, Germany; n = 10), or EFA+CLA (◆; n = 10) from wk 9 antepartum until wk 8 postpartum.
Data are presented as the LSM ± SE. LSM with different superscripts (a–c) differ ($P < 0.05$) at the respective time point. X = EFA effect at respective time point. Y = CLA effect at respective time point. Significant ($P < 0.05$) effects for ALA concentration during late and early lactation (time; EFA; CLA; EFA × CLA; EFA × time; CLA × time interactions). Significant ($P < 0.05$) effects for EPA concentration during late and early lactation (time; EFA; CLA; EFA × CLA; EFA × time; CLA × time interactions). Significant ($P < 0.05$) effects for DPA concentration during late lactation (time; EFA; CLA; EFA × time; CLA × time interactions) and early lactation (time; EFA; EFA × time interaction). Significant ($P < 0.05$) effects for LA concentration during late and early lactation [time; EFA; CLA; EFA × CLA (only early lactation); EFA × time; CLA × time interactions]. Significant ($P < 0.05$) effects for DGLA concentration during late lactation (time) and early lactation (EFA). Significant ($P < 0.05$) effects for ARA concentration during late lactation (time; CLA; CLA × time interaction) and early lactation (time; EFA; EFA × time interaction). Significant ($P < 0.05$) effects for cis-9,trans-11 CLA and trans-10,cis-12 CLA concentrations during late and early lactation, respectively (time; CLA; CLA × time interaction).

3.4.4 Plasma Metabolites and Liver Triglycerides

Plasma concentration of NEFA increased rapidly ($P < 0.001$; **Figure 3.4A**) with the onset of lactation; showed a CLA effect and a CLA × time interaction during the transition, PP, and entire period; and was lower ($P < 0.05$) in both CLA-treated groups than in CTRL (d 21 and 28 PP) and EFA (d 21 PP). Concentration of NEFA at d −42 AP was lower ($P < 0.05$) in cows of both CLA groups than in CTRL and EFA cows. The plasma TG concentration was highest during the dry period and decreased after calving with ongoing lactation in all groups ($P < 0.001$; **Figure 3.4B**). We detected a CLA effect at d 14, 28, and 35 PP with lower ($P < 0.05$) TG concentration in CLA- than in non-CLA-treated groups. Liver TG increased in all groups after calving and decreased again until d 63 PP ($P < 0.001$; **Figure 3.4C**). The increase in liver TG was less pronounced following CLA and EFA+CLA supplementation on d 28 PP compared with the CTRL ($P < 0.01$) and EFA cows ($P < 0.05$).

Plasma concentrations of TC, LDL-C, and HDL-C decreased ($P < 0.001$; **Figure 3.5**) after drying off and rose in all groups ($P < 0.001$) after calving, with the highest concentrations seen at the end of the study. Time changes in TC concentration were affected by CLA treatment (CLA × time interaction AP, $P < 0.05$; CLA × time interaction PP, $P < 0.1$). The increase in plasma TC PP tended to be more pronounced in EFA+CLA than in EFA (EFA × CLA × time interaction; $P < 0.1$) and tended to be higher ($P = 0.06$) in EFA+CLA than in EFA on d 56 PP (**Figure 3.5A**). We detected a significant CLA effect ($P < 0.05$) for plasma LDL-C, and the concentration in EFA+CLA was higher ($P < 0.05$) than in EFA on d −42 AP, higher ($P < 0.05$) than in CTRL at 28 PP, and higher ($P < 0.05$) than in CTRL and EFA cows from d 42 to 56 PP (**Figure 3.5B**). The plasma concentration of HDL-C in CTRL was higher ($P < 0.05$) than in CLA on d 35, 42, and 56 and was higher ($P < 0.05$) than in EFA+CLA on d 42 (**Figure 3.5C**).

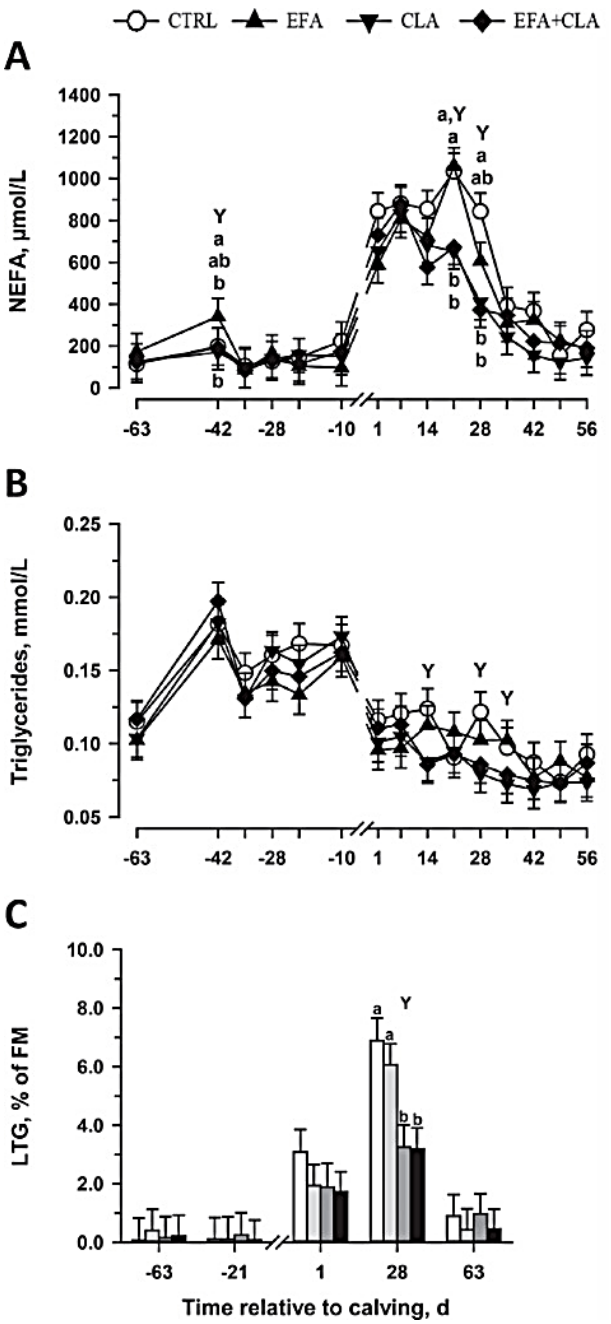

Figure 3.4 Plasma concentrations of nonesterified fatty acids (NEFA; A), triglycerides (TG; B), and liver triglycerides (LTG; C) in cows supplemented abomasally daily with coconut oil (○ CTRL; white bars in panel C; n = 9), linseed and safflower oil (▲ EFA; light gray bars in panel C; n = 9), Lutalin (▼ CLA *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; dark gray bars in panel C; n = 10), or EFA+CLA (◆; black bars in panel C; n = 10) from d 63 antepartum until d 56 postpartum.
Data are presented as LSM ± SE. LSM with different lowercase letters (a, b) differ ($P < 0.05$) at the respective time point. Y = CLA effect at respective time point. Significant ($P < 0.05$) effects for NEFA concentration during antepartum (time), transition (time; CLA; CLA × time interaction), postpartum (time; CLA; CLA × time interaction), and during the entire study (time; CLA; CLA × time interaction). Significant ($P < 0.05$) effects for TG concentration in plasma during antepartum, transition, postpartum, and during the entire study (time), respectively. Significant ($P < 0.05$) effects for TG concentration in liver during the entire study (time; CLA × time interaction).

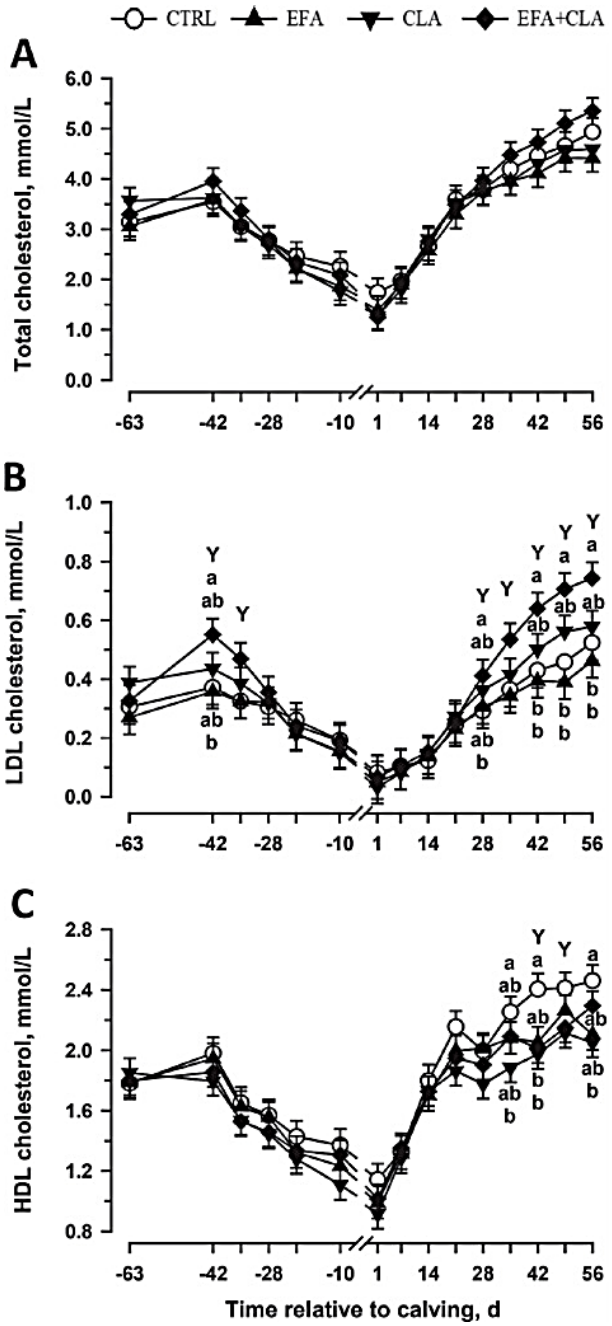

Figure 3.5 Plasma concentrations of total cholesterol (TC; A), low-density lipoprotein cholesterol (LDL; B), and high-density lipoprotein cholesterol (HDL; C) in cows supplemented abomasally daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; n = 10), or EFA+CLA (◆;n = 10) from d 63 antepartum until d 56 postpartum.
Data are presented as LSM ± SE. LSM with different lowercase letters (a, b) differ ($P < 0.05$) at the respective time point. Y = CLA effect at respective time point. Significant ($P < 0.05$) effects for total TC antepartum (time; EFA × time interaction; CLA × time interaction), transition (time), postpartum (time; CLA × time interaction), and during the entire study (time). Significant ($P < 0.05$) effects for LDL concentration antepartum (time; CLA × time interaction), transition (time), postpartum (time; CLA × time interaction), and during the entire study (time; CLA × time interaction). Significant ($P < 0.05$) effects for HDL antepartum (time), transition (time), postpartum (time), and during the entire study (time; EFA × CLA × time interaction).

Table 3.6 Body weight, hot carcass weight (HCW), cold carcass weight (CCW), organ weights, adipose depot weights, and their proportion of BW and total fat at slaughter, in cows daily abomasally supplemented either with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10), or the combination (EFA+CLA; n = 10) from wk 9 antepartum until slaughter on d 63 postpartum

	Treatment				*P*-values		
Variable[1]	**CTRL**	**EFA**	**CLA**	**EFA+CLA**	**EFA**	**CLA**	**EFA × CLA**
BW, kg	624 ±19	584 ±19	617 ±18	618 ±17	0.3	0.5	0.3
HCW, kg	260 ± 9	249 ± 9	266 ± 9	263 ± 9	0.4	0.2	0.6
CCW, kg	254 ± 9	243 ± 9	260 ± 9	258 ± 9	0.5	0.2	0.6
Liver, kg	11.0 ± 0.5	10.0 ± 0.5	10.3 ± 0.5	10.6 ± 0.5	0.5	1.0	0.2
Kidney (left and right), kg	1.89± 0.15	1.85± 0.14	1.58± 0.13	1.81± 0.13	0.5	0.2	0.3
Spleen, kg	0.98± 0.04	1.02± 0.04	1.00± 0.04	1.04± 0.04	0.3	0.6	1.0
Pancreas, kg	0.71± 0.05	0.62± 0.05	0.65± 0.05	0.65± 0.05	0.4	0.8	0.4
Mammary gland, kg	26.1 ± 1.4	24.7 ± 1.3	24.3 ± 1.2	26.1 ± 1.2	0.8	0.9	0.2
Subcutaneous fat							
Weight, kg	0.72± 0.12	0.92± 0.12	0.85± 0.11	0.97± 0.11	0.2	0.5	0.8
Proportion of BW, %	0.11± 0.02	0.16± 0.02	0.13± 0.02	0.15± 0.02	0.08	0.6	0.5
Proportion of total fat, %	4.92± 0.74	6.68± 0.71	4.37± 0.67	5.80± 0.67	0.03	0.3	0.8
Retroperitoneal fat							
Weight, kg	5.85± 0.85	5.43± 0.82	7.21± 0.78	7.22± 0.77	0.8	0.06	0.8
Proportion of BW, %	0.92± 0.12	0.91± 0.12	1.15± 0.11	1.15± 0.11	1.0	0.05	0.9
Proportion of total fat, %	39.8 ± 3.1	37.5 ± 2.9	36.5 ± 2.8	42.1 ± 2.8	0.6	0.8	0.18

Table 3.6 Continuation

Variable[1]	Treatment				*P*-values		
	CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA × CLA
Omental fat							
Weight, kg	4.62± 0.96	4.86± 0.92	7.17± 0.87	6.30± 0.87	0.7	0.03	0.5
Proportion of BW, %	0.72± 0.14[b]	0.83± 0.14[ab]	1.14± 0.13[a]	1.01± 0.13[ab]	0.9	0.03	0.4
Proportion of total fat, %	30.4 ± 1.9	32.9 ± 1.8	35.4 ± 1.7	33.7 ± 1.7	0.8	0.1	0.3
Mesenteric fat							
Weight, kg	3.75± 0.65	3.98± 0.67	4.78± 0.59	4.76± 0.63	0.9	0.16	0.8
Proportion of BW, %	0.59± 0.10	0.67± 0.10	0.75± 0.09	0.75± 0.09	0.7	0.19	0.7
Proportion of total fat, %	25.0 ± 2.7	26.1 ± 2.8	24.1 ± 2.5	21.3 ± 2.7	0.8	0.3	0.5
Total fat[2]							
Weight, kg	14.9 ± 2.4	16.6 ± 2.2	20.1 ± 1.9	18.5 ± 2.2	0.7	0.05	0.9
Proportion of BW, %	2.35± 0.29	2.44± 0.34	3.16± 0.32	2.96± 0.32	0.9	0.05	0.7

[a,b]Means within a row with different lowercase superscripts differ ($P < 0.05$).
[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE.
[3]Sum of subcutaneous, retroperitoneal, omental, and mesenteric fat.

3.4.5 Body Composition

The data regarding body composition and dissected fat depots are shown in **Table 3.6**. Body weight, hot carcass weight, and cold carcass weight, and weights of liver, kidneys, spleen, pancreas, and mammary gland did not differ between treatments. Body fat (absolute and relative to BW) was higher ($P < 0.05$) in CLA-treated than in non-CLA-treated cows. Omental and retroperitoneal fat (absolute weight and weight relative to BW) were higher ($P < 0.05$; trend for absolute retroperitoneal fat, $P < 0.1$) in CLA-treated than in non-CLA-treated groups, and omental fat relative to BW was higher ($P < 0.05$) in CLA than in CTRL cows. Subcutaneous fat (weight relative to BW and relative to total fat) was higher ($P < 0.05$ relative to total fat; $P < 0.1$ relative to BW) in EFA-treated than in non-EFA-treated groups.

3.5 Discussion

3.5.1 Animal Performance and Body Composition

An effect of EFA on DMI has been shown by previous investigations, which could not be observed in the present study (Drackley et al. 1992; Bremmer et al. 1998). The reduction in DMI known to occur with PUFA probably could not be recorded because of the moderate doses of linseed oil applied. Furthermore, effects of EFA on DMI were less when abomasal infusion of fat was provided as TG instead of as free FA (Litherland et al. 2005). In contrast, we detected a hypophagic effect of CLA at the end of the study; DMI and NE_L intake were lower in CLA-supplemented cows in early lactation. A reduction in DMI after CLA treatment has already been observed in other studies (Baumgard et al. 2000; Moallem et al. 2010; Schäfers et al. 2017). An important reason for the decrease in DMI and energy intake is certainly the reduction in energy requirement due to lower milk fat production and ECM in the CLA-treated cows, which additionally leads to a significantly improved EB in these cows. Voluntary feed intake decreased during CLA-induced milk fat depression (Harvatine et al. 2009). The CLA effect on EB during early lactation is inconsistent, and enhancing as well as lowering effects on EB were observed (Bernal-Santos et al. 2003; Moallem et al. 2010; Schäfers et al. 2017). The variation in DMI, milk production, and calculated EB due to CLA feeding might depend on the study design (e.g., the amount and time of *trans*-10,*cis*-12 CLA isomer fed). In the present study, the long infusion period of 18 wk certainly contributed to the hypophagic CLA effect.

Calculations of EB do not consider the CLA effects on body composition, presumably via reduced fat mobilization, or on inflammatory status that may result in changes in maintenance requirements and an improved tissue energy level after CLA supplementation (Trevisi et al. 2008; von Soosten et al. 2012). In the current study, improved EB in the CLA and EFA+CLA groups resulted in less BW reduction PP and more body and omental fat in CLA-supplemented

cows at the end of the study. On the other hand, CLA supplementation caused reduced body fat accretion in growing pigs (Ostrowska et al. 2003). However, in dairy cows an inhibitory effect of CLA on body fat accretion was not observed, and energy spared from reduction of milk fat synthesis is partitioned toward adipose tissue fat storage during short-term milk fat depression (Baumgard et al. 2002; Harvatine et al. 2009; von Soosten et al. 2012). Therefore, the huge milk fat depression and reduction in body fat mobilization due to CLA treatment supports enhanced accretion of body fat in these cows. Interestingly, the proportion of subcutaneous fat relative to total fat was higher and the subcutaneous fat relative to BW tended to be higher in EFA-treated groups. This may indicate less mobilization of subcutaneous fat compared with other fat depots after calving in cows supplemented with EFA. These findings were not supported by different changes in BFT or BCS among treatment groups PP. Therefore, EFA treatment may have affected the relative degree of fat mobilization in different fat depots but not the overall body fat mobilization.

Despite the reduction in milk fat and ECM in late lactation, we found only a weak effect of CLA feeding on EB due to a reduction of DMI in CLA-supplemented cows. Furthermore, we detected no increase in EB in the EFA group during late lactation, as ECM did not decline in this group in late lactation. The effects of linseed oil treatment on milk production and EB are inconsistent and may depend on the dosage, method of administration, and method of linseed processing; lactation stage could also bias the results (Zachut et al. 2010; Moallem 2018). However, linseed oil supplementation has possibly improved the persistence of milk production. Several studies have shown higher milk production according to linseed oil feeding (Petit et al. 2004; Hurtaud et al. 2010; Moallem 2018). However, to our knowledge, no study has addressed the effects of EFA supplementation on milk production before the onset of the dry period.

3.5.2 Milk Composition

The *trans*-10,*cis*-12 CLA isomer is responsible for milk fat reduction in the CLA and EFA+CLA groups (Baumgard et al. 2000). Milk fat reduction was particularly obtained by reduced *de novo* FA synthesis in the mammary gland, as indicated by the decrease in FA < C16 (Mackle et al. 2003; Harvatine and Bauman 2011). Because mammary epithelium is impermeable to citrate in both directions (Linzell et al. 1976), increased citrate in milk underlines the CLA-inhibiting effect on FA synthesis in the mammary gland. The citrate-isocitrate pathway is responsible for generating NADPH for *de novo* milk FA synthesis and is indirectly associated with FA synthesis in the mammary glands of ruminants; hence, elevated citrate concentrations in milk represent a decline in *de novo* fat synthesis (Mackle et al. 2003; Garnsworthy et al. 2006). The increased

milk citrate in the CLA group confirmed the results of Haubold et al. (2020). However, in contrast to the study of Haubold et al. (2020), elevation of milk citrate was weak in EFA+CLA despite significant milk fat reduction in this group. Furthermore, milk acetone was elevated in EFA+CLA. Milk acetone is positively correlated with BHB in plasma (Klein et al. 2013). However, plasma BHB was not higher in the EFA+CLA group compared with levels in the other groups (data not shown). Whether and how the low citrate and high acetone in milk after EFA+CLA supplementation are connected is somewhat speculative. Due to the influence of CLA, milk fat is low after EFA+CLA supplementation, and generating NADPH through the citrate-isocitrate pathway could not explain the lower milk citrate in this group compared with that in the CLA group. However, enhanced synthesis of amino acids or carbohydrates in the mammary gland is able to reduce citrate and to increase acetone in milk, but further studies are necessary to strengthen this hypothesis.

In previous studies, abomasal or duodenal infusion of linseed, free ALA, or PUFA (mainly oleic acid and LA) in dairy cows resulted in higher milk fat compared with control groups (Benson et al. 2001; Khas-Erdene et al. 2010; Côrtes et al. 2011). In contrast, in most studies where linseed was fed as extruded flaxseed, the milk fat content declined due to diet-induced milk fat depression (Petit et al. 2007; Zachut et al. 2010; Mach et al. 2013). However, the lack of changes in milk fat in EFA in the present study was consistent with findings in mid-lactating cows using the same EFA dose (Haubold et al. 2020), and Moallem et al. (2012) indicated only a trend for an increasing milk fat content after infusing higher doses of linseed oil than in the present study. In late lactation, milk protein was higher in CLA-supplemented cows. An increase in milk protein during CLA feeding was also mentioned by Bauman et al. (2008). In contrast, in early lactation, milk protein in the CLA groups was reduced. Milk protein reduction in early lactation was also determined by others (Moallem et al. 2010; von Soosten et al. 2011). The different results of CLA supplementation on the milk protein concentration in late and early lactation might be a consequence of the lactation stage. The protein balance was positive during late lactation but turned to negative results during early lactation, which could have affected CLA responses to milk protein content. Because cows in early lactation received CLA supplementation for a much longer time than did cows in late lactation, the differences in milk protein content in early and late lactation due to CLA treatment were confounded by time of treatment. Therefore, further studies are needed to clarify whether CLA effects on milk protein content depend on lactation stage.

In accordance with the study of Haubold et al. (2020), milk urea was diminished in CLA groups. Moreover, we also found a reduction in milk urea in CTRL. The urea concentration in milk reflects the efficiency of protein utilization and is, in general, positively correlated with crude protein intake and, to a lesser extent, negatively correlated with available energy (Nousiainen

et al. 2004). Therefore, the slight reduction in DMI in the CLA group cannot explain the reduction in urea, because milk urea is also lowered in the CTRL group. Reduced milk protein and urea concentrations in early lactation after CLA administration have previously been reported by Moallem et al. (2010) and von Soosten et al. (2011). Higher body protein accretion and nitrogen retention after CLA supplementation are supposed to cause milk protein reduction (von Soosten et al. 2012). Other studies could not confirm an effect of CLA on milk protein and urea or showed an increase in these parameters (Bauman et al. 2008). The reduction in milk urea in CTRL also provides evidence that urea reduction might not be a factor in CLA treatment. Further studies are needed to clarify whether there are direct effects of CLA on protein synthesis in the mammary gland or on whole-body protein accretion in cows.

3.5.3 Milk Fatty Acid Pattern

According to previous research, the milk FA pattern in response to CLA changed as expected (Chouinard et al. 1999; Perfield II et al. 2002). The altered milk FA composition with CLA supplementation was characterized by lower *de novo* synthesized FA (<16 carbons), resulting in a shift to longer-chain FA. The differences in the proportions of n-3 and n-6 FA in milk fat are reflected by the composition of the infused FA in EFA and EFA+CLA (Petit 2002; Kazama et al. 2010; Moallem et al. 2012). The accumulation of ALA and LA was higher in EFA+CLA than in EFA due to the lower milk fat content and reduction of *de novo* FA synthesis in the mammary gland following CLA supplementation. Therefore, an increase in the LA content in CLA (26% in early lactation) was also measurable. Nevertheless, EPA and DPA as well as ARA were higher AP in the EFA group than in the EFA+CLA group, which points to a *trans*-10,*cis*-12 CLA–related inhibition of FA desaturation in dairy cows (Harvatine and Bauman 2011; Haubold et al. 2020). Other studies have determined an inhibition of ARA synthesis from LA but not an inhibition of EPA from ALA due to *trans*-10,*cis*-12 CLA treatment (Loor and Herbein 2003). Correspondingly, ARA decreased due to CLA treatment (CLA and EFA+CLA) in late lactation and was higher in CTRL and EFA. In early lactation, ARA decreased equally in all groups. However, because cows in early lactation had a much longer treatment time than those in late lactation, the present study does not allow us to conclude an effect of the lactation stage on ARA in milk fat. The lower transfer efficiencies of ALA and of the infused CLA isomers after parturition were probably a consequence of the enrichment of these FA in colostrum during the dry period that has reached a plateau at the end of colostrogenesis. With ongoing milk production, efficiency rates of the infused FA increased again. Whether differences in transfer efficiencies between late and early lactation were a consequence of lactation stage or of infusion time cannot be ascertained by the present study. The transfer efficiency for ALA was comparable to efficiency rates in early studies with infused linseed oil (Hagemeister et al. 1991;

Moallem et al. 2012). The transfer efficiency in our study during early lactation of *trans*-10,*cis*-12 CLA was lower than the transfer efficiency published recently (Urrutia et al. 2018). According to the FA composition of EFA and EFA+CLA, supplementation led to a higher content of n-3 FA compared with CTRL and CLA. Such a shift in the n-6/n-3 ratio in the milk FA profile has also been shown several times before (Moallem 2018). As food enriched with n-3 FA is in high demand for human nutrition due to its beneficial health effects (Simopoulos 2002), the supplementation of dairy cows with a combination of CLA and EFA improves both the energy status of the dairy cow due to CLA supplementation and the nutrient value of the milk fat due to EFA supplementation. Keeping animals on pasture provides cows with EFA and CLA, as in the present study (Lahlou et al. 2014), and this is important for consumer acceptance of both dairy production and dairy products (Kühl et al. 2017).

3.5.4 Metabolites in Plasma and Liver

A reduced severity of negative EB should reduce the mobilization of adipose tissue and, therefore, leads to a lower increase of plasma NEFA concentration around calving (Bauman and Currie 1980; Weber et al. 2013). Indeed, in the present study, improved EB and reduced BW loss in the CLA groups led to a reduction in circulating NEFA concentration, as also noted previously (Kay et al. 2006; Odens et al. 2007; Galamb et al. 2017). Fatty acids in the liver can be oxidized but also esterified into TG. Re-esterified FA and newly synthesized TG can either be packed into VLDL and delivered into blood or stored as cytosolic lipid droplets. In dairy cows, release of VLDL by the liver is relatively low (Drackley 1999). Therefore, in CTRL and EFA, higher plasma NEFA leads to increased liver TG at d 28. Several studies have shown that elevated liver TG is associated with lipomobilization and high plasma NEFA levels (Bobe et al. 2004; Overton and Waldron 2004; Weber et al. 2013). However, the decrease in NEFA concentration in the current experiment is not in accord with other CLA trials during the transition period (Bernal-Santos et al. 2003; Hötger et al. 2013; Schäfers et al. 2017), probably a consequence of the long-lasting CLA treatment in the current study.

The VLDL are processed in circulation into intermediate-density lipoproteins, which can be further metabolized to LDL. From extrahepatic tissues, cholesterol is returned to the liver in HDL (Drackley 1999). In accordance with the present data, research has demonstrated that the total cholesterol concentration and individual lipoprotein-associated cholesterol fractions in plasma were dramatically decreased at the onset of lactation and steadily increased thereafter (Kessler et al. 2014). The presented results contribute to the minor effects of EFA supplementation on total cholesterol and associated fractions in blood plasma. The higher plasma LDL cholesterol concentration after EFA+CLA supplementation possibly indicated a lower mam-

mary uptake of cholesterol and not an enhanced export rate of cholesterol from the liver, particularly because liver TG was diminished in the CLA groups. The lower HDL cholesterol concentration in CLA at the end of the trial may be a consequence of reduced reverse cholesterol transport from extrahepatic tissues, as hormone-sensitive lipase and perilipin 1 are decreased by CLA supplementation (Urrutia and Harvatine 2017). In addition, the highest HDL cholesterol concentration at the end of the study was observed in the CTRL group, and lauric acid that is enriched in coconut oil has been shown to stimulate HDL cholesterol in humans (Mensink et al. 2003).

3.6 Conclusions

Our results indicate a reduced milk fat content after CLA with or without EFA supplementation during late and early lactation. An elevated milk protein content after CLA supplementation was observed only in late lactation, whereas the energy status of the cows was improved, especially during early lactation in both CLA-supplemented groups. The different degrees of CLA effects on milk performance during late and early lactation were probably not only a consequence of the different lactation stage but also due to the fact that cows in early lactation received the treatments for much longer time. Increased milk citrate concentration in cows in the CLA group points to reduced *de novo* FA synthesis in the mammary gland, but milk citrate was less affected by combined EFA and CLA treatment, indicating that EFA supplementation may influence changes in mammary gland FA metabolism achieved by CLA. However, very few effects of the EFA treatment alone were evident with regard to milk performance and fat metabolism, indicating low importance of an enhanced EFA supply for milk production.

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3.8 Supplementary Material

Table 3.7 Fatty acid composition of the daily infused supplements during lactation[1]

	CTRL[2]	EFA[3]			CLA[4]	EFA+CLA[5]
Fatty acid (%)	**Coconut oil**	**Linseed oil**	**Safflower oil**	**Total**	**Lutalin®**	**Total**
SFA	89.4	10.4	12.3	10.5	11.1	10.7
6:0	0.93	–	–	–	–	–
8:0	9.85	–	–	–	–	–
10:0	6.10	–	–	–	–	–
12:0	45.5	–	–	–	–	–
14:0	16.9	0.02	0.09	0.02	–	0.02
16:0	6.87	5.78	7.63	5.87	6.23	5.98
17:0	–	0.03	–	0.03	–	0.02
18:0	3.10	4.11	3.71	4.10	4.19	4.13
20:0	0.11	0.19	0.42	0.20	0.22	0.21
22:0	–	0.13	0.25	0.14	0.37	0.21
24:0	–	0.09	0.16	0.10	0.10	0.10
MUFA	8.35	21.5	23.5	21.6	29.5	24.1
16:1, *cis*-9	–	0.07	0.12	0.07	0.09	0.08
18:1, *cis*-9	8.35	20.7	22.4	20.8	28.3	23.1
18:1, *cis*-11	–	0.61	0.66	0.61	0.67	0.63
20:1, *cis*-11	–	0.16	0.23	0.16	0.51	0.27
24:1, *cis*-15	–	–	0.11	0.01	–	< 0.01

Table 3.7 Continuation

	CTRL[2]	EFA[3]			CLA[4]	EFA+CLA[5]
Fatty acid (%)	**Coconut oil**	**Linseed oil**	**Safflower oil**	**Total**	**Lutalin®**	**Total**
PUFA	1.83	67.1	62.5	66.9	3.75	46.9
18:2, *cis*-9,*cis*-12	1.83	15.9	62.0	18.2	3.52	13.5
18:2, *cis*-9,*trans*-12	–	–	–	–	0.08	0.03
18:2, *trans*-9,*cis*-12	–	–	–	–	0.07	0.02
18:3, *cis*-6,*cis*-9,*cis*-12	–	–	0.05	< 0.01	0.07	0.02
18:3, *cis*-9,*cis*-12,*cis*-15	–	51.1	0.24	48.7	–	33.3
20:2, *cis*-11,*cis*-14	–	0.06	–	0.06	–	0.04
20:3, *cis*-11,*cis*-14,*cis*-17	–	0.02	–	0.02	–	0.02
22:5, *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19	–	–	0.28	0.01	–	0.01
CLA	< 0.05	0.02	0.32	0.04	54.4	17.2
18:2, *cis*-9,*trans*-11 CLA	–	–	0.12	0.01	27.2	8.61
18:2, *trans*-10,*cis*-12 CLA	–	0.02	0.20	0.03	27.0	8.56
18:2, *cis*-9,*cis*-11 CLA	–	–	–	–	0.23	0.07

[1]Dosage of oil supplementation was halved during the dry period in all groups, respectively.
[2]Control (CTRL, n=9): 76 g/d coconut oil (Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard KG, Bad Ditzenbach, Germany) and 0.06 g/d Vitamin E (Covitol®1360, BASF SE, Ludwigshafen, Germany), 1.48 MJ NE_L/d.
[3]Essential fatty acids (EFA, n = 9): 78 g/d linseed (DERBY® Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany) and 4 g/d safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany), comprised 0.06 g/d Vitamin E, 1.57 MJ NE_L/d.
[4]Conjugated linoleic acid (CLA, n = 10): 38 g/d Lutalin® (BASF SE, Ludwigshafen, Germany) and 0.06 g/d Vitamin E (Covitol®1360, BASF SE, Ludwigshafen, Germany), 0.69 MJ NE_L/d.
[5]Essential fatty acids and conjugated linoleic acid (EFA+CLA, n = 10): 78 g/d linseed (DERBY® Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany),4 g/d safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany) and 38 g/d Lutalin® (BASF SE, Ludwigshafen, Germany), comprised 0.06 g/d Vitamin E, 2.26 MJ NE_L/d.

Table 3.8 Concentrations of fatty acids in milk fat of cows daily abomasally supplemented either with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10) or the combination (EFA+CLA; n = 10) from wk 9 antepartum until wk 8 postpartum

		Treatment				*P*-values[3]			
Fatty acid[2], %	Time[4], wk	CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
4:0	-10	3.42±0.19	3.65±0.17	3.53±0.16^{A}	3.53±0.16^{A}	<0.001	<0.001	<0.001	
	-7	3.10±0.19^{a}	3.35±0.17^{a}	1.61±0.16^{c,B}	2.35±0.16^{b,B}				
	1	2.15±0.25^{B}	2.32±0.24^{B}	2.38±0.23^{C}	2.33±0.23^{C}				
	4	3.86±0.25^{A}	3.91±0.24^{A}	4.22±0.23^{A}	4.23±0.23^{A}	0.9	<0.001	0.2	
	8	3.67±0.25^{A}	3.89±0.24^{A}	3.28±0.23^{B}	3.42±0.23^{B}				
6:0	-10	2.28±0.12	2.40±0.11	2.48±0.10^{A}	2.39±0.10^{A}	<0.001	<0.001	<0.001	
	-7	2.09±0.12^{a}	2.34±0.11^{a}	1.03±0.10^{b,B}	1.33±0.10^{b,B}				
	1	1.35±0.12^{B}	1.40±0.12^{B}	1.35±0.12^{B}	1.32±0.12^{B}				
	4	2.31±0.12^{a,A}	2.51±0.12^{a,A}	1.80±0.12^{b,A}	1.77±0.12^{b,A}	<0.001	<0.001	<0.001	
	8	2.38±0.12^{a,A}	2.79±0.12^{a,A}	1.58±0.12^{b,AB}	1.68±0.12^{b,AB}				
8:0	-10	1.48±0.08	1.54±0.07	1.65±0.07^{A}	1.54±0.07^{A}	<0.001	<0.001	<0.001	
	-7	1.34±0.08^{a}	1.52±0.07^{a}	0.67±0.07^{b,B}	0.77±0.07^{b,B}				
	1	0.88±0.08^{B}	0.84±0.07^{C}	0.74±0.07^{B}	0.73±0.07^{B}				
	4	1.47±0.08^{a,A}	1.49±0.07^{a,B}	0.98±0.07^{b,A}	0.92±0.07^{b,AB}	<0.001	<0.001	<0.001	a
	8	1.56±0.08^{a,A}	1.84±0.07^{a,A}	0.97±0.07^{b,AB}	1.04±0.07^{b,A}				
10:0	-10	3.26±0.19^{b}	3.60±0.17^{ab}	3.94±0.16^{a,A}	3.56±0.16^{ab,A}	0.001	<0.001	<0.001	a
	-7	2.98±0.19^{a}	3.55±0.17^{a}	1.72±0.16^{b,B}	1.82±0.16^{b,B}				
	1	1.99±0.17^{B}	1.93±0.16^{C}	1.82±0.17	1.69±0.16^{B}				
	4	2.90±0.17^{a,A}	3.00±0.16^{a,B}	1.97±0.16^{b}	1.81±0.16^{b,AB}	<0.001	<0.001	<0.001	a
	8	3.42±0.17^{b,A}	4.26±0.16^{a,A}	2.06±0.16^{c}	2.26±0.16^{c,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
10:1	-10	0.37±0.02	0.36±0.02	0.38±0.02^{A}	0.38±0.02^{A}	<0.001	<0.001	<0.001	c
	-7	0.37±0.02^{a}	0.33±0.02^{a}	0.14±0.02^{b,B}	0.13±0.02^{b,B}				
	1	0.13±0.02^{a,C}	0.11±0.02^{ab,C}	0.06±0.02^{b}	0.05±0.02^{b,B}				
	4	0.24±0.02^{a,B}	0.25±0.02^{a,B}	0.09±0.02^{b}	0.07±0.02^{b,AB}	<0.001	<0.001	<0.001	c
	8	0.30±0.02^{a,A}	0.34±0.02^{a,A}	0.11±0.02^{b}	0.12±0.02^{b,A}				
11:0	-10	0.08±0.01^{ab}	0.07±0.01^{b}	0.12±0.01^{a,A}	0.08±0.01^{b,A}	0.2	<0.001	<0.001	
	-7	0.06±0.01^{ab}	0.07±0.01^{a}	0.04±0.01^{ab,B}	0.03±0.01^{b,B}				
	1	0.03±0.01^{B}	0.02±0.01^{C}	0.02±0.01	0.02±0.01				
	4	0.07±0.01^{a,A}	0.07±0.01^{a,B}	0.04±0.01^{b}	0.03±0.01^{b}	<0.001	<0.001	<0.01	
	8	0.09±0.01^{a,A}	0.11±0.01^{a,A}	0.04±0.01^{b}	0.04±0.01^{b}				
12:0	-10	4.28±0.23^{B}	4.50±0.20	4.78±0.19^{A}	4.27±0.19^{A}	<0.001	<0.001	<0.001	a
	-7	5.37±0.23^{a,A}	4.38±0.20^{b}	2.79±0.19^{c,B}	2.64±0.19^{c,B}				
	1	4.02±0.20^{a,B}	3.13±0.20^{b,B}	2.96±0.20^{b}	2.76±0.19^{b,AB}				
	4	4.37±0.20^{a,B}	3.41±0.20^{b,B}	2.46±0.19^{c}	2.29±0.19^{c,B}	<0.001	<0.001	<0.001	a
	8	5.31±0.20^{a,A}	4.87±0.20^{a,A}	2.93±0.19^{b}	2.96±0.19^{b,A}				
12:1	-10	0.11±0.01^{B}	0.11±0.01	0.12±0.01^{A}	0.11±0.01^{A}	<0.001	0.10	<0.001	c
	-7	0.17±0.01^{a,A}	0.10±0.01^{b}	0.09±0.01^{bc,B}	0.06±0.01^{c,B}				
	1	0.09±0.01^{a,AB}	0.05±0.01^{b,B}	0.04±0.01^{b}	0.03±0.01^{b}				
	4	0.07±0.01^{a,B}	0.06±0.01^{a,AB}	0.02±0.01^{b}	0.02±0.01^{b}	<0.001	<0.001	0.2	
	8	0.10±0.01^{a,A}	0.08±0.01^{a,A}	0.03±0.01^{b}	0.04±0.01^{b}				

Table 3.8 Continuation

		Treatment				*P*-values[3]			
Fatty acid[2], %	Time[4], wk	CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
13:0	-10	0.14±0.01^{ab}	0.12±0.01^{ab}	0.16±0.01^{a,A}	0.12±0.01^{b,A}	0.14	<0.001	<0.01	
	-7	0.11±0.01	0.12±0.01	0.09±0.01^{B}	0.08±0.01^{B}				
	1	0.07±0.01^{B}	0.06±0.01^{B}	0.05±0.01^{B}	0.04±0.01^{B}				
	4	0.12±0.01^{A}	0.11±0.01^{A}	0.10±0.01^{A}	0.08±0.01^{A}	<0.05	<0.001	0.8	
	8	0.14±0.01^{A}	0.14±0.01^{A}	0.11±0.01^{A}	0.11±0.01^{A}				
14:0	-10	12.4 ±0.4	12.7 ±0.4	13.3 ±0.3^{A}	12.2 ±0.3^{A}	0.2	<0.001	0.13	a
	-7	12.3 ±0.4	11.9 ±0.4	11.8 ±0.3^{B}	11.1 ±0.3^{B}				
	1	14.3 ±0.5^{A}	13.6 ±0.5^{A}	14.6 ±0.5^{A}	13.5 ±0.5^{A}				
	4	10.7 ±0.5^{a,B}	9.62±0.53^{ab,B}	9.62±0.53^{ab,C}	8.71±0.51^{b,C}	<0.05	<0.001	0.6	a
	8	12.4 ±0.5^{B}	12.6 ±0.5^{A}	11.9 ±0.5^{B}	11.1 ±0.5^{B}				
iso-14:0	-10	0.10±0.01^{B}	0.10±0.01	0.08±0.01	0.10±0.01	0.3	<0.05	0.6	a
	-7	0.13±0.01^{A}	0.11±0.01	0.09±0.01	0.11±0.01				
	1	0.06±0.01	0.06±0.01	0.06±0.01^{B}	0.05±0.01^{B}				
	4	0.08±0.01	0.07±0.01	0.09±0.01^{A}	0.09±0.01^{A}	0.7	<0.001	0.4	
	8	0.10±0.01	0.07±0.01	0.10±0.01^{A}	0.09±0.01^{A}				
14:1, *cis*-9	-10	1.62±0.15^{B}	1.42±0.14	1.48±0.13^{B}	1.45±0.13	<0.05	<0.01	<0.001	
	-7	1.97±0.15^{a,A}	1.24±0.14^{b}	2.18 ±0.13^{a,A}	1.41±0.13 ^{b}				
	1	1.77±0.11^{a,A}	1.47±0.11^{ab,A}	1.37 ±0.11^{bc,A}	1.04 ±0.10^{c,A}				
	4	1.09±0.11^{a,B}	0.90±0.11^{ab,B}	0.69 ±0.10^{b,C}	0.54 ±0.10^{b,B}	<0.001	<0.001	0.7	
	8	1.33±0.11^{a,B}	1.00±0.11^{ab,B}	1.01 ±0.10^{ab,B}	0.86 ±0.10^{b,A}				

Table3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
15:0	-10	1.39±0.08	1.25±0.07	1.47±0.07^{A}	1.28±0.07	0.15	<0.01	0.6	a
	-7	1.26±0.08	1.17±0.07	1.24±0.07^{B}	1.15±0.07				
	1	0.84±0.08^{B}	0.83±0.08^{B}	0.74±0.08^{B}	0.71±0.08^{C}				
	4	1.05±0.08^{AB}	1.04±0.08^{AB}	1.04±0.08^{A}	1.00±0.08^{B}	0.9	<0.001	1.0	
	8	1.24±0.08^{A}	1.24±0.08^{A}	1.23±0.08^{A}	1.23±0.08^{A}				
iso-15:0	-10	0.21±0.01	0.18±0.01	0.18±0.01	0.19±0.01	0.05	0.4	0.3	
	-7	0.23±0.01^{a}	0.17±0.01^{b}	0.20±0.01^{ab}	0.18±0.01^{ab}				
	1	0.20±0.01^{a,A}	0.17±0.01^{ab}	0.13±0.01^{b}	0.12±0.01^{b}				
	4	0.15±0.01^{B}	0.14±0.01	0.15±0.01	0.14±0.01	0.06	0.3	<0.05	a
	8	0.16±0.01^{AB}	0.14±0.01	0.17±0.01	0.15±0.01				
anteiso-15:0	-10	0.60±0.03	0.60±0.02	0.61±0.02^{A}	0.60±0.02^{A}	0.4	0.05	0.07	a
	-7	0.62±0.03^{a}	0.59±0.02^{ab}	0.53 ±0.02^{b,B}	0.55 ±0.02^{ab,B}				
	1	0.27±0.04^{B}	0.27±0.04^{B}	0.21±0.03^{B}	0.19±0.03^{B}				
	4	0.42±0.04^{A}	0.42±0.04^{A}	0.50±0.03^{A}	0.46±0.03^{A}	0.6	<0.001	0.08	
	8	0.48±0.04^{A}	0.48±0.04^{A}	0.58±0.03^{A}	0.53±0.03^{A}				
16:0	-10	32.0 ±1.3	31.1 ±1.2	30.3 ±1.1	31.3 ±1.1^{A}	0.3	0.14	0.06	
	-7	30.9 ±1.3^{ab}	29.3 ±1.2^{ab}	32.4 ±1.1^{a}	27.4 ±1.1^{b,B}				
	1	40.4 ±1.6^{b,A}	41.8 ±1.6^{ab,A}	47.0 ±1.6^{a,A}	44.3 ±1.5^{ab,A}				
	4	29.0 ±1.6^{B}	26.5 ±1.6^{B}	25.5 ±1.5^{B}	24.1 ±1.5^{B}	0.4	<0.001	<0.01	
	8	30.1 ±1.6^{B}	30.2 ±1.6^{B}	29.0 ±1.5^{B}	25.9 ±1.5^{B}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-16:0	-10	0.26±0.03^{B}	0.29±0.03	0.22±0.02	0.30±0.02	0.09	0.07	0.5	a
	-7	0.32±0.03^{A}	0.30±0.03	0.24±0.02	0.30±0.02				
	1	0.18±0.03	0.20±0.03	0.14±0.02^{B}	0.16±0.02^{B}				
	4	0.21±0.03	0.22±0.03	0.22±0.02^{A}	0.25±0.02^{A}	1.0	<0.001	<0.05	
	8	0.22±0.03	0.19±0.03	0.26±0.02^{A}	0.24±0.02^{A}				
16:1, *cis*-9	-10	1.95±0.26	1.74±0.24	1.96±0.22^{B}	1.90±0.22	<0.01	<0.001	<0.001	
	-7	2.35±0.26^{b}	1.53±0.24^{b}	3.55±0.22^{a,A}	1.77±0.22^{b}				
	1	3.15±0.20^{a,A}	2.79±0.20^{ab,A}	2.91±0.20^{ab,A}	2.35±0.19^{b,A}				
	4	2.27±0.20^{a,B}	1.92±0.20^{ab,B}	1.63±0.19^{ab,B}	1.29±0.19^{b,B}	<0.01	<0.001	0.8	
	8	1.93±0.20^{a,B}	1.39±0.20^{ab,B}	1.39±0.19^{ab,B}	1.10±0.19^{b,B}				
16:1, *trans*-9	-10	0.05±0.00	0.05±0.00	0.05±0.00^{B}	0.05±0.00	0.2	0.13	<0.01	a, b
	-7	0.05±0.00^{ab}	0.05±0.00^{b}	0.07±0.00^{a,A}	0.05±0.00^{ab}				
	1	0.04±0.00	0.04±0.00	0.04±0.00^{B}	0.04±0.00				
	4	0.04±0.00^{b}	0.04±0.00^{b}	0.06±0.00^{a,A}	0.05±0.00^{ab}	<0.001	<0.05	<0.01	a
	8	0.05±0.00^{bc}	0.03±0.00^{c}	0.07±0.00^{a,A}	0.05±0.00^{ab}				
17:0	-10	0.82±0.05	0.76±0.05	0.83±0.05	0.79±0.05	0.4	0.5	0.5	a
	-7	0.77±0.05	0.77±0.05	0.87±0.05	0.88±0.05				
	1	0.64±0.07^{B}	0.75±0.06^{B}	0.72±0.06^{B}	0.73±0.06^{B}				
	4	0.83±0.07^{c,A}	0.93±0.06^{bc,A}	1.17±0.06^{a,A}	1.09±0.06^{ab,A}	<0.01	<0.001	<0.05	a
	8	0.80±0.07^{b,AB}	0.80±0.06^{b,AB}	1.10±0.06^{a,A}	1.12±0.06^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-17:0	-10	0.45±0.03	0.45±0.02	0.42±0.02^{B}	0.50±0.02	0.11	<0.05	0.07	a
	-7	0.50±0.03	0.44±0.02	0.52±0.02^{A}	0.52±0.02				
	1	0.33±0.03	0.35±0.03	0.28±0.03^{B}	0.28±0.03^{B}				
	4	0.41±0.03	0.43±0.03	0.52±0.03^{A}	0.50±0.03^{A}	0.11	<0.001	<0.001	a
	8	0.42±0.03^{bc}	0.38±0.03^{c}	0.56±0.03^{a,A}	0.52±0.03^{ab,A}				
anteiso-17:0	-10	0.63±0.03	0.64±0.03	0.65±0.03	0.66±0.03	1.0	0.9	0.9	a
	-7	0.65±0.03	0.65±0.03	0.64±0.03	0.65±0.03				
	1	0.42±0.05^{B}	0.43±0.05^{B}	0.36±0.05^{B}	0.40±0.05^{B}				
	4	0.59±0.05^{A}	0.57±0.05^{A}	0.67±0.05^{A}	0.61±0.05^{A}	0.5	<0.001	<0.05	
	8	0.56±0.05^{b,A}	0.55±0.05^{b,AB}	0.75±0.05^{a,A}	0.70±0.05^{ab,A}				
17:1, *cis*-9	-10	0.29±0.02	0.24±0.02	0.28±0.02^{B}	0.27±0.02^{A}	<0.01	0.4	<0.001	
	-7	0.30±0.02^{ab}	0.22±0.02^{c}	0.37±0.02^{a,A}	0.23±0.02^{bc,B}				
	1	0.34±0.02^{a,B}	0.28±0.02^{ab,B}	0.25±0.02^{ab,B}	0.22±0.02^{b}				
	4	0.42±0.02^{a,A}	0.37±0.02^{a,A}	0.34±0.02^{ab,A}	0.28±0.02^{b}	<0.001	<0.001	0.5	
	8	0.34±0.02^{a,B}	0.23±0.02^{b,B}	0.27±0.02^{ab,AB}	0.24±0.02^{b}				
18:0	-10	6.91±0.59	7.39±0.54	6.83±0.51	7.33±0.51^{B}	<0.05	<0.001	<0.01	
	-7	6.73±0.59^{b}	8.23±0.54^{ab}	7.79±0.51^{b}	10.2 ±0.5^{a,A}				
	1	4.82±0.73^{B}	5.65±0.71^{B}	5.56±0.71^{B}	6.72±0.67^{C}				
	4	7.48±0.73^{c,A}	8.55±0.71^{bc,A}	10.9 ±0.7^{ab,A}	12.1 ±0.7^{a,A}	<0.001	<0.001	<0.05	
	8	6.34±0.73^{b,AB}	7.01±0.71^{b,AB}	10.1 ±0.7^{a,A}	9.93 ±0.67^{a,B}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-18:0	-10	0.04±0.00^{B}	0.04±0.00	0.04±0.00^{B}	0.05±0.00^{B}	<0.05	<0.001	0.09	a
	-7	0.05±0.00^{ab,A}	0.04±0.00^{b}	0.06±0.00^{a,A}	0.05±0.00^{a,A}				
	1	0.03±0.01^{B}	0.03±0.01^{B}	0.03±0.01^{B}	0.04±0.01^{B}				
	4	0.06±0.01^{A}	0.05±0.01^{A}	0.06±0.01^{A}	0.06±0.01^{A}	0.2	<0.001	0.4	a
	8	0.05±0.01^{ab,AB}	0.04±0.01^{b,AB}	0.07±0.01^{a,A}	0.06±0.01^{ab,AB}				
18:1, *cis*-9	-10	17.8 ±0.9	17.6 ±0.8	17.2 ±0.8^{B}	18.3 ±0.8	0.3	<0.05	0.05	a
	-7	18.0 ±0.9	17.4 ±0.8	20.4 ±0.8^{A}	19.9 ±0.8				
	1	16.0 ±1.2^{a,B}	14.4 ±1.2^{ab,B}	11.3 ±1.2^{b,C}	12.5 ±1.1^{ab,B}				
	4	23.0 ±1.2^{A}	23.6 ±1.2^{A}	24.9 ±1.1^{A}	23.2 ±1.1^{A}	0.7	<0.001	<0.05	a
	8	18.4 ±1.2^{B}	16.2 ±1.2^{B}	19.9 ±1.1^{B}	19.4 ±1.1^{A}				
18:1, *cis*-11	-10	0.75±0.08	0.71±0.07	0.78±0.07	0.71±0.07	0.2	0.3	0.2	
	-7	0.68±0.08	0.69±0.07	0.93±0.07	0.80±0.07				
	1	0.66±0.08^{B}	0.66±0.08^{B}	0.50±0.08^{C}	0.54±0.08^{C}				
	4	1.09±0.08^{A}	1.06±0.08^{A}	1.24±0.08^{A}	1.24±0.08^{A}	0.6	<0.001	<0.05	a
	8	0.95±0.08^{A}	0.80±0.08^{B}	1.01±0.08^{B}	1.04±0.08^{B}				
18:1, *cis*-12	-10	0.33±0.02	0.35±0.02^{A}	0.31±0.02	0.35±0.02	0.4	0.2	<0.05	
	-7	0.30±0.02	0.28±0.02^{B}	0.35±0.02	0.35±0.02				
	1	0.16±0.02^{B}	0.16±0.02^{B}	0.12±0.02^{B}	0.13±0.02^{C}				
	4	0.26±0.02^{b,A}	0.26±0.02^{b,A}	0.41±0.02^{a,A}	0.34±0.02^{ab,B}	<0.001	<0.001	<0.001	a, b
	8	0.23±0.02^{b,AB}	0.27±0.02^{b,A}	0.41±0.02^{a,A}	0.41±0.02^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
18:1, *trans*-vaccenic	-10	1.79±0.24	1.69±0.22	2.00±0.21	1.57±0.21^{B}	0.5	0.06	0.2	
(*trans*-9 + 10 + 11)	-7	2.19±0.24	1.59±0.22	2.04±0.21	2.17±0.21^{A}				
	1	0.62±0.55^{B}	0.68±0.54^{B}	0.61±0.54^{B}	0.59±0.51^{B}				
	4	2.04±0.55^{AB}	2.26±0.54^{A}	2.80±0.51^{A}	2.25±0.51^{A}	0.7	<0.001	0.3	
	8	3.57±0.55^{A}	1.63±0.54^{AB}	2.95±0.51^{A}	3.22±0.51^{A}				
18:2, *cis*-9,*cis*-12	-10	2.57±0.18	2.56±0.17^{B}	2.26±0.16^{B}	2.42±0.16^{B}	<0.001	<0.001	<0.001	
	-7	2.41±0.18^{c}	3.42±0.17^{b,A}	2.90±0.16^{bc,A}	4.56±0.16^{a,A}				
	1	2.63±0.16^{b}	2.99±0.16^{ab}	2.40±0.16^{b,B}	3.27±0.15^{a,B}				
	4	2.37±0.16^{c}	2.98±0.16^{b}	3.49±0.15^{b,A}	4.78±0.15^{a,A}	<0.001	<0.001	<0.001	a
	8	2.31±0.16^{c}	3.12±0.16^{b}	3.33±0.15^{b,A}	4.69±0.15^{a,A}				
18:2, *cis*-9,*trans*-11	-10	0.54±0.05	0.55±0.05	0.55±0.05^{B}	0.54±0.05^{B}	<0.001	<0.001	<0.001	
CLA	-7	0.61±0.05^{b}	0.44±0.05^{b}	1.19±0.05^{a,A}	1.16±0.05^{a,A}				
	1	0.27±0.06^{B}	0.23±0.05^{B}	0.33±0.05^{B}	0.33±0.05^{C}				
	4	0.38±0.06^{b,A}	0.37±0.05^{b,A}	1.09±0.05^{a,A}	0.94±0.05^{a,B}	<0.001	<0.001	<0.001	
	8	0.41±0.06^{b,A}	0.33±0.05^{b,AB}	1.17±0.05^{a,A}	1.06±0.05^{a,A}				
18:2, *trans*-10,*cis*-12	-10	0.10±0.03	0.17±0.03	0.14±0.02^{B}	0.15±0.02^{B}	<0.001	<0.001	<0.001	a
CLA	-7	0.15±0.03^{b}	0.12±0.03^{b}	0.47±0.02^{a,A}	0.53±0.02^{a,A}				
	1	0.10±0.03	0.11±0.03	0.17±0.03^{B}	0.21±0.03^{B}				
	4	0.06±0.03^{b}	0.11±0.03^{b}	0.53±0.03^{a,A}	0.51±0.03^{a,A}	<0.001	<0.001	<0.001	a
	8	0.09±0.03^{b}	0.09±0.03^{b}	0.53±0.03^{a,A}	0.53±0.03^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
18:3, *cis*-6,*cis*-9,	-10	0.06±0.01	0.05±0.01^{A}	0.06±0.01	0.06±0.00^{A}	0.4	<0.01	0.4	a
cis-12	-7	0.05±0.01	0.04±0.01^{B}	0.05±0.01	0.04±0.00^{B}				
	1	0.06±0.01^{a}	0.03±0.01^{b}	0.04±0.01^{ab}	0.03±0.01^{b}				
	4	0.05±0.01	0.04±0.01	0.06±0.01	0.04±0.01	<0.05	0.2	0.3	a
	8	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01				
18:3, *cis*-9,*cis*-12,	-10	0.31±0.15	0.51±0.14^{B}	0.32±0.13	0.33±0.13^{B}	<0.001	<0.001	<0.001	
cis-15	-7	0.32±0.15^{c}	2.92±0.14^{b,A}	0.36±0.13^{c}	4.02±0.13^{a,A}				
	1	0.07±0.11^{b}	1.34±0.10^{a,B}	0.09±0.10^{b}	1.55±0.10^{a,B}				
	4	0.13±0.11^{c}	2.20±0.10^{b,A}	0.33±0.10^{c}	3.52±0.10^{a,A}	<0.001	<0.001	<0.001	a
	8	0.11±0.11^{c}	2.17±0.10^{b,A}	0.28±0.10^{c}	3.33±0.10^{a,A}				
20:0	-10	0.09±0.01	0.11±0.01	0.10±0.01^{B}	0.10±0.01^{B}	<0.01	<0.01	<0.01	
	-7	0.10±0.01^{b}	0.10±0.01^{b}	0.13±0.01^{ab,A}	0.15±0.01^{a,A}				
	1	0.08±0.01	0.09±0.01	0.08±0.01^{B}	0.09±0.01^{B}				
	4	0.07±0.01^{b}	0.09±0.01^{b}	0.15±0.01^{a,A}	0.15±0.01^{a,A}	<0.001	<0.001	<0.001	
	8	0.08±0.01^{b}	0.08±0.01^{b}	0.15±0.01^{a,A}	0.15±0.01^{a,A}				
20:1, *cis*-11	-10	0.06±0.00	0.07±0.00	0.07±0.00^{B}	0.07±0.00^{B}	<0.001	<0.001	<0.001	a
	-7	0.06±0.00^{b}	0.07±0.00^{b}	0.09±0.00^{a,A}	0.09±0.00^{a,A}				
	1	0.05±0.01^{B}	0.05±0.01^{B}	0.04±0.01^{B}	0.06±0.01^{B}				
	4	0.07±0.01^{A}	0.08±0.01^{A}	0.10±0.01^{A}	0.10±0.01^{A}	<0.05	<0.001	<0.01	a
	8	0.07±0.01^{b,AB}	0.06±0.01^{b,AB}	0.10±0.01^{a,A}	0.10±0.01^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
20:2, *cis*-11,*cis*-14	-10	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00^{B}	0.8	0.3	0.2	a
	-7	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00^{A}				
	1	0.03±0.01	0.04±0.01	0.02±0.01	0.04±0.01				
	4	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01	0.3	0.15	0.4	a
	8	0.03±0.01^{b}	0.03±0.01^{ab}	0.04±0.01^{ab}	0.05±0.01^{a}				
20:3, *cis*-8,*cis*-11,	-10	0.11±0.01	0.10±0.01	0.09±0.01^{A}	0.10±0.01^{A}	<0.05	<0.001	0.13	
cis-14	-7	0.11±0.01^{a}	0.10±0.01^{ab}	0.07±0.01^{b,B}	0.07±0.01^{b,B}				
	1	0.24±0.01^{a,A}	0.13±0.01^{b,A}	0.19±0.01^{a,A}	0.13±0.01^{b,A}				
	4	0.08±0.01^{B}	0.05±0.01^{B}	0.05±0.01^{B}	0.05±0.01^{B}	<0.001	<0.001	<0.001	
	8	0.08±0.01^{B}	0.08±0.01^{B}	0.06±0.01^{B}	0.05±0.01^{B}				
20:4, *cis*-5,*cis*-8,	-10	0.14±0.01	0.12±0.01	0.12±0.01^{A}	0.13±0.01^{A}	<0.01	<0.001	0.01	a
cis-11,*cis*-14	-7	0.12±0.01^{a}	0.13±0.01^{a}	0.08±0.01^{b,B}	0.09±0.01^{b,B}				
	1	0.26±0.02^{a,A}	0.17±0.02^{b,A}	0.23±0.02^{a,A}	0.20±0.02^{ab,A}				
	4	0.12±0.02^{B}	0.10±0.02^{B}	0.10±0.02^{B}	0.09±0.02^{B}	<0.05	<0.001	<0.05	a
	8	0.10±0.02^{B}	0.11±0.02^{B}	0.08±0.02^{B}	0.07±0.02^{B}				
20:5, *cis*-5,*cis*-8,	-10	0.02±0.00	0.02±0.00^{B}	0.02±0.00	0.02±0.00^{B}	<0.001	<0.001	<0.001	
cis-11,*cis*-14,*cis*-17	-7	0.02±0.00^{c}	0.07±0.00^{a,A}	0.02±0.00^{c}	0.04±0.00^{b,A}				
	1	0.03±0.01^{b}	0.10±0.01^{a,A}	0.03±0.01^{b}	0.10±0.01^{a,A}				
	4	0.01±0.01^{c}	0.07±0.01^{a,B}	0.01±0.01^{c}	0.04±0.01^{b,B}	<0.001	<0.001	<0.001	a, b
	8	0.01±0.01^{c}	0.10±0.01^{a,A}	0.01±0.01^{c}	0.05±0.01^{b,B}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
21:0	-10	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.05	0.4	0.2	a
	-7	0.03±0.00^{ab}	0.04±0.00^{a}	0.03±0.00^{b}	0.03±0.00^{b}				
	1	0.03±0.01^{ab}	0.04±0.01^{ab}	0.02±0.01^{b}	0.05±0.01^{a}				
	4	0.02±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.06	0.3	0.5	
	8	0.02±0.01	0.02±0.01	0.03±0.01	0.04±0.01				
22:0	-10	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00^{B}	0.3	<0.01	0.8	a, c
	-7	0.03±0.00	0.04±0.00	0.03±0.00	0.04±0.00^{A}				
	1	0.03±0.00^{A}	0.03±0.00	0.02±0.00^{B}	0.03±0.00				
	4	0.02±0.00^{B}	0.02±0.00	0.03±0.00^{AB}	0.03±0.00	0.6	0.7	<0.01	a
	8	0.02±0.00^{AB}	0.02±0.00	0.03±0.00^{A}	0.03±0.00				
22:5, *cis*-7,*cis*-10,	-10	0.07±0.01	0.05±0.01^{B}	0.06±0.01	0.06±0.01^{B}	<0.001	<0.001	<0.001	
cis-13,*cis*-16,*cis*-19	-7	0.06±0.01^{c}	0.12±0.01^{a,A}	0.04±0.01^{c}	0.09±0.01^{b,A}				
	1	0.12±0.01^{c,A}	0.21±0.01^{b,A}	0.11±0.01^{c,A}	0.28±0.01^{a,A}				
	4	0.05±0.01^{b,B}	0.09±0.01^{ab,C}	0.06±0.01^{b,B}	0.10±0.01^{a,B}	<0.001	<0.001	<0.001	
	8	0.04±0.01^{b,B}	0.13±0.01^{a,B}	0.05±0.01^{b,B}	0.11±0.01^{a,B}				
24:0	-10	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00^{B}	0.6	<0.05	0.4	a
	-7	0.05±0.00	0.04±0.00	0.04±0.00	0.05±0.00^{A}				
	1	0.06±0.01^{A}	0.05±0.01^{A}	0.05±0.01	0.05±0.01				
	4	0.03±0.01^{B}	0.02±0.01^{B}	0.04±0.01	0.04±0.01	0.2	<0.001	0.3	a
	8	0.03±0.01^{B}	0.02±0.01^{B}	0.04±0.01	0.04±0.01				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
Summation									
SFA[6]	-10	70.9 ±1.2	71.5 ±1.1	71.8 ±1.0^{A}	71.0 ±1.0^{A}	<0.01	<0.001	<0.001	a
	-7	69.7 ±1.2^{a}	69.2 ±1.1^{a}	64.5 ±1.0^{b,B}	62.4 ±1.0^{b,B}				
	1	73.1 ±1.5^{b,A}	74.0 ±1.5^{ab,A}	79.2 ±1.5^{a,A}	76.3 ±1.4^{ab,A}				
	4	66.1 ±1.5^{a,B}	63.2 ±1.5^{ab,B}	61.9 ±1.4^{ab,C}	60.5 ±1.4^{b,B}	<0.05	<0.001	<0.001	a
	8	69.5 ±1.5^{a,AB}	71.8 ±1.5^{a,A}	67.1 ±1.4^{ab,B}	63.4 ±1.4^{b,B}				
MUFA[7]	-10	23.3 ±0.9	22.6 ±0.9	22.5 ±0.8^{B}	23.6 ±0.8	<0.05	<0.01	<0.001	a
	-7	24.2 ±0.9^{b}	21.8 ±0.9^{b}	28.1 ±0.8^{a,A}	24.8 ±0.8^{b}				
	1	22.4 ±1.2^{a,B}	19.9 ±1.2^{ab,B}	16.6 ±1.2^{b,C}	16.9 ±1.2^{b,B}				
	4	28.5 ±1.2^{A}	28.5 ±1.2^{A}	29.4 ±1.2^{A}	27.1 ±1.2^{A}	0.10	<0.001	<0.05	a
	8	23.7 ±1.2^{B}	20.4 ±1.2^{B}	24.3 ±1.2^{B}	23.3 ±1.2^{A}				
PUFA[8]	-10	3.31±0.31	3.45±0.28^{B}	2.95±0.27	3.14±0.27^{B}	<0.001	<0.001	<0.001	
	-7	3.12±0.31^{c}	6.82±0.28^{b,A}	3.57±0.27^{c}	8.95±0.27^{a,A}				
	1	3.43±0.24^{b}	5.02±0.23^{a,B}	3.12±0.23^{b,B}	5.59±0.22^{a,B}				
	4	2.84±0.24^{d}	5.56±0.23^{b,AB}	4.12±0.22^{c,A}	8.66±0.22^{a,A}	<0.001	<0.001	<0.001	a, b
	8	2.71±0.24^{d}	5.78±0.23^{b,A}	3.89±0.22^{c,A}	8.39±0.22^{a,A}				
EFA[9]	-10	2.88±0.30	3.07±0.27^{B}	2.58±0.26^{B}	2.75±0.26^{B}	<0.001	<0.001	<0.001	
	-7	2.73±0.30^{c}	6.34±0.27^{b,A}	3.27±0.26^{c,A}	8.58±0.26^{a,A}				
	1	2.69±0.22^{b}	4.33±0.22^{a,B}	2.49±0.22^{b,B}	4.82±0.21^{a,B}				
	4	2.50±0.22^{d}	5.18±0.22^{b,A}	3.82±0.21^{c,A}	8.30±0.21^{a,A}	<0.001	<0.001	<0.001	a
	8	2.42±0.22^{d}	5.28±0.22^{b,A}	3.61±0.21^{c,A}	8.02±0.21^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
CLA[10]	-10	0.64±0.08	0.72±0.07	0.69±0.07^{B}	0.69±0.07^{B}	<0.001	<0.001	<0.001	a
	-7	0.75±0.08^{b}	0.56±0.07^{b}	1.66±0.07^{a,A}	1.69±0.07^{a,A}				
	1	0.37±0.08	0.34±0.08	0.50±0.08^{B}	0.54±0.08^{B}				
	4	0.44±0.08^{b}	0.48±0.08^{b}	1.61±0.08^{a,A}	1.45±0.08^{a,A}	<0.001	<0.001	<0.001	
	8	0.50±0.08^{b}	0.42±0.08^{b}	1.70±0.08^{a,A}	1.60±0.08^{a,A}				
trans-fatty acids[11]	-10	1.85±0.24	1.74±0.22	2.05±0.21	1.62±0.21^{B}	0.5	0.05	0.2	
	-7	2.25±0.24	1.64±0.22	2.11±0.21	2.22±0.21^{A}				
	1	0.67±0.55^{B}	0.72±0.54^{B}	0.65±0.54^{B}	0.63±0.51^{B}				
	4	2.08±0.55^{AB}	2.29±0.54^{A}	2.85±0.51^{A}	2.30±0.51^{A}	0.6	<0.001	0.3	
	8	3.61±0.55^{B}	1.67±0.54^{AB}	3.01±0.51^{A}	3.27±0.51^{A}				
Sum of n-3	-10	0.40±0.16	0.58±0.14^{B}	0.40±0.14	0.41±0.14^{B}	<0.001	<0.001	<0.001	
fatty acids[12]	-7	0.39±0.16^{c}	3.11±0.14^{b,A}	0.42±0.14^{c}	4.15±0.14^{a,A}				
	1	0.22±0.11^{b}	1.66±0.11^{a,B}	0.23±0.11^{b}	1.92±0.10^{a,B}				
	4	0.20±0.11^{c}	2.36±0.11^{b,A}	0.40±0.10^{c}	3.66±0.10^{a,A}	<0.001	<0.001	<0.001	a
	8	0.16±0.11^{c}	2.39±0.11^{b,A}	0.34±0.10^{c}	3.49±0.10^{a,A}				
Sum of n-6	-10	2.92±0.19	2.87±0.17^{B}	2.56±0.16^{B}	2.74±0.16^{B}	<0.001	<0.001	<0.001	
fatty acids[13]	-7	2.73±0.19^{c}	3.71±0.17^{b,A}	3.15±0.16^{bc,A}	4.80±0.16^{a,A}				
	1	3.21±0.17$^{ab,\ A}$	3.36±0.17^{ab}	2.89±0.17^{b,B}	3.67±0.16^{a,B}				
	4	2.65±0.17^{c,B}	3.20±0.17^{bc}	3.72±0.16^{b,A}	5.00±0.16^{a,A}	<0.001	<0.001	<0.001	a
	8	2.55±0.17^{c,B}	3.38±0.17^{b}	3.55±0.16^{b,A}	4.90±0.16^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
Ratio n-6 to n-3	-10	7.22±0.40	6.93±0.37^{A}	6.43±0.35^{B}	6.92±0.35^{A}	<0.001	<0.001	<0.001	a
	-7	6.85±0.40^{a}	1.47±0.37^{b,B}	8.22±0.35^{a,A}	1.04±0.35^{b,B}				
	1	8.82±0.42^{a}	2.34±0.42^{b}	10.19±0.40^{a,A}	1.82±0.39^{b}				
	4	7.67±0.42^{a}	1.62±0.42^{b}	8.41±0.40^{a,B}	1.24±0.39^{b}	<0.001	<0.001	0.6	a
	8	8.34±0.42^{a}	1.60±0.42^{b}	9.05±0.40^{a,AB}	1.28±0.39^{b}				
<16 carbons	-10	31.7 ±1.0	32.5 ±0.9	34.2 ±0.9^{A}	31.8 ±0.9^{A}	<0.001	<0.001	<0.001	a, c
	-7	32.1 ±1.0^{a}	30.9 ±0.9^{a}	24.2 ±0.9^{b,B}	23.7 ±0.9^{b,B}				
	1	28.2 ±1.0^{B}	26.2 ±1.0^{B}	26.5 ±1.0	24.6 ±1.0^{AB}				
	4	28.9 ±1.0^{a,B}	27.0 ±1.0^{ab,B}	23.5 ±1.0^{bc}	22.2 ±1.0^{c,B}	<0.001	<0.001	<0.01	a
	8	32.7 ±1.0^{a,A}	33.8 ±1.0^{a,A}	26.1 ±1.0^{b}	25.7 ±1.0^{b,A}				
16 carbons	-10	34.2 ±1.4	33.5 ±1.3	32.6 ±1.2^{B}	33.6 ±1.2^{A}	0.13	0.4	0.01	
	-7	33.7 ±1.4^{ab}	31.1 ±1.3^{b}	36.2 ±1.2^{a,A}	29.5 ±1.2^{b,B}				
	1	43.8 ±1.7^{b,A}	44.8 ±1.7^{ab,A}	50.1 ±1.7^{a,A}	46.9 ±1.6^{ab,A}				
	4	31.5 ±1.7^{B}	28.7 ±1.7^{B}	27.4 ±1.6^{B}	25.7 ±1.6^{B}	0.3	<0.001	0.01	
	8	32.3 ±1.7^{B}	31.8 ±1.7^{B}	30.7 ±1.6^{B}	27.3 ±1.6^{B}				
>16 carbons	-10	34.1 ±1.6	34.3 ±1.5^{B}	33.2 ±1.4^{B}	34.7 ±1.4^{B}	<0.01	<0.001	<0.001	
	-7	34.3 ±1.6^{b}	37.9 ±1.5^{b,A}	39.5 ±1.4^{b,A}	46.8 ±1.4^{a,A}				
	1	28.1 ±2.0^{B}	28.9 ±2.0^{B}	23.4 ±2.0^{B}	28.6 ±1.9^{B}				
	4	39.7 ±2.0^{c,A}	44.3 ±2.0^{bc,A}	49.1 ±1.9^{ab,A}	52.1 ±1.9^{a,A}	<0.001	<0.001	<0.001	a
	8	35.1 ±2.0^{b,A}	34.3 ±2.0^{b,B}	43.1 ±1.9^{a,A}	47.0 ±1.9^{a,A}				

Table 3.8 Continuation

Fatty acid[2], %	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
Desaturase index[14]									
14:1, *cis*-9	-10	0.11±0.01^{B}	0.10±0.01	0.10±0.01^{B}	0.11±0.01	<0.05	<0.001	<0.001	
	-7	0.14 ±0.01^{ab,A}	0.09±0.01^{c}	0.16±0.01^{a,A}	0.11±0.01^{bc}				
	1	0.11±0.01^{a,A}	0.10±0.01^{ab,A}	0.08±0.01^{bc}	0.07±0.01^{c}				
	4	0.09±0.01^{a,B}	0.09±0.01^{ab,AB}	0.07±0.01^{bc}	0.06±0.01^{c}	<0.001	<0.001	0.2	
	8	0.10±0.01^{a,AB}	0.07±0.01^{ab,B}	0.08±0.01^{ab}	0.07±0.01^{b}				
16:1, *cis*-9	-10	0.06±0.01^{B}	0.05±0.01	0.06±0.01^{B}	0.06±0.01	<0.01	<0.001	<0.001	
	-7	0.07±0.01^{b,A}	0.05±0.01^{b}	0.10±0.01^{a,A}	0.06±0.01^{b}				
	1	0.07±0.00^{a}	0.06±0.00^{ab,A}	0.06±0.00^{ab,AB}	0.05±0.00^{b}				
	4	0.07±0.00^{a}	0.07±0.00^{ab,A}	0.06±0.00^{ab,A}	0.05±0.00^{b}	<0.001	<0.001	0.7	
	8	0.06±0.00^{a}	0.04±0.00^{ab,B}	0.05±0.00^{ab,B}	0.04±0.00^{b}				
18:1, *cis*-9	-10	0.72±0.01	0.70±0.01	0.72±0.01	0.71±0.01^{A}	<0.05	<0.05	0.01	a
	-7	0.72±0.01^{ab}	0.68±0.01^{bc}	0.73±0.01^{a}	0.66±0.01^{c,B}				
	1	0.77±0.01^{a}	0.71±0.01^{ab}	0.69±0.01^{b}	0.66±0.01^{b}				
	4	0.75±0.01^{a}	0.73±0.01^{ab}	0.70±0.01^{bc}	0.66±0.01^{c}	<0.001	<0.05	0.6	a
	8	0.74±0.01^{a}	0.70±0.01^{ab}	0.66±0.01^{b}	0.66±0.01^{b}				
18:2, *cis*-9,*trans*-11	-10	0.23±0.03	0.26±0.03	0.22±0.03^{B}	0.31±0.03	<0.01	0.13	0.06	a
CLA	-7	0.22±0.03^{b}	0.23±0.03^{b}	0.37±0.03^{a,A}	0.35±0.03^{a}				
	1	0.30±0.03^{A}	0.26±0.03^{A}	0.32±0.02	0.35±0.02^{A}				
	4	0.19±0.03^{b,B}	0.19±0.03^{b,B}	0.31±0.02^{a}	0.30±0.02^{a,AB}	<0.001	<0.001	0.09	
	8	0.18±0.03^{b,B}	0.18±0.03^{b,B}	0.31±0.02^{a}	0.26±0.02^{ab,B}				

[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE.
Least squares means within a row with different lowercase letters (a-d) differ ($P < 0.05$).
Least squares means within a column with different uppercase letters (A-C) differ ($P < 0.05$).
[3]Data were analyzed for each observation period (late and early lactation), separately.
[4]Time as wk relative to calving; wk 1 represents the colostrum sample.
[5]Significant effect ($P < 0.05$): a = block; b=calving interval, c = projected milk yield during the 2nd lactation
[6]Sum of 4:0; 6:0; 8:0; 10:0; 11:0; 12:0; 13:0; 14:0; *iso*-14:0; 15:0; *iso*-15:0; *anteiso*-15:0; 16:0; *iso*-16:0; 17:0; *iso*-17:0; *anteiso*-17:0; 18:0; *iso*-18:0; 20:0; 21:0; 22:0 and 24:0.
[7]Sum of 10:1; 12:1; 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *cis*-12 and 20:1 *cis*-11.
[8]Sum of 18:2 *cis*-9,*cis*-12; 18:3 *cis*-6,*cis*-9,*cis*-12; 18:3 *cis*-9,*cis*-12,*cis*-15; 20:2 *cis*-11,*cis*-14; 20:3 *cis*-8,*cis*-11,*cis*-14; 20:4 *cis*-5,*cis*-8,*cis*-11,*cis*-14; 20:5 *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 and 22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[9]Sum of essential fatty acids, consisting of 18:2 *cis*-9,*cis*-12 and 18:3 *cis*-9,*cis*-12,*cis*-15.
[10]Sum of 18:2 *cis*-9,*trans*-11and 18:2 *trans*-10,*cis*-12.
[11]Sum of 16:1 *trans*-9 and 18:1 *trans*-vaccenic (*trans*-9 + 10 + 11).
[12]Sum of 18:3 *cis*-9,*cis*-12,*cis*-15; 20:5 *cis*-5,*cis*-8,*cis*-11, *cis*-14,*cis*-17 and 22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[13]Sum of 18:2 *cis*-9,*cis*-12; 18:3 *cis*-6,*cis*-9,*cis*-12; 20:2 *cis*-11,*cis*-14; 20:3 *cis*-8,*cis*-11,*cis*-14 and 20:4 *cis*-5,*cis*-8,*cis*-11,*cis*-14.
[14]Defined as [product of Δ^9-desaturase] ÷ [product of Δ^9-desaturase + substrate of Δ^9-desaturase]

Table 3.9 Yield of fatty acids in milk of cows daily abomasally supplemented either with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10) or the combination (EFA+CLA; n = 10) from wk 9 antepartum until wk 8 postpartum

		Treatment				*P*-values[3]			
Fatty acid[2], g/kg milk	Time[4], wk	CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
4:0	-10	1.54±0.15	1.65±0.14	1.49±0.13^{A}	1.60±0.13^{a}	<0.01	<0.001	<0.001	
	-7	1.34±0.15^{a}	1.47±0.14^{a}	0.38±0.13^{b,B}	0.63±0.13^{b,B}				
	1	1.19±0.17	0.68±0.17^{B}	0.79±0.17	0.72±0.16				
	4	1.52±0.17^{a}	1.49±0.17^{Aa}	0.74±0.16^{b}	0.94±0.16^{ab}	<0.001	0.01	0.11	
	8	1.31±0.17^{a}	1.32±0.17^{Aa}	0.55±0.16^{b}	0.64±0.16^{b}				
6:0	-10	1.02±0.10	1.08±0.09	1.05±0.08^{A}	1.08±0.08^{A}	0.001	<0.001	<0.001	a
	-7	0.90±0.10^{a}	1.03±0.09^{a}	0.25±0.08^{b,B}	0.37±0.08^{b,B}				
	1	0.71±0.10	0.40±0.09^{B}	0.46±0.09	0.42±0.09				
	4	0.91±0.10^{a}	0.95±0.09^{a,A}	0.32±0.09^{b}	0.40±0.09^{b}	<0.001	0.06	0.001	
	8	0.86±0.10^{a}	0.94±0.09^{a,A}	0.26±0.09^{b}	0.33±0.09^{b}				
8:0	-10	0.65±0.06	0.69±0.05	0.69±0.05^{A}	0.69±0.05^{A}	<0.001	<0.001	<0.001	
	-7	0.56±0.06^{a}	0.67±0.05^{a}	0.17±0.05^{b,B}	0.21±0.05^{b,B}				
	1	0.44±0.05^{a}	0.24±0.05^{b,B}	0.27±0.05^{ab}	0.24±0.05^{b}				
	4	0.56±0.05^{a}	0.56±0.05^{a,A}	0.17±0.05^{b}	0.21±0.05^{b}	<0.001	<0.05	<0.001	
	8	0.55±0.05^{a}	0.62±0.05^{a,A}	0.16±0.05^{b}	0.20±0.05^{b}				
10:0	-10	1.44±0.14	1.62±0.13	1.65±0.12^{A}	1.60±0.12^{A}	<0.01	<0.001	<0.001	a
	-7	1.26±0.14^{a}	1.56±0.13^{a}	0.43±0.12^{b,B}	0.51±0.12^{b,B}				
	1	0.98±0.12	0.55±0.12^{B}	0.65±0.12	0.56±0.11				
	4	1.14±0.12^{a}	1.12±0.12^{a,A}	0.35±0.11^{b}	0.42±0.11^{b}	<0.001	0.07	<0.001	
	8	1.25±0.12^{a}	1.44±0.12^{a,A}	0.35±0.11^{b}	0.44±0.11^{b}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
10:1	-10	0.16±0.02	0.16±0.01	0.16±0.01^{A}	0.17±0.01^{A}	0.001	<0.001	<0.001	
	-7	0.16±0.02^{a}	0.15±0.01^{a}	0.04±0.01^{b,B}	0.04±0.01^{b,B}				
	1	0.06±0.01^{a,B}	0.03±0.01^{ab,B}	0.02±0.01^{b}	0.02±0.01^{b}				
	4	0.10±0.01^{a,A}	0.09±0.01^{a,A}	0.02±0.01^{b}	0.02±0.01^{b}	<0.001	<0.001	<0.001	
	8	0.11±0.01^{a,A}	0.11±0.01^{a,A}	0.02±0.01^{b}	0.02±0.01^{b}				
11:0	-10	0.04±0.01^{A}	0.03±0.01	0.05±0.01^{A}	0.03±0.01^{A}	0.25	<0.001	<0.001	a
	-7	0.03±0.01^{ab,B}	0.03±0.01^{a}	0.01±0.01^{b,B}	0.01±0.01^{b,B}				
	1	0.01±0.00^{B}	0.01±0.00^{C}	0.01±0.00	0.01±0.00				
	4	0.03±0.00^{a,A}	0.03±0.00^{a,B}	0.01±0.00^{b}	0.01±0.00^{b}	<0.001	<0.001	<0.001	
	8	0.03±0.00^{a,A}	0.04±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}				
12:0	-10	1.88±0.17^{B}	1.99±0.15	1.99±0.15^{A}	1.93±0.15^{A}	<0.001	<0.001	<0.001	a
	-7	2.29±0.17^{a,A}	1.90±0.15^{a}	0.71±0.15^{b,B}	0.74±0.15^{b,B}				
	1	2.00±0.19^{a}	0.88±0.18^{b,B}	1.06±0.18^{b,A}	0.92±0.17^{b}				
	4	1.71±0.19^{a}	1.25±0.18^{a,AB}	0.44±0.17^{b,B}	0.54±0.17^{b}	<0.001	0.12	<0.05	
	8	1.90±0.19^{a}	1.64±0.18^{a,A}	0.51±0.17^{b,AB}	0.57±0.17^{b}				
12:1	-10	0.05±0.01^{B}	0.05±0.00	0.05±0.00^{A}	0.05±0.00^{A}	<0.001	<0.001	<0.001	a
	-7	0.07±0.01^{a,A}	0.04±0.00^{b}	0.02±0.00^{c,B}	0.02±0.00^{c,B}				
	1	0.04±0.00^{a}	0.01±0.00^{b,B}	0.01±0.00^{b}	0.01±0.00^{b}				
	4	0.03±0.00^{a}	0.02±0.00^{a,AB}	0.00±0.00^{b}	0.00±0.00^{b}	<0.001	<0.05	<0.05	
	8	0.04±0.00^{a}	0.03±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
13:0	-10	0.06±0.01^{A}	0.05±0.01	0.07±0.01^{A}	0.05±0.01^{A}	0.10	<0.001	<0.001	a
	-7	0.05±0.01^{a,B}	0.05±0.01^{a}	0.02±0.01^{b,B}	0.02±0.01^{b,B}				
	1	0.03±0.00^{a,B}	0.02±0.00^{b,B}	0.02±0.00^{ab}	0.01±0.00^{b}				
	4	0.04±0.00^{a,AB}	0.04±0.00^{a,A}	0.02±0.00^{b}	0.02±0.00^{b}	<0.001	<0.001	<0.01	
	8	0.05±0.00^{a,A}	0.05±0.00^{a,A}	0.02±0.00^{b}	0.02±0.00^{b}				
14:0	-10	5.46±0.44	5.67±0.40	5.51±0.38^{A}	5.55±0.38^{A}	0.01	<0.001	<0.01	a
	-7	5.22±0.44^{a}	5.17±0.40^{a}	2.94±0.38^{b,B}	3.06±0.38^{b,B}				
	1	7.10±0.68^{a,A}	3.79±0.67^{b}	5.18±0.68^{ab,A}	4.40±0.64^{b,A}				
	4	4.18±0.68^{a,B}	3.63±0.67^{ab}	1.67±0.64^{b,B}	2.03±0.64^{ab,B}	0.001	<0.001	0.11	
	8	4.46±0.68^{B}	4.27±0.67	2.08±0.64^{B}	2.07±0.64^{B}				
iso-14:0	-10	0.04±0.01	0.04±0.01	0.04±0.01	0.05±0.01^{A}	0.11	0.35	0.01	a
	-7	0.06±0.01^{a}	0.05±0.01^{ab}	0.02±0.01^{b}	0.03±0.01^{ab,B}				
	1	0.03±0.00^{a}	0.02±0.00^{ab}	0.02±0.00^{b}	0.01±0.00^{b}				
	4	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	<0.01	0.41	0.68	
	8	0.04±0.00^{a}	0.02±0.00^{ab}	0.02±0.00^{b}	0.02±0.00^{b}				
14:1, *cis*-9	-10	0.68±0.07	0.63±0.07	0.62±0.06	0.67±0.06^{A}	0.09	<0.05	<0.01	a
	-7	0.81±0.07^{a}	0.54±0.07^{ab}	0.55±0.06^{ab}	0.40±0.06^{b,B}				
	1	0.77±0.06^{a,A}	0.41±0.06^{b}	0.45±0.06^{b,A}	0.33±0.05^{b,A}				
	4	0.42±0.06^{a,B}	0.34±0.06^{ab}	0.13±0.05^{c,B}	0.13±0.05^{bc,AB}	<0.001	<0.001	0.23	
	8	0.46±0.06^{a,B}	0.34±0.06^{ab}	0.18±0.05^{b,B}	0.15±0.05^{b,B}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
15:0	-10	0.65±0.06	0.55±0.05	0.60±0.05^{A}	0.58±0.05^{A}	0.06	<0.001	0.01	a
	-7	0.54±0.06^{a}	0.52±0.05^{a}	0.31±0.05^{b,B}	0.32±0.05^{b,B}				
	1	0.44±0.05^{a}	0.23±0.05^{b,B}	0.26±0.05^{b}	0.23±0.04^{b}				
	4	0.41±0.05^{a}	0.38±0.05^{ab,A}	0.19±0.04^{c}	0.22±0.04^{bc}	<0.001	0.58	0.08	
	8	0.44±0.05^{a}	0.42±0.05^{a,A}	0.21±0.04^{b}	0.22±0.04^{b}				
iso-15:0	-10	0.09±0.01	0.08±0.01	0.08±0.01^{A}	0.08±0.01^{A}	<0.05	<0.001	<0.01	a
	-7	0.10±0.01^{a}	0.07±0.01^{ab}	0.05±0.01^{b,B}	0.05±0.01^{b,B}				
	1	0.11±0.01^{a,A}	0.05±0.01^{b}	0.05±0.01^{b}	0.04±0.01^{b}				
	4	0.06±0.01^{B}	0.05±0.01	0.03±0.01	0.03±0.01	<0.001	0.01	0.11	a
	8	0.06±0.01^{B}	0.05±0.01	0.03±0.01	0.03±0.01				
anteiso-15:0	-10	0.27±0.02	0.26±0.02	0.26±0.02^{A}	0.28±0.02^{A}	<0.01	<0.001	<0.001	a
	-7	0.27±0.02^{a}	0.26±0.02^{a}	0.13±0.02^{b,B}	0.15±0.02^{b,B}				
	1	0.14±0.02^{a}	0.07±0.02^{b,B}	0.07±0.02^{b}	0.06±0.02^{b}				
	4	0.17±0.02^{a}	0.16±0.02^{ab,A}	0.09±0.02^{c}	0.10±0.02^{bc}	<0.001	<0.001	0.30	
	8	0.17±0.02^{a}	0.16±0.02^{a,A}	0.10±0.02^{b}	0.10±0.02^{b}				
16:0	-10	14.4 ±1.4	14.1 ±1.3	13.0 ±1.2^{A}	14.48±1.20^{A}	<0.05	<0.001	<0.05	a
	-7	13.4 ±1.4^{a}	12.7 ±1.3^{ab}	8.03±1.20^{bc,B}	7.54±1.20^{c,B}				
	1	19.5 ±1.9^{a,A}	11.9 ±1.8^{b}	16.4 ±1.8^{ab,A}	14.7 ±1.7^{ab,A}				
	4	11.3 ±1.9^{a,B}	10.2 ±1.8^{ab}	4.62±1.73^{b,B}	5.56±1.73^{ab,B}	<0.01	<0.001	0.09	
	8	11.1 ±1.9^{B}	10.3 ±1.8	5.09±1.73^{B}	4.75±1.73^{B}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-16:0	-10	0.12±0.02	0.12±0.01	0.10±0.01^{A}	0.14±0.01^{A}	<0.01	0.06	<0.01	a
	-7	0.14±0.02^{a}	0.13±0.01^{ab}	0.06±0.01^{c,B}	0.08±0.01^{bc,B}				
	1	0.10±0.01^{a}	0.06±0.01^{b}	0.05±0.01^{b}	0.05±0.01^{b}				
	4	0.09±0.01^{a}	0.08±0.01^{ab}	0.04±0.01^{b}	0.06±0.01^{ab}	<0.001	0.72	0.67	
	8	0.08±0.01	0.07±0.01	0.04±0.01	0.05±0.01				
16:1, *cis*-9	-10	0.85±0.12	0.76±0.11	0.84±0.11	0.88±0.11^{A}	0.33	0.16	<0.05	
	-7	0.95±0.12^{a}	0.65±0.11^{ab}	0.90±0.11^{ab}	0.50±0.11^{b,B}				
	1	1.51±0.12^{a,A}	0.79±0.12^{b}	1.01±0.12^{b,A}	0.75±0.12^{b,A}				
	4	0.88±0.12^{a,B}	0.73±0.12^{ab}	0.30±0.12^{b,B}	0.31±0.12^{b,B}	<0.001	<0.001	0.18	a
	8	0.69±0.12^{a,B}	0.48±0.12^{ab}	0.25±0.12^{ab,B}	0.21±0.12^{b,B}				
16:1, *trans*-9	-10	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00^{A}	0.27	<0.01	0.22	a, b
	-7	0.02±0.00	0.02±0.00	0.02±0.00	0.01±0.00^{B}				
	1	0.02±0.00^{a}	0.01±0.00^{b}	0.02±0.00^{ab}	0.01±0.00^{b}				
	4	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	<0.01	<0.05	0.70	a
	8	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00				
17:0	-10	0.37±0.03	0.33±0.03	0.34±0.03^{A}	0.35±0.03^{A}	0.07	<0.001	0.01	a
	-7	0.31±0.03^{ab}	0.34±0.03^{a}	0.20±0.03^{c,B}	0.23±0.03^{bc,B}				
	1	0.39±0.04^{a}	0.23±0.04^{b}	0.26±0.04^{ab}	0.25±0.04^{ab}				
	4	0.32±0.04	0.34±0.04	0.21±0.04	0.24±0.04	<0.05	0.13	0.31	a
	8	0.27±0.04	0.27±0.04	0.18±0.04	0.19±0.04				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-17:0	-10	0.20±0.02	0.20±0.02	0.18±0.01^{A}	0.23±0.01^{A}	<0.01	<0.01	0.01	a
	-7	0.21±0.02^{a}	0.19±0.02^{ab}	0.13±0.01^{c,B}	0.14±0.01^{bc,B}				
	1	0.19±0.02^{a}	0.10±0.02^{b}	0.10±0.02^{b}	0.09±0.02^{b}				
	4	0.16±0.02^{a}	0.16±0.02^{ab}	0.09±0.02^{b}	0.11±0.02^{ab}	<0.001	0.50	0.45	a
	8	0.15±0.02	0.13±0.02	0.10±0.02	0.09±0.02				
anteiso-17:0	-10	0.28±0.02	0.28±0.02	0.27±0.02^{A}	0.30±0.02^{A}	0.01	<0.001	<0.01	a
	-7	0.28±0.02^{a}	0.29±0.02^{a}	0.15±0.02^{b,B}	0.18±0.02^{b,B}				
	1	0.24±0.03^{a}	0.12±0.02^{b,B}	0.12±0.02^{b}	0.13±0.02^{b}				
	4	0.23±0.03^{a}	0.21±0.02^{ab,A}	0.12±0.02^{c}	0.14±0.02^{bc}	<0.001	0.36	0.23	
	8	0.19±0.03	0.18±0.02^{AB}	0.12±0.02	0.12±0.02				
17:1, *cis*-9	-10	0.13±0.01	0.10±0.01	0.12±0.01^{A}	0.12±0.01^{AB}	0.11	<0.001	<0.01	
	-7	0.12±0.01^{a}	0.09±0.01^{ab}	0.09±0.01^{ab,B}	0.06±0.01^{b,}				
	1	0.19±0.02^{a,A}	0.08±0.02^{b,B}	0.09±0.02^{b}	0.07±0.02^{b}				
	4	0.16±0.02^{a,AB}	0.14±0.02^{a,A}	0.06±0.02^{b}	0.06±0.02^{b}	<0.001	<0.01	0.18	
	8	0.12±0.02^{a,B}	0.08±0.02^{ab,AB}	0.05±0.02^{b}	0.04±0.02^{b}				
18:0	-10	3.12±0.39	3.31±0.35	3.02±0.34^{A}	3.45±0.33	0.08	0.05	0.16	a
	-7	2.90±0.39^{ab}	3.57±0.35^{a}	1.88±0.34^{b,B}	2.72±0.33^{ab}				
	1	2.85±0.42	1.61±0.41	1.85±0.41^{B}	1.93±0.39				
	4	3.02±0.42	3.31±0.41	1.90±0.39^{A}	2.70±0.39	0.07	<0.05	0.39	
	8	2.43±0.42	2.42±0.41	1.72±0.39^{AB}	1.83±0.39				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
iso-18:0	-10	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00^{A}	0.55	0.18	0.16	a, c
	-7	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00^{B}				
	1	0.02±0.00^{a}	0.01±0.00^{b,B}	0.01±0.00^{ab}	0.01±0.00^{ab}				
	4	0.03±0.00^{a}	0.02±0.00^{ab,A}	0.01±0.00^{c}	0.02±0.00^{bc}	<0.001	<0.01	0.39	a
	8	0.02±0.00	0.01±0.00^{AB}	0.01±0.00	0.01±0.00				
18:1, *cis*-9	-10	7.65±0.68	7.56±0.62	7.26±0.59^{A}	8.35±0.59^{A}	0.10	<0.001	<0.05	
	-7	7.35±0.68^{a}	7.55±0.62^{a}	4.89±0.59^{b,B}	5.23±0.59^{ab,B}				
	1	9.15±0.96^{a}	4.21±0.95^{b,B}	4.03±0.95^{b}	3.76±0.90^{b}				
	4	9.06±0.96^{a}	8.97±0.95^{a,A}	4.50±0.90^{b}	5.12±0.90^{b}	<0.001	<0.01	0.19	
	8	6.52±0.96	5.51±0.95^{B}	3.39±0.90	3.43±0.90				
18:1, *cis*-11	-10	0.33±0.03	0.30±0.02	0.32±0.02^{A}	0.32±0.02^{A}	0.32	<0.001	0.06	a
	-7	0.28±0.03	0.30±0.02	0.22±0.02^{B}	0.21±0.02^{B}				
	1	0.40±0.04^{a}	0.19±0.04^{b,B}	0.19±0.04^{b}	0.17±0.04^{b}				
	4	0.43±0.04^{a}	0.39±0.04^{ab,A}	0.22±0.04^{c}	0.27±0.04^{bc}	<0.001	<0.01	0.33	
	8	0.31±0.04	0.27±0.04^{AB}	0.17±0.04	0.18±0.04				
18:1, *cis*-12	-10	0.15±0.01	0.15±0.01	0.13±0.01^{A}	0.16±0.01^{A}	0.08	<0.001	0.38	a
	-7	0.12±0.01	0.12±0.01	0.08±0.01^{B}	0.10±0.01^{B}				
	1	0.09±0.01^{a}	0.05±0.01^{b,B}	0.05±0.01^{b}	0.04±0.01^{b}				
	4	0.10±0.01	0.09±0.01^{A}	0.07±0.01	0.07±0.01	<0.01	<0.001	0.21	a
	8	0.08±0.01	0.09±0.01^{A}	0.07±0.01	0.07±0.01				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
18:1, *trans*-vaccenic	-10	0.83±0.08	0.76±0.08	0.79±0.07^{A}	0.71±0.07	0.11	0.01	<0.05	a
(*trans*-9 + 10 + 11)	-7	0.90±0.08^{a}	0.70±0.08^{ab}	0.50±0.07^{b,B}	0.60±0.07^{b}				
	1	0.41±0.11^{B}	0.16±0.11^{B}	0.23±0.11	0.20±0.11^{B}				
	4	0.82±0.11^{A}	0.76±0.11^{A}	0.52±0.11	0.48±0.11^{AB}	<0.05	<0.001	0.41	
	8	0.93±0.11^{Aa}	0.53±0.11^{ab,A}	0.51±0.11^{b}	0.55±0.11^{ab,A}				
18:2, *cis*-9,*cis*-12	-10	1.09±0.10	1.11±0.09^{B}	0.94±0.09^{A}	1.10±0.09	<0.001	0.44	<0.01	a
	-7	0.97±0.10^{bc}	1.50±0.09^{a,A}	0.71±0.09^{c,B}	1.23±0.09^{ab}				
	1	1.44±0.14^{a,A}	0.84±0.13^{ab}	0.82±0.13^{b}	1.03±0.13^{b}				
	4	0.93±0.14^{ab,B}	1.14±0.13^{a}	0.63±0.13^{b}	1.04±0.13^{ab}	<0.01	0.06	<0.05	a
	8	0.78±0.14^{B}	1.06±0.13	0.58±0.13	0.83±0.13				
18:2, *cis*-9,*trans*-11	-10	0.24±0.02	0.23±0.02	0.22±0.02^{B}	0.24±0.02^{B}	0.06	<0.05	0.01	
CLA	-7	0.25±0.02^{ab}	0.19±0.02^{b}	0.29±0.02^{a,A}	0.31±0.02^{a,A}				
	1	0.15±0.02^{a}	0.06±0.02^{b,B}	0.10±0.02^{ab,B}	0.10±0.02^{ab,B}				
	4	0.16±0.02	0.14±0.02^{A}	0.19±0.02^{A}	0.20±0.02^{A}	<0.01	<0.001	0.08	
	8	0.14±0.02^{ab}	0.11±0.02^{b,AB}	0.20±0.02^{a,A}	0.18±0.02^{ab,A}				
18:2, *trans*-10,*cis*-12	-10	0.04±0.01	0.06±0.01	0.05±0.01^{B}	0.07±0.01^{B}	<0.001	<0.01	<0.05	
CLA	-7	0.05±0.01^{b}	0.04±0.01^{b}	0.11±0.01^{a,A}	0.13±0.01^{a,A}				
	1	0.05±0.01	0.03±0.01	0.06±0.01^{B}	0.06±0.01^{B}				
	4	0.02±0.01^{b}	0.03±0.01^{b}	0.09±0.01^{a,A}	0.10±0.01^{a,A}	<0.001	0.18	<0.05	
	8	0.02±0.01^{b}	0.03±0.01^{b}	0.09±0.01^{a,AB}	0.09±0.01^{a,AB}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
18:3, *cis*-6,*cis*-9,	-10	0.03±0.00	0.02±0.00^{A}	0.02±0.00^{A}	0.02±0.00^{A}	0.29	<0.001	0.06	a
cis-12	-7	0.02±0.00	0.02±0.00^{B}	0.01±0.00^{B}	0.01±0.00^{B}				
	1	0.03±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}				
	4	0.02±0.00^{a,B}	0.02±0.00^{ab}	0.01±0.00^{ab}	0.01±0.00^{b}	<0.001	0.01	<0.01	a
	8	0.01±0.00^{B}	0.01±0.00	0.01±0.00	0.01±0.00				
18:3, *cis*-9,*cis*-12,	-10	0.13±0.07	0.20±0.07^{B}	0.13±0.06	0.15±0.06^{B}	<0.001	<0.001	<0.001	
cis-15	-7	0.13±0.07^{b}	1.31±0.07^{a,A}	0.09±0.06^{b}	1.08±0.06				
	1	0.08±0.06^{b}	0.38±0.06^{a,B}	0.03±0.06^{b}	0.48±0.05^{a,B}				
	4	0.07±0.06^{b}	0.86±0.06^{a,A}	0.06±0.05^{b}	0.76±0.05^{a,A}	<0.001	<0.001	<0.001	a
	8	0.05±0.06^{b}	0.76±0.06^{a,A}	0.05±0.05^{b}	0.57±0.05^{a,B}				
20:0	-10	0.04±0.01	0.05±0.00	0.04±0.00	0.05±0.00	0.18	0.13	0.73	a
	-7	0.04±0.01	0.04±0.00	0.03±0.00	0.04±0.00				
	1	0.04±0.01^{a}	0.02±0.01^{b}	0.02±0.01^{b}	0.02±0.00^{b}				
	4	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00	0.23	0.49	0.19	a
	8	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00				
20:1, *cis*-11	-10	0.03±0.00	0.03±0.00	0.03±0.00^{A}	0.03±0.00^{A}	0.19	<0.01	0.20	a
	-7	0.03±0.00^{ab}	0.03±0.00^{a}	0.02±0.00^{b,B}	0.02±0.00^{ab,B}				
	1	0.03±0.00^{a}	0.01±0.00^{b,B}	0.01±0.00^{b}	0.02±0.00^{b}				
	4	0.03±0.00^{ab}	0.03±0.00^{a,A}	0.02±0.00^{b}	0.02±0.00^{ab}	<0.01	<0.05	0.11	a
	8	0.02±0.00	0.02±0.00^{AB}	0.02±0.00	0.02±0.00				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
20:2, *cis*-11,*cis*-14	-10	0.02±0.00	0.01±0.00	0.02±0.00^{A}	0.02±0.00	0.29	<0.01	0.05	a
	-7	0.02±0.00	0.02±0.00	0.01±0.00^{B}	0.01±0.00				
	1	0.02±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}				
	4	0.01±0.00^{AB}	0.01±0.00	0.01±0.00	0.01±0.00	<0.01	0.72	0.25	a
	8	0.01±0.00^{B}	0.01±0.00	0.01±0.00	0.01±0.00				
20:3, *cis*-8,*cis*-11,	-10	0.05±0.00	0.05±0.00	0.04±0.00^{A}	0.04±0.00^{A}	0.001	<0.001	<0.01	
cis-14	-7	0.04±0.00^{a}	0.04±0.00^{a}	0.02±0.00^{b,B}	0.02±0.00^{b,B}				
	1	0.13±0.01^{a,A}	0.04±0.01^{b}	0.07±0.01^{b,A}	0.05±0.01^{b}				
	4	0.03±0.01^{B}	0.02±0.01	0.01±0.01^{B}	0.01±0.01	<0.01	<0.001	0.01	
	8	0.03±0.01^{B}	0.03±0.01	0.01±0.01^{B}	0.01±0.01				
20:4, *cis*-5,*cis*-8,	-10	0.06±0.01	0.05±0.01	0.05±0.01^{A}	0.06±0.01^{A}	<0.01	<0.001	<0.01	a
cis-11,*cis*-14	-7	0.05±0.01^{a}	0.05±0.01^{a}	0.02±0.01^{b,B}	0.02±0.01^{b,B}				
	1	0.15±0.02^{a,A}	0.05±0.02^{b}	0.08±0.02^{b,A}	0.08±0.02^{b,A}				
	4	0.05±0.02^{B}	0.04±0.02	0.02±0.02^{B}	0.02±0.02^{AB}	<0.05	<0.001	0.11	a
	8	0.03±0.02^{B}	0.04±0.02	0.01±0.02^{B}	0.01±0.02^{B}				
20:5, *cis*-5,*cis*-8,	-10	0.01±0.00	0.01±0.00^{B}	0.01±0.00	0.01±0.00^{B}	<0.001	<0.001	<0.001	a
cis-11,*cis*-14,*cis*-17	-7	0.01±0.00^{bc}	0.03±0.00^{a,A}	0.00±0.00^{c}	0.01±0.00^{b,A}				
	1	0.02±0.00^{bc}	0.03±0.00^{ab}	0.01±0.00^{c}	0.04±0.00^{a,A}				
	4	0.01±0.00^{b}	0.03±0.00^{a}	0.00±0.00^{b}	0.01±0.00^{b,B}	<0.001	<0.001	0.01	a
	8	0.00±0.00^{b}	0.03±0.00^{a}	0.00±0.00^{b}	0.01±0.00^{b,B}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
21:0	-10	0.01±0.00	0.01±0.00	0.01±0.00^{A}	0.01±0.00^{A}	<0.001	<0.001	<0.001	
	-7	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{b,B}	0.01±0.00^{b,B}				
	1	0.01±0.00^{A}	0.01±0.00	0.01±0.00	0.01±0.00^{A}				
	4	0.01±0.00^{AB}	0.01±0.00	0.00±0.00	0.01±0.00^{B}	<0.05	<0.01	0.33	a
	8	0.01±0.00^{B}	0.01±0.00	0.00±0.00	0.01±0.00^{B}				
22:0	-10	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00^{A}	<0.05	0.08	0.22	a, c
	-7	0.01±0.00^{ab}	0.02±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{ab,B}				
	1	0.02±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}				
	4	0.01±0.00^{B}	0.01±0.00	0.00±0.00	0.01±0.00	<0.05	<0.001	0.08	a
	8	0.01±0.00^{B}	0.01±0.00	0.01±0.00	0.01±0.00				
22:5, *cis*-7,*cis*-10,	-10	0.03±0.00	0.02±0.00^{B}	0.02±0.00^{A}	0.03±0.00	<0.001	0.45	<0.001	c
cis-13,*cis*-16,*cis*-19	-7	0.02±0.00^{b}	0.05±0.00^{a,A}	0.01±0.00^{c,B}	0.02±0.00^{b}				
	1	0.07±0.01^{ab,A}	0.06±0.01^{ab}	0.03±0.01^{b}	0.09±0.01^{a,A}				
	4	0.02±0.01^{B}	0.03±0.01	0.01±0.01	0.02±0.01^{B}	<0.01	<0.001	0.12	
	8	0.02±0.01^{ab,B}	0.05±0.01^{a}	0.01±0.01^{b}	0.02±0.01^{ab,B}				
24:0	-10	0.02±0.00	0.02±0.00	0.02±0.00^{A}	0.02±0.00	0.01	0.10	<0.05	a
	-7	0.02±0.00^{a}	0.02±0.00^{ab}	0.01±0.00^{c,B}	0.01±0.00^{bc}				
	1	0.03±0.00^{a,A}	0.01±0.00^{b}	0.01±0.00^{b}	0.01±0.00^{b}				
	4	0.01±0.00^{B}	0.01±0.00	0.01±0.00	0.01±0.00	<0.01	<0.01	0.19	a
	8	0.01±0.00^{B}	0.01±0.00	0.01±0.00	0.01±0.00				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
Summation									
SFA[6]	-10	31.8 ±2.7	32.2 ±2.5	30.5 ±2.4^{A}	32.6 ±2.4^{A}	0.01	<0.001	<0.01	a
	-7	29.9 ±2.7^{a}	30.1 ±2.5^{a}	15.9 ±2.4^{b,B}	17.1 ±2.4^{b,B}				
	1	36.6 ±3.5^{a}	21.0 ±3.5^{b}	27.7 ±3.5^{ab,A}	24.8 ±3.3^{ab,A}				
	4	26.0 ±3.5^{a}	24.0 ±3.5^{a}	11.1 ±3.3^{b,B}	13.8 ±3.3^{ab,B}	<0.001	<0.001	0.07	
	8	25.4 ±3.5^{a}	24.4 ±3.5^{ab}	11.6 ±3.3^{c,B}	11.7 ±3.3^{bc,B}				
MUFA[7]	-10	10.0 ±0.8	9.74±0.76	9.52±0.72^{A}	10.7 ±0.7^{A}	0.12	<0.001	0.01	
	-7	9.90±0.83^{a}	9.48±0.76^{ab}	6.81±0.72^{bc,B}	6.58±0.72^{c,B}				
	1	12.2 ±1.2^{a}	5.78±1.15^{b,B}	5.87±1.15^{b}	5.17±1.09^{b}				
	4	11.2 ±1.2^{a}	10.8 ±1.1^{a,A}	5.32±1.09^{b}	6.00±1.09^{b}	<0.001	<0.05	0.16	
	8	8.35±1.16^{a}	6.9 ±1.1^{ab,AB}	4.15±1.09^{b}	4.13±1.09^{b}				
PUFA[8]	-10	1.41±0.17	1.48±0.15^{B}	1.22±0.14	1.42±0.14^{B}	<0.001	<0.001	<0.001	a
	-7	1.26±0.17^{c}	3.02±0.15^{a,A}	0.87±0.14^{c}	2.41±0.14^{b,A}				
	1	1.93±0.21^{a,A}	1.41±0.21^{ab,B}	1.07±0.21^{b}	1.78±0.20^{ab}				
	4	1.13±0.21^{bc,B}	2.14±0.21^{a,A}	0.73±0.20^{c}	1.90±0.20^{ab}	<0.001	0.14	<0.01	a
	8	0.93±0.21^{bc,B}	1.98±0.21^{a,AB}	0.67±0.20^{c}	1.47±0.20^{ab}				
EFA[9]	-10	1.21±0.16	1.31±0.14^{B}	1.07±0.14	1.24±0.13^{B}	<0.001	<0.001	<0.001	a
	-7	1.10±0.16^{b}	2.81±0.14^{a,A}	0.80±0.14^{b}	2.31±0.13^{a,A}				
	1	1.52±0.18^{a,A}	1.22±0.17^{ab,B}	0.86±0.17^{b}	1.51±0.16^{a}				
	4	0.99±0.18^{b,AB}	1.99±0.17^{a,A}	0.68±0.16^{b}	1.80±0.16^{a}	<0.001	0.25	<0.01	a
	8	0.82±0.18^{bc,B}	1.82±0.17^{a,A}	0.62±0.16^{c}	1.40±0.16^{ab}				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
CLA[10]	-10	0.27±0.03	0.29±0.03	0.27±0.03^{B}	0.31±0.03^{B}	<0.01	0.01	0.01	
	-7	0.30±0.03^{bc}	0.23±0.03^{c}	0.41±0.03^{ab,A}	0.44±0.03^{a,A}				
	1	0.20±0.03	0.10±0.03	0.16±0.03^{B}	0.16±0.03^{B}				
	4	0.18±0.03^{b}	0.17±0.03^{b}	0.29±0.03^{a,A}	0.30±0.03^{a,A}	<0.001	<0.001	<0.05	
	8	0.16±0.03^{b}	0.14±0.03^{b}	0.29±0.03^{a,A}	0.27±0.03^{a,A}				
trans-fatty acids[11]	-10	0.85±0.09	0.79±0.08	0.81±0.07^{A}	0.73±0.07	0.10	0.01	<0.05	a
	-7	0.92±0.09^{a}	0.72±0.08^{ab}	0.51±0.07^{b,B}	0.61±0.07^{b}				
	1	0.43±0.11^{B}	0.18±0.11^{B}	0.25±0.11	0.21±0.11^{B}				
	4	0.83±0.11^{A}	0.78±0.11^{A}	0.53±0.11	0.49±0.11^{AB}	<0.05	<0.001	0.42	
	8	0.95±0.11^{a,A}	0.54±0.11^{ab,A}	0.52±0.11^{b}	0.56±0.11^{ab,A}				
Sum of n-3	-10	0.17±0.08	0.23±0.07^{B}	0.16±0.07	0.18±0.07^{B}	<0.001	<0.001	<0.001	
fatty acids[12]	-7	0.16±0.08^{c}	1.40±0.07^{a,A}	0.11±0.07^{c}	1.11±0.07^{a,A}				
	1	0.17±0.07^{b}	0.47±0.07^{a,B}	0.08±0.07^{b}	0.61±0.06^{a}				
	4	0.09±0.07^{b}	0.92±0.07^{a,A}	0.07±0.06^{b}	0.79±0.06^{a}	<0.001	0.01	0.001	a
	8	0.07±0.07^{b}	0.84±0.07^{a,A}	0.05±0.06^{b}	0.61±0.06^{a}				
Sum of n-6	-10	1.24±0.11	1.25±0.10^{B}	1.06±0.09^{A}	1.24±0.09	<0.001	0.97	<0.01	
fatty acids[13]	-7	1.11±0.11^{bc}	1.63±0.10^{a,A}	0.77±0.09^{c,B}	1.29±0.09^{ab}				
	1	1.77±0.16^{a,A}	0.94±0.16^{b}	0.99±0.16^{b}	1.17±0.15^{b}				
	4	1.03±0.16^{B}	1.22±0.16	0.67±0.15	1.10±0.15	<0.01	0.01	<0.05	a
	8	0.86±0.16^{B}	1.15±0.16	0.61±0.15	0.87±0.15				

Table 3.9 Continuation

Fatty acid[2], g/kg milk	Time[4], wk	Treatment				*P*-values[3]			
		CTRL	EFA	CLA	EFA+CLA	Treatment	Time	Treatment × time	Additional effect[5]
<16 carbons	-10	14.0 ±1.1	14.6 ±1.0	14.3 ±1.0^{A}	14.4 ±1.0^{A}	<0.01	<0.001	<0.001	a
	-7	13.7 ±1.1^{a}	13.5 ±1.0^{a}	6.03±0.98^{b,B}	6.55±0.98^{b,B}				
	1	14.1 ±1.4^{a}	7.39±1.35^{b}	9.33±1.35^{ab,A}	8.00±1.27^{b}				
	4	11.3 ±1.4^{a}	10.1 ±1.3^{a}	4.17±1.28^{b,B}	5.09±1.27^{b}	<0.001	0.06	<0.05	
	8	11.7 ±1.4^{a}	11.4 ±1.3^{a}	4.51±1.28^{b,B}	4.83±1.27^{b}				
16 carbons	-10	15.4 ±1.5	15.1 ±1.4	14.0 ±1.3^{A}	15.5 ±1.3^{A}	0.06	<0.001	<0.05	a
	-7	14.5 ±1.5^{a}	13.5 ±1.4^{ab}	9.01±1.29^{bc,B}	8.14±1.29^{c,B}				
	1	21.1 ±2.0^{a,A}	12.8 ±1.9^{b}	17.5 ±1.9^{ab,A}	15.5 ±1.8^{ab,A}				
	4	12.3 ±2.0^{a,B}	11.0 ±1.9^{ab}	4.97±1.84^{b,B}	5.94±1.83^{ab,B}	<0.01	<0.001	0.09	
	8	11.9 ±2.0^{B}	10.9 ±1.9	5.40±1.84^{B}	5.01±1.83^{B}				
>16 carbons	-10	14.9 ±1.3	14.9 ±1.2	14.0 ±1.1^{A}	15.9 ±1.1^{A}	<0.05	<0.05	<0.05	
	-7	14.2 ±1.3^{a}	16.6 ±1.2^{a}	9.50±1.12^{b,B}	12.4 ±1.1^{ab,B}				
	1	16.2 ±1.7^{a}	8.30±1.71^{b,B}	8.23±1.71^{b}	8.65±1.62^{b}				
	4	15.7 ±1.7^{a}	16.8 ±1.7^{a,A}	8.79±1.62^{b}	11.5 ±1.6^{ab}	<0.001	<0.05	0.18	
	8	12.2 ±1.7	11.7 ±1.7^{AB}	7.33±1.62	8.32±1.62				

[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE.
Least squares means within a row with different lowercase letters (a-d) differ ($P < 0.05$).
Least squares means within a column with different uppercase letters (A-C) differ ($P < 0.05$).
[3]Data were analyzed for each observation period (late and early lactation), separately.
[4]Time as wk relative to calving; wk 1 represents the colostrum sample.
[5]Significant effect ($P < 0.05$): a = block; b=calving interval, c = projected milk yield during the 2nd lactation
[6]Sum of 4:0; 6:0; 8:0; 10:0; 11:0; 12:0; 13:0; 14:0; *iso*-14:0; 15:0; *iso*-15:0; *anteiso*-15:0; 16:0; *iso*-16:0; 17:0; *iso*-17:0; *anteiso*-17:0; 18:0; *iso*-18:0; 20:0; 21:0; 22:0 and 24:0.

[7]Sum of 10:1; 12:1; 14:1 *cis*-9; 16:1 *cis*-9; 17:1 *cis*-9; 18:1 *cis*-9; 18:1 *cis*-11; 18:1 *cis*-12 and 20:1 *cis*-11.
[8]Sum of 18:2 *cis*-9,*cis*-12; 18:3 *cis*-6,*cis*-9,*cis*-12; 18:3 *cis*-9,*cis*-12,*cis*-15; 20:2 *cis*-11,*cis*-14; 20:3 *cis*-8,*cis*-11,*cis*-14; 20:4 *cis*-5,*cis*-8,*cis*-11,*cis*-14; 20:5 *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 and 22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[9]Sum of essential fatty acids, consisting of 18:2 *cis*-9,*cis*-12 and 18:3 *cis*-9,*cis*-12,*cis*-15.
[10]Sum of 18:2 *cis*-9,*trans*-11and 18:2 *trans*-10,*cis*-12.
[11]Sum of 16:1 *trans*-9 and 18:1 *trans*-vaccenic (*trans*-9 + 10 + 11).
[12]Sum of 18:3 *cis*-9,*cis*-12,*cis*-15; 20:5 *cis*-5,*cis*-8,*cis*-11, *cis*-14,*cis*-17 and 22:5 *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19.
[13]Sum of 18:2 *cis*-9,*cis*-12; 18:3 *cis*-6,*cis*-9,*cis*-12; 20:2 *cis*-11,*cis*-14; 20:3 *cis*-8,*cis*-11,*cis*-14 and 20:4 *cis*-5,*cis*-8,*cis*-11,*cis*-14.

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4. MANUSCRIPT 2

Glucose metabolism and the somatotropic axis in dairy cows after abomasal infusion of essential fatty acids together with conjugated linoleic acid during late gestation and early lactation

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4.1 Abstract

Sufficient glucose availability is crucial for exploiting the genetic potential of milk production during early lactation, and endocrine changes are mainly related to repartitioning of nutrient supplies towards the mammary gland. Long-chain FA such as EFA and CLA have the potential to improve negative EB and modify endocrine changes. In the present study, the hypothesis that combined CLA and EFA treatment supports glucose metabolism around the time of calving and stimulates insulin action and the somatotropic axis in cows in an additive manner was tested. Rumen-cannulated German Holstein cows (n = 40) were investigated from wk 9 AP until wk 9 PP. The cows were abomasally supplemented with coconut oil (CTRL, 76 g/d), 78 g/d linseed and 4 g/d safflower oil (EFA), Lutalin (CLA, isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, each 10 g/d) or the combination of EFA+CLA. Blood samples were collected several times AP and PP to determine the concentrations of plasma metabolites and hormones related to glucose metabolism and the somatotropic axis. Liver tissue samples were collected several d AP and PP to measure glycogen concentration and the mRNA abundance of genes related to gluconeogenesis and the somatotropic axis. On d 28 AP and 21 PP, eGP and GOx were measured via tracer technique. The concentration of plasma glucose was higher in CLA than in non-CLA-treated cows, and the plasma BHB concentration was higher in EFA than in non-EFA cows on d 21 PP. The eGP increased from AP to PP with elevated eGP in EFA- and decreased eGP in CLA-treated cows; GOx was lower in CLA than in CTRL on d 21 PP. The plasma insulin concentration decreased after calving in all groups and was higher in CLA than in non-CLA cows at several time points. Plasma glucagon and cortisol concentrations on d 21 PP were lower in CLA than non-CLA groups. The glucagon/insulin and glucose/insulin ratios were higher in CTRL than in CLA group during the transition period. Plasma IGF-I concentration was lower in EFA than non-EFA cows on d 42 AP and was higher during the dry period and early lactation in CLA than in non-CLA cows. The IGFBP-3/-2 ratio in blood plasma was higher in CLA than in non-CLA cows. Hepatic glycogen concentration on d 28 PP was higher, but the mRNA abundance of *PC* and *IGFBP2* was lower in CLA than non-CLA cows on d 1 PP. The EFA treatment decreased the mRNA abundance of *IGFBP3 AP and PCK1, PCK2, G6PC, PCCA, HMGCS2, IGFBP2*, and *INSR* at several time points PP. Results indicated elevated concentrations of plasma glucose and insulin along with the stimulation of the somatotropic axis in cows treated with CLA, whereas EFA treatment stimulated eGP but not mRNA abundance related to eGP PP. The systemic effects of the combined EFA+CLA treatment were very similar to those of CLA treatment, but the effects on hepatic gene expression partially corresponded to those of EFA treatment.

Key words: α-linolenic acid, conjugated linoleic acid, glucose metabolism, somatotropic axis

4.2 Introduction

The time period from late gestation to early lactation involves substantial metabolic and endocrine changes in dairy cows that are related to the repartitioning of the nutrient supply for milk production (Bauman 2000; Drackley et al. 2001; Gross and Bruckmaier 2019). Providing sufficient glucose is an important prerequisite for exploiting the genetic potential for milk synthesis (Bauman 2000; Drackley et al. 2001). Glucose is needed for the synthesis of lactose, which is the major osmoregulator of mammary water uptake and, consequently, milk volume (Linzell 1972) as well as milk fat synthesis (Grummer and Carroll 1991). Postcalving, glucose metabolism adapts by increasing eGP and decreasing peripheral glucose utilization in tissues other than the mammary gland (Drackley et al. 2001; Aschenbach et al. 2010; Hammon et al. 2016). There are marked changes in the hepatic gene expression of enzymes related to gluconeogenesis that reflect the increased glucose demands, and changes in substrate availability associated with the onset of lactation (Aschenbach et al. 2010; Donkin 2016; Hammon et al. 2016). The endocrine regulation of nutrition partitioning during the transition period and the glucose supply for milk synthesis involves insulin action and the somatotropic axis (Bauman 2000; Drackley et al. 2001; Lucy 2004). Insulin sensitivity is decreased, and the GH–IGF-I axis is uncoupled during early lactation to favor the mobilization of body energy reserves, and the provision of substrates, such as glucose, for milk production (Etherton and Bauman 1998; Drackley et al. 2001; De Koster and Opsomer 2013).

The feeding of various FA can relieve the energy load in dairy cows during early lactation. The supplementation of *trans*-10,*cis*-12 CLA causes milk fat depression, which has the potential to improve the EB in early lactation (Baumgard et al 2000; Odens et al. 2007). The CLA supplementation leads to nutrient repartitioning towards increased lactose release and decreased eGP, resulting in a glucose-sparing effect during early lactation (Hötger et al. 2013). Interestingly, *trans*-10,*cis*-12 CLA causes an insulin resistant state in rodent and human (Riserus et al. 2002; Halade et al. 2010; Bezan et al. 2018). The effects of CLA treatment on endocrine changes associated with nutrient partitioning and the gene expression of gluconeogenic enzymes in the liver of dairy cows are less clear. In addition, common rations for dairy cows contain high levels of corn silage in the TMR, providing forage with a high energy density but low amounts of fat and EFA with a high n-6/n-3 FA ratio (Chilliard et al. 2001; Barkema et al. 2015). Interestingly, n-3 FA supplementation improves insulin sensitivity in mice and in cattle (Pires and Grummer 2008; Fortin et al. 2010; Fan et al. 2020). The gene expression of enzymes related to gluconeogenesis seems to be under the control of long-chain FA (White et al. 2011), and n-3 FA stimulate whole-body glycogen storage (Clarke 2001).

The aim of the present study was to investigate the effect of combined CLA and EFA supplementation on glucose metabolism and the regulation of nutrition partitioning by the somatotropic axis in dairy cows during late gestation and early lactation. Previous findings within this project confirmed the improvement of the EB around the time of calving in cows associated with combined CLA and EFA supplementation (Vogel et al. 2020). Therefore, the tested hypothesis was that CLA and EFA treatments during the transition from late pregnancy to early lactation affect glucose metabolism and stimulate insulin action and the somatotropic axis, respectively, and that the combined EFA and CLA treatment may support these endocrine changes in an additive manner.

4.3 Materials and Methods

4.3.1 Animals, Husbandry, Fatty Acid Supplementation and Feeding

All experimental procedures were carried out in compliance with the German Animal Welfare Act and were approved by the animal ethics committee of the state of Mecklenburg-Western Pomerania, Germany (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern; LALLF M-V/TSD/7221.3-1-038/15).

A detailed description of the study design, feeding management, and diet composition was published recently (Vogel et al. 2020). Briefly, from December 2015 to September 2017 German Holstein cows (n = 40) were investigated in 5 blocks consisting of 8 cows (2 cows per group; 2 cows were removed from the evaluation because of premature calving). The German Holstein cows were purchased from a local farm (Agrarprodukte Dedelow GmbH, Prenzlau, Germany) in approximately wk 18 of gestation during their 2nd lactation and were kept in a free-stall barn at the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany. Before the beginning of the trial the cows were surgically fitted with rumen cannulas (#2C or #1C 4 in, Bar Diamond Inc., Parma, ID) as described previously (Haubold et al. 2020; Vogel et al. 2020). The cows were assigned to 4 supplementation groups exhibiting comparable projected milk production, BW, and calving interval. The cows were supplemented daily from 63 d AP until slaughter on d 63 PP with 1 of the 4 following treatments: 76 g/d coconut oil (**CTRL**, n = 9; Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard KG, Bad Ditzenbach, Germany); 78 g/d linseed plus 4 g/d safflower oil (**EFA**, n = 9; linseed oil, DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH, Münster, Germany; safflower oil, GEFRO Distelöl, GEFRO Reformversand Frommlet KG, Memmingen, Germany; linseed/safflower oil ratio = 19.5:1; n-6/n-3 FA ratio = 1:3); 38 g/d of Lutalin® (**CLA**, n = 10; 27.2% *cis*-9,*trans*-11 and 27.0% *trans*-10,*cis*-12 CLA in Lutalin®, BASF SE, Ludwigshafen, Germany); or 120 g/d of the mixture of linseed and safflower oil plus Lutalin® in the same mentioned quantities (**EFA+CLA**, n = 10).

During the dry period, each dose was halved. The amounts and FA composition of the daily infused supplements which are shown in **Table 3.1** and **Table 3.7** were recently evaluated in a companion dose-response study in mid-lactating dairy cows (Haubold et al. 2020). The treatments were abomasally infused twice a day (2 equal portions) at 0700 and 1630 h via infusion lines using 60-mL catheter tip syringes. All supplements were liquefied by heating to 38°C to allow infusion. The placement of the abomasal infusion line was confirmed weekly by palpation. Observations and sampling were performed from wk 10 before calving until wk 9 during the third lactation. At 40 ± 6 d AP (mean ± SD), the cows were dried off and from 10 d before until 1 d after parturition, the cows were housed in straw bedded calving boxes. The cows were slaughtered on d 63 ± 3 PP (mean ± SD).

The cows were fed a corn silage based TMR during late and early lactation (wk 22-6 AP and wk 1-9 PP) and during the dry period (wk 6-1 AP). We recently published the details of the feed sampling procedure and analyses in a companion paper (Vogel et al. 2020). The ingredients and chemical composition of the diets are shown **Table 3.2**. The major FA concentrations in the diets are shown in **Table 3.3**. The diets were provided *ad libitum* beginning at 0600 h and the cows had free access to water as well as tracemineralized salt blocks. After calving a calcium bolus (RUMINCa^{DL}, Wirtschaftsgenossenschaft Deutscher Tierärzte eG; Garbsen, Germany), and 300 mL/d 1,2-propanediol (Propylenglykol USP; Dr. Pieper Technologie- und Produktentwicklung GmbH, Wuthenow, Germany) were administered intraruminally on 3 consecutive days. Individual daily feed intake was recorded as the disappearance of feed from troughs connected to an electronic scale to which access was controlled by individual transponders (Institute for Agricultural Engineering and Animal Husbandry ILT, Bavarian State Research Center for Agriculture LfL, Freising, Germany). The cows were milked twice daily at 0630 and 1800 h, and the milk yield was recorded electronically.

4.3.2 Blood and Liver Tissue Sampling and Analyses

A detailed description of the sampling procedures was published in a companion paper (Vogel et al. 2020). Blood samples were collected 63, 42, 35, 28, 21, and 10 d before expected calving, 1 d after calving and then once weekly up to d 56 immediately after morning milking and before feeding via jugular vein puncture using a Vacuette system (Greiner Bio-One International GmbH, Kremsmünster, Austria) containing either K_3EDTA (1.8 g/L) for the analysis of hormones of the somatotropic axis or sodium fluoride (2-4 g/L) in combination with potassium oxalate (1-3 g/L) as an anticoagulant for the measurement of plasma metabolites. Immediately after collection, the samples were cooled on crushed ice and centrifuged at 1,500 × *g* (4°C, 20 min). The supernatant was harvested and stored at -20°C until analysis.

Plasma metabolites were analyzed using an automatic spectrophotometer (ABX Pentra 400; HORIBA ABX SAS, Montpellier, France) and specific kits for glucose (#A11A01667, hexokinase–glucose-6-phosphate dehydrogenase method; HORIBA ABX SAS, Montpellier, France) and BHB (#RB 1008, 3-hydroxybutyrate dehydrogenase method; Randox Laboratories Ltd., Crumlin, UK). The interassay variations were < 4% for glucose and < 5% for BHB when testing for control plasma with low, medium, and high concentrations. The concentrations of plasma insulin (#RIA-1257) and glucagon (#RIA-1258) were determined via RIA using kits from DRG Instruments GmbH (Marburg, Germany) adapted for cattle (Hammon et al. 2009). The intra- and interassay coefficients of variation were 3.7 and 5.5% for insulin and 4.6 and 13.4% for glucagon. Plasma cortisol concentrations were analyzed using a commercially available ELISA kit (#EIA1887; DRG Instruments GmbH, Marburg, Germany) according to the manufacturer's instructions. The assay was validated for use with bovine plasma (Weber et al. 2013b). The test sensitivity was 3.5 ng/mL, and the intra- and interassay coefficients of variation were 4.7 and 12.7%, respectively. Plasma GH and IGF-I were measured by radioimmunoassay as described previously (Vicari et al. 2008). Intra- and interassay coefficients of variation for GH and IGF-I RIA were below 10 and 15%, respectively. Concentrations of plasma IGFBP were analyzed via quantitative Western ligand blot analysis as previously described using plasma samples containing K_3-EDTA (Wirthgen et al. 2016). The intra- and interassay coefficients of variation were < 15% and < 20.0% for all IGFBP, respectively.

Liver biopsy samples were obtained after morning milking by needle biopsy under local anesthesia on d 63 and 21 before calving and d 1 and 28 PP by using a tailor-made biopsy needle (length 400 mm; outer diameter of 6 mm; Weber et al. 2013b). Additional samples were collected during slaughter on d 63 PP. Liver samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Liver tissue was ground in liquid nitrogen, and glycogen content was determined using a commercial photometric kit based on the amyloglucosidase-catalyzed release of glucose (ENZYTEC™ Starch #E1268, R-Biopharm AG, Darmstadt, Germany).

For gene expression analysis liver tissue was homogenized using a FastPrep 120 centrifuge (Thermo Electron Corporation, Waltham, MA), and total RNA was isolated from the liver samples with TRIzol Reagent (Life Technologies, Darmstadt, Germany), cleaned with an RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany), and transcribed into cDNA as described by Hammon et al. (2009). The integrity, quantity, and quality of total RNA were confirmed according to Haubold et al. (2020). The mean RNA integrity number for liver tissue was 6 ± 1. The quantity and purity of the total RNA were assessed on the basis of optical density measurements, and the A260:280 ratio ranged from 1.8 to 2.0. The relative mRNA abundance of genes related to glucose metabolism and the somatotropic axis was quantified as described by Saremi et al. (2012). The primer sequences and PCR conditions used for the reference genes low-density

lipoprotein 10 (***LRP10***) and RNA polymerase II (***POLR2A***) and the target genes related to glucose metabolism and the somatotropic axis are reported in **Table 4.3**. The selected targets were genes encoding enzymes involved in glucose metabolism, such as pyruvate carboxylase (***PC***), cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (cytosolic ***PCK1***; mitochondrial ***PCK2***), glucose-6-phosphatase (***G6PC***), and propionyl-CoA-carboxylase α (***PCCA***), or ketogenesis, such as 3-hydroxy-3-methyl-glutaryl-CoA synthase 2 (***HMGCS2***). Additionally, genes encoding hormones or receptors involved in the somatotropic axis such as GH receptor 1A (***GHR1A***), IGF-I (***IGF1***), insulin receptor (***INSR***), and IGFBP-2 and -3 (***IGFBP2***; ***IGFBP3)*** were investigated. The primer products were verified by sequencing with the BigDye™ Terminator v1.1 Cycle Sequencing Kit and an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific Inc., Waltham, MA).

The mRNA expression relative to reference genes was performed by real-time reversetranscription PCR with the use of a LightCycler 96 (Roche Diagnostics GmbH, Mannheim, Germany); SYBR Green I was used as the fluorescent dye. Duplicate measurements were performed for all samples and each block included 2 negative controls (no cDNA and no RT) and 2 inter-run calibrators. Melting curve analysis and agarose gel electrophoreses were used to confirm the specificity of the PCR products. The quantification cycle values and amplification efficiencies obtained with LinRegPCR version 2017.0 (Ruijter et al. 2013) were imported into qBASE+ version 3.1 (Biogazelle, Gent, Belgium) for all subsequent calculations and quality controls. The geometric mean of the reference gene abundance was applied for normalization. The data are presented as the ratio of the copy number of the gene of interest to the geometric mean reference gene abundance.

4.3.3 Tracer Studies

On d 28 before expected calving and d 21 PP, eGP and GOx were determined after feed withdrawal for 12 h via the primed continuous intravenous infusion of [U-^{13}C]-glucose [99 atom% ^{13}C, Euriso-Top SAS, Staint-Aubin Cedex, France; prime: 5.38 µmol/kg of BW; infusion: 7.53 µmol/(kg of BW × h); dissolved in 0.9% saline] for 4 h (Hammon et al. 2008; Hötger et al. 2013; Weber et al. 2016). Cows were fitted with 2 jugular catheters (Cavafix® Certo® with Splittocan®, B. Braun Vet Care GmbH, Tuttlingen, Germany) for tracer infusion and blood sampling. Blood samples were collected 30 and 20 min before tracer infusion and at 60, 120, 150, 180, 210, and 240 min after the initiation of infusion in tubes containing Li-heparin (14-15 IU/mL; S-Monovette, Sarstedt, Nürnberg, Germany). The enrichment of [U-^{13}C]-glucose was determined by GC-MS (QP2010, coupled with GC 2010; Shimadzu, Duisburg, Germany) to calculate eGP as described (Hammon et al. 2008; Steinhoff-Wagner et al. 2011). Whole blood in K_3EDTA tubes collected before and at regular intervals between 60 and 240 min after the initiation of

tracer infusion was used to isolate CO_2, for the measurement of the $^{13}C/^{12}C$ ratio by isotope ratio mass spectrometry and calculate GOx (Hammon et al. 2008; Weber et al. 2016).
Additional blood samples were collected hourly until 6 h after the initiation of tracer infusion to measure concentrations of plasma glucose, BHB, insulin, glucagon, cortisol, and GH. Blood samples were treated, and measurements were performed as described above.

4.3.4 Statistical Analyses

Statistical analyses were performed with SAS for Windows, release 9.4 (SAS Institute Inc., Cary, NC). The basal concentrations of plasma metabolites and hormones and gene expression in the liver were analyzed by repeated-measures ANOVA using the MIXED procedure and a model including EFA (levels: yes, no), CLA (levels: yes, no), time (level: d relative to calving), block (levels: 1-5), and the respective interactions as fixed effects, and the calving interval and projected milk yield during the second lactation as covariates. Repeated measures of each cow were considered by using the repeated statement of the MIXED procedure with a compound symmetry covariance structure. The ranges of the repeated variable time for the metabolite and hormone data were as follows: AP (d 63-10 AP), the transition period (d 21 AP to 28 PP), PP (d 1-56 PP), and the entire period (d 63 AP to 56 PP). The data were analyzed separately for each observation period. The liver glycogen concentration and gene expression data were analyzed considering only the entire period (d 63 AP to 63 PP). The concentrations of plasma metabolites and hormones during profiling were analyzed by repeated-measures ANOVA using the MIXED procedure and a model including EFA (levels: yes, no), CLA (levels: yes, no), d (levels: d 28 AP, d 21 PP), hour (levels: 0-6 h), block (levels: 1-5), and the respective interactions as fixed effects, as well as the calving interval and projected milk yield during the second lactation as covariates. Due to large differences between d 28 AP and d 21 PP, the data on whole body glucose metabolism determined via the tracer technique were analyzed separately for d 28 AP and d 21 PP by ANOVA using the MIXED procedure and with a model containing EFA (levels: yes, no), CLA (levels: yes, no), block (levels: 1-5), and the respective interactions as fixed effects, as well as the calving interval and projected milk yield during the second lactation as covariates. For the analysis at d 21 PP milk yield on the day of measurement was used as an additional covariate. The differences over time between d 28 AP and d 21 PP were calculated in a separate model by repeated-measures ANOVA. The LSM and their SE were computed for each fixed effect in the ANOVA models to display the results. All group differences of these LSM were tested using the TukeyKramer procedure. The SLICE statement of the MIXED procedure was used to assess the partitioned analyses of the LSM for interactions. All differences with $P < 0.05$ were considered significant.

4.4 Results

4.4.1 Plasma Glucose and Related Hormones as well as Whole-Body Glucose Metabolism

The plasma glucose concentration (**Figure 4.1A**) peaked ($P < 0.001$) on d 28 AP, dropped down with calving and slightly increased thereafter ($P < 0.001$). On d 21 PP, plasma glucose concentration indicated a CLA effect ($P < 0.05$) and was 15% higher in CLA-treated than in EFA-treated cows ($P < 0.05$). During the 6-h time profile plasma glucose concentration remained constant on d 28 AP but on d 21 PP glucose concentration slightly decreased ($P < 0.001$) with a 17% higher ($P < 0.05$) glucose concentration in EFA+CLA than in EFA after 5 h and a 11 to 12% higher glucose concentration ($P < 0.05$) in CLA- than in non-CLA treated cows 5 and 6 h after beginning of the measurement (**Figure 4.1B**). The plasma BHB concentration (**Figure 4.1C**) increased after calving, with an EFA effect on d 21 PP ($P < 0.05$) and a 75% higher concentration ($P = 0.05$) in EFA+CLA than in CTRL. Accordingly, the BHB concentration during profiling was lower ($P < 0.001$) on d 28 AP than on d 21 PP and declined ($P < 0.001$) on d 21 PP in CLA and EFA+CLA (**Figure 4.1D**). During plasma profiling on d 21 PP BHB concentration was 43% higher ($P < 0.05$) in EFA- than in non-EFA-treated cows, 70% higher ($P < 0.05$) in EFA+CLA than in CTRL at the beginning of the profiling, and 80% higher ($P < 0.05$) in EFA+CLA and EFA than in CTRL at 1 h after the start.

The plasma insulin concentration increased after drying off ($P < 0.001$) and decreased ($P < 0.001$) markedly after calving in all groups (**Figure 4.2A**). Plasma insulin was 26 to 44 % higher ($P < 0.05$) in CLA-treated than non-CLA cows from d 21 AP to d 1 PP, as well as on d 21 PP and was highest ($P < 0.05$) on d 1 PP in CLA. During profiling plasma insulin was lower ($P < 0.05$) PP than AP and dropped ($P < 0.001$) after beginning within the first hours AP and PP (**Figure 4.2B**). On d 28 AP, plasma insulin was 24% lower ($P < 0.05$) at the beginning of the profiling in EFA than in non-EFA groups, and on d 21 PP was 79% higher ($P < 0.05$) in CLA than in the non-CLA groups at the beginning. In addition, there was a trend ($P = 0.1$) on d 21 PP for 57% higher plasma insulin in CLA than non-CLA cows across all time points. There were no significant differences over time or treatment effects for basal glucagon concentration (**Figure 4.2C**). With respect to profiling plasma glucagon concentration increased ($P < 0.05$) in non-CLA -cows but decreased ($P < 0.05$) in CLA from d 28 AP to d 21 PP (**Figure 4.2D**). On d 21 PP plasma glucagon was 21 to 34% lower ($P < 0.05$) in CLA than in the non-CLA groups from 4 to 6 h and was higher ($P < 0.05$) in EFA than in EFA+CLA and CLA at 4 and 5 h after the beginning of blood sampling.

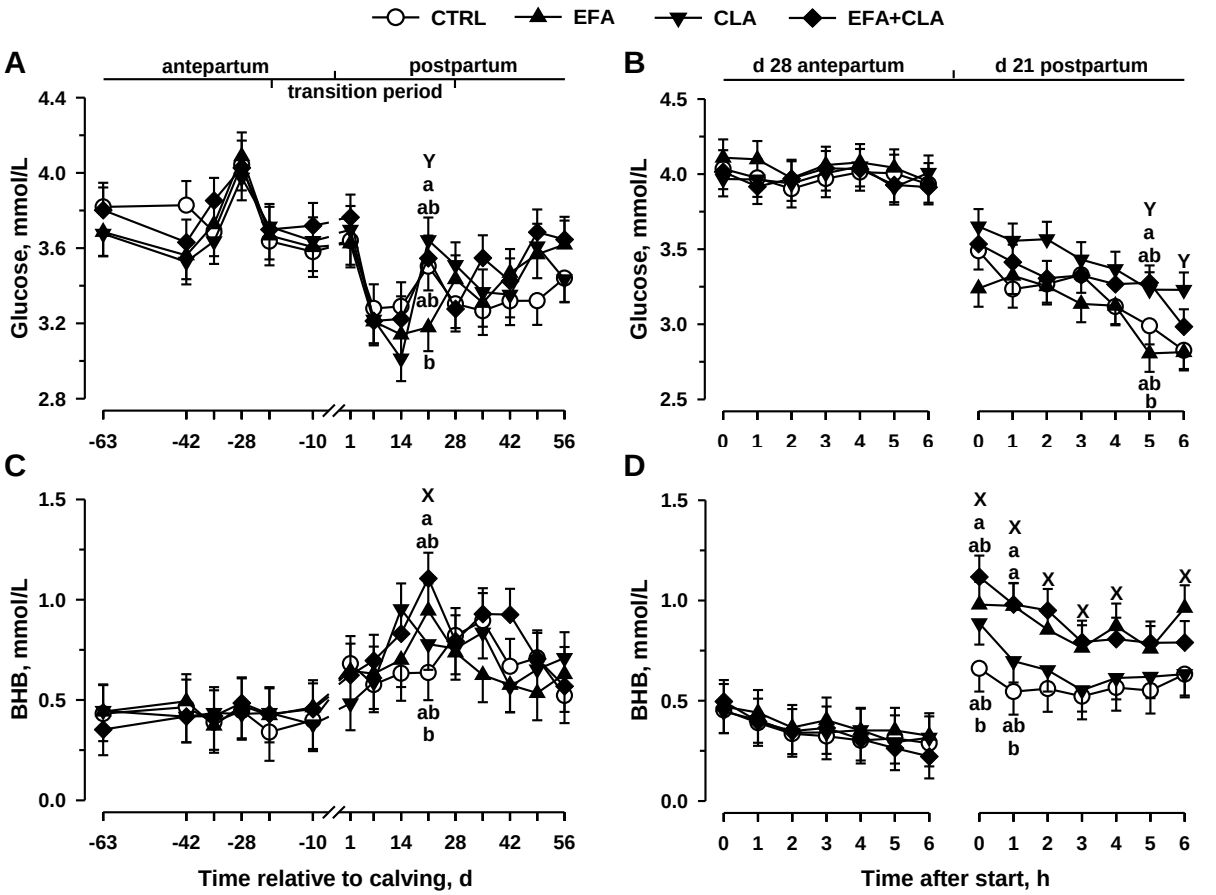

Figure 4.1 Concentrations of plasma glucose (A, B) and BHB (C, D) during the entire study (A, C) and during 6-h metabolic profiling with feed withdrawal on d 28 antepartum and d 21 postpartum (B, D) in cows supplemented daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA;n = 9), Lutalin (▼ CLA; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany; n = 10), and EFA+CLA (◆; n = 10) from d 63 antepartum until d 56 postpartum.
Data are presented as LSM ± SE, LSM with different superscripts (a, b) differ ($P < 0.05$) at the respective time point. X: EFA effect at the respective time point. Y: CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects of the basal plasma glucose concentration during the antepartum (time), transition (time), and postpartum (time) periods, during the entire study (time) and during profiling (day, hour, EFA × day, CLA × day). Statistically significant ($P < 0.05$) effects for the basal plasma BHB concentration during the transition (time) and postpartum (time) periods, during the entire study (time) and during profiling (day, hour, EFA × day).

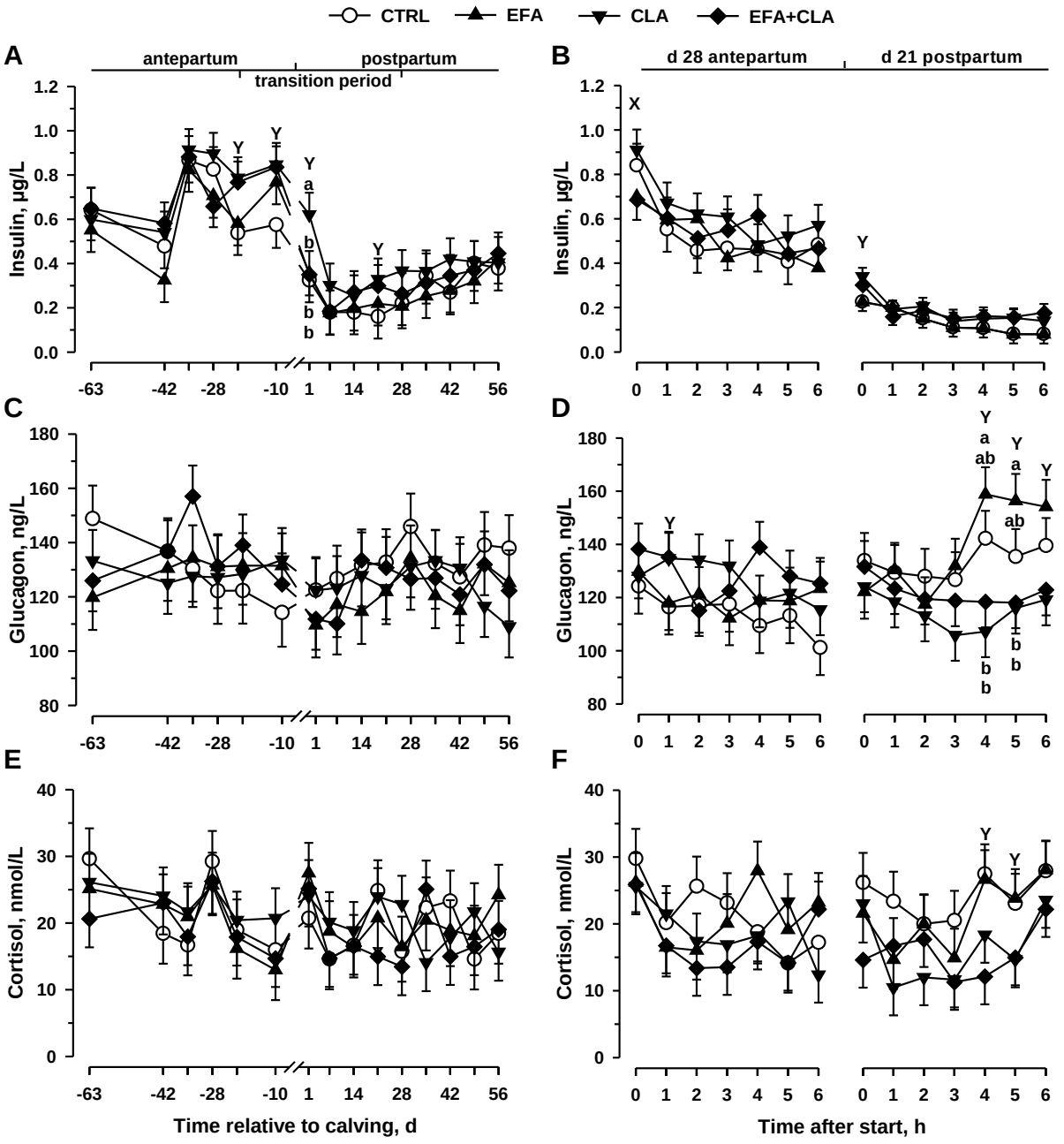

Figure 4.2 Concentrations of plasma insulin (A, B), glucagon (C, D) and cortisol (E, F) during the entire study (A, C, E) and during 6-h metabolic profiling with feed withdrawal on d 28 antepartum and d 21 postpartum (B, D, F) in cows supplemented daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany; n = 10), and EFA+CLA (◆; n = 10) from d 63 antepartum until d 56 postpartum.
Data are presented as LSM ± SE, LSM with different superscripts (a, b) differ ($P < 0.05$) at the respective time point. X: EFA effect at the respective time point. Y: CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects for the basal plasma insulin concentration during the antepartum (time), transition (time; CLA), and postpartum (time; CLA) periods, during the entire study (time) and during profiling (day, hour, day × hour). Statistically significant ($P < 0.05$) effects for the plasma glucagon concentration during profiling (day, day × hour, CLA × day, CLA × day × hour). Statistically significant ($P < 0.05$) effects for the basal plasma cortisol concentration during the antepartum (time) and transition (time) periods, during the entire study (time) and during profiling (CLA, CLA × day, hour; day × hour).

The glucagon/insulin and glucose/insulin ratios increased after calving ($P < 0.01$) in all groups, were 4.5- and 3.8-fold higher ($P < 0.05$) in CTRL than in CLA during the transition period (**Table 4.1**), and peaked on d 21 PP ($P < 0.05$) in CTRL (data not shown). During the profiling studies, the 2 ratios were higher ($P < 0.05$) on d 21 PP than on d 28 AP (data not shown). On d 21 PP the glucagon/insulin ratio in all groups except for EFA+CLA and the glucose/insulin ratio in EFA and CLA cows increased ($P < 0.05$) during blood sampling and both ratios were 77% (glucagon/insulin) and 33% (glucose/insulin) lower ($P < 0.05$) in CLA than in the non-CLA groups. The glucagon/insulin ratio on d 21 PP was higher ($P < 0.05$) in CTRL than in CLA and EFA+CLA and was higher ($P < 0.05$) in EFA than in EFA+CLA (LSM ± SE for CTRL, EFA, CLA, and EFA+CLA were 8.46 ± 0.91, 7.27 ± 0.89, 5.11 ± 0.84, and 3.76 ± 0.84 mol/mol, respectively). The glucose/insulin ratio was higher ($P < 0.05$) in CTRL than in EFA+CLA on d 21 PP (LSM ± SE for CTRL, EFA, CLA, and EFA+CLA were 681 ± 78, 518 ± 75, 505 ± 72, and 394 ± 71 mmol/nmol, respectively). The glucose/glucagon ratio decreased ($P < 0.05$) after calving in EFA+CLA but indicated no further time and treatment differences (data not shown). During the profiling studies, the glucose/glucagon ratio was higher ($P < 0.05$) on d 28 AP than on d 21 PP (data not shown). On d 21 PP, the glucose/glucagon ratio was higher ($P < 0.05$) in CLA than in EFA and was higher ($P < 0.05$) in CLA- than non-CLA groups (LSM ± SE for CTRL, EFA, CLA, and EFA+CLA were 88.7 ± 6.9, 83.5 ± 6.6, 109.1 ± 6.3, and 98.8 ± 6.2 mmol/nmol, respectively).

Plasma cortisol varied AP ($P < 0.001$) and during the transition period ($P < 0.05$) with peaks ($P < 0.05$ or less) at d 28 AP and d 1 after calving but without differences between groups (**Figure 4.2E**). The 6-h profile of the plasma cortisol concentration indicated no differences over time between AP and PP but plasma cortisol was 60% lower ($P < 0.05$) on d 21 PP in CLA than in the non-CLA cows, especially 4 and 5 h after the beginning of blood sampling (**Figure 4.2F**).

The results for eGP and GOx are shown in **Table 4.2**. Endogenous glucose production increased from AP to PP ($P < 0.001$) by 63%, indicating an EFA and CLA effect with 6% elevated eGP ($P < 0.05$) in EFA compared with the non-EFA groups and 11% decreased eGP ($P < 0.01$) in CLA compared with the non-CLA groups, and was higher ($P < 0.05$) in EFA than in CLA (18%) and EFA+CLA cows (12%) on d 21 PP ($P < 0.05$). On the other hand, GOx decreased from d 28 AP to d 21 PP ($P < 0.001$) by 130% and was 38% lower in CLA cows than in CTRL cows PP ($P < 0.05$). The percentage of GOx relative to eGP declined ($P < 0.001$) from d 28 AP to d 21 PP 3.6-fold, indicating an EFA × CLA interaction ($P = 0.05$) on d 21 PP. There was a trend ($P < 0.1$) of a decreased GOx/eGP ratio in EFA and CLA compared with CTRL cows.

Table 4.1 Glucagon/insulin and glucose/insulin ratios in blood plasma from late gestation (antepartum; AP) to early lactation (postpartum; PP) in cows supplemented daily with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10) or the combination of EFA and CLA (EFA+CLA; n = 10)

		Treatment				Fixed effect, *P*-values					
Variable[2]	Time	CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA × CLA	Time	EFA × time	CLA × time
Glucagon/Insulin, mol/mol											
Basal Values[3]	Antepartum	0.38± 0.13	0.70± 0.13	0.30± 0.12	0.37± 0.12	0.13	0.11	0.30	0.17	0.58	0.45
	Transition period	3.05± 0.59[a]	1.69± 0.56[ab]	0.66± 0.55[b]	1.56± 0.53[ab]	0.69	0.03	0.05	0.01	0.75	0.74
	Postpartum	2.82± 0.63	1.94± 0.61	1.10± 0.58	1.84± 0.58	0.91	0.14	0.19	0.08	0.80	0.35
	Entire study	1.85± 0.39	1.45± 0.37	0.78± 0.35	1.25± 0.35	0.92	0.09	0.24	0.001	0.87	0.34
Glucose/Insulin, mmol/nmol											
Basal Values[3]	Antepartum	39.0 ± 15.4	77.1 ± 14.7	30.3 ± 14.2	27.6 ± 13.8	0.14	0.10	0.29	0.28	0.43	0.44
	Transition period	226.2 ± 43.6[a]	174.1 ± 41.6[ab]	60.6 ± 40.5[b]	172.7 ± 39.2[ab]	0.48	0.05	0.05	0.01	0.66	0.81
	Postpartum	215.6 ± 55.0	198.4 ± 52.8	102.2 ± 50.4	192.4 ± 49.9	0.50	0.25	0.31	0.14	0.78	0.33
	Entire study	145.4 ± 34.0	149.9 ± 32.6	73.4 ± 31.1	130.4 ± 30.7	0.36	0.16	0.42	0.001	0.89	0.37

[a,b,c]Means within a row with different lowercase superscripts differ (P < 0.05).
[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE.
[3]Time relative to calving: Antepartum (d 63-10 AP), transition period (d 21 AP to 28 PP), postpartum (d 1-56 PP), and the entire period (d 63 AP to d 56 PP).

Table 4.2 Endogenous glucose production (eGP) and glucose oxidation (GOx) on d 28 antepartum (AP) and d 21 postpartum (PP) in cows supplemented daily with coconut oil (CTRL; n = 9), linseed and safflower oil (EFA; n = 9), Lutalin[1] (CLA; n = 10) or the combination of EFA and CLA (EFA+CLA; n = 10) during late gestation and early lactation

		Treatment				Fixed, effect, *P*-values		
Variable[2]	Time	CTRL	EFA	CLA	EFA+CLA	EFA	CLA	EFA × CLA
eGP,	d 28 AP	0.69±0.03^{B}	0.70±0.02^{B}	0.69±0.02^{B}	0.72±0.02^{B}	0.37	0.56	0.62
mmol/(kg × h)	d 21 PP	1.14±0.04^{A,ab}	1.23±0.03^{A,a}	1.04±0.03^{A,b}	1.10±0.03^{A,b}	0.05	0.002	0.63
GOx,	d 28 AP	0.37±0.03^{A}	0.32±0.03^{A}	0.36±0.02^{A}	0.37±0.02^{A}	0.58	0.39	0.25
mmol/(kg × h)	d 21 PP	0.18±0.02^{B,a}	0.15±0.02^{B,ab}	0.13±0.02^{B,b}	0.16±0.02^{B,ab}	0.86	0.19	0.09
GOx, % of eGP	d 28 AP	52.7 ±2.6^{A}	46.3 ±2.5^{A}	51.5 ±2.4^{A}	51.6 ±2.4^{A}	0.22	0.41	0.19
	d 21 PP	16.3 ±1.6^{B}	12.1 ±1.4^{B}	12.6 ±1.4^{B}	14.4 ±1.4^{B}	0.43	0.63	0.05

^{a,b}Means within a row with different lowercase superscripts differ ($P < 0.05$).
^{A,B}Means of a particular parameter within a column with different uppercase superscripts differ ($P < 0.05$).
[1]Conjugated linoleic acid, *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany.
[2]Values are presented as LSM ± SE.

4.4.2 Somatotropic Axis in Blood Plasma

The GH concentration in blood plasma increased during early lactation ($P < 0.05$) and was 49% higher ($P < 0.05$) in CLA than in non-CLA cows on d 49 PP (**Figure 4.3A**). The 6-h time profile of plasma GH showed only minor changes by d with slightly higher GH concentrations being observed on d 21 PP than d 28 AP, especially in EFA (**Figure 4.3B**). On d 21 PP, plasma GH increased ($P < 0.05$) in EFA+CLA and showed a tendency to increase ($P < 0.1$) in EFA up to 2 h after the beginning of blood sampling. At 2 h on d 21 PP, 87% higher plasma GH was observed in EFA than in non-EFA cows and plasma GH was higher ($P < 0.05$) in EFA+CLA than in CTRL and EFA. The plasma IGF-I concentration was highest ($P < 0.05$) on d -35 AP and decreased ($P < 0.001$) in all groups during the transition period until d 14 PP (**Figure 4.3C**). The plasma IGF-I results indicated an EFA effect ($P < 0.05$) on d 42 AP with 46% higher concentrations in CLA than in EFA cows ($P < 0.05$). Beginning on d 28 AP in the CLA group and d -21 AP in the EFA group plasma IGF-I was higher ($P < 0.05$) than in CTRL until calving (25-46% difference). After calving plasma IGF-I was 25-37% higher ($P < 0.05$) at several time points in CLA than in non-CLA cows and at the end of the study plasma IGF-I was higher ($P < 0.05$) in the CLA than in CTRL.

The concentration of plasma IGFBP-2 increased ($P < 0.05$) from the AP to the PP period by 158%, and the results indicated a 35 to 43% decreased plasma concentration ($P < 0.05$) with CLA treatment on d 42 AP and d 56 PP, and a lower ($P < 0.05$) concentration in the CLA group than in the EFA group on d 56 PP (**Figure 4.4A**). The plasma IGFBP-3 concentration decreased ($P < 0.001$) during AP by 46%, reached the lowest concentration on d 1 PP, and slowly increased ($P < 0.001$) thereafter (**Figure 4.4B**). Elevated concentrations ($P < 0.05$) were observed on d -42 and -21 AP and d 28 and 56 PP in CLA (by 23-34%) and on d -21 AP in EFA groups (by 23%). Plasma IGFBP-3 was higher ($P < 0.05$) in EFA+CLA than in CTRL on d -21 AP and higher ($P < 0.05$) in CLA than in CTRL on d 28 PP. The concentration of IGFBP-3 was 23% higher ($P < 0.05$) in CLA than in non-CLA cows during the transition and PP periods. The IGFBP-3/-2 ratio was 60 to 170% higher ($P < 0.05$) in CLA than in CTRL and EFA during the entire study, reached the lowest point on d 14 PP, and increased ($P < 0.001$) thereafter (**Figure 4.4C**). A decreasing effect by 32% ($P < 0.05$) of EFA treatment was observed on d 42 AP. The concentration of plasma IGFBP-4 slightly decreased AP ($P < 0.01$) and was higher ($P < 0.05$) in CLA than in the non-CLA groups on d 56 PP by 32% (**Figure 4.4D**).

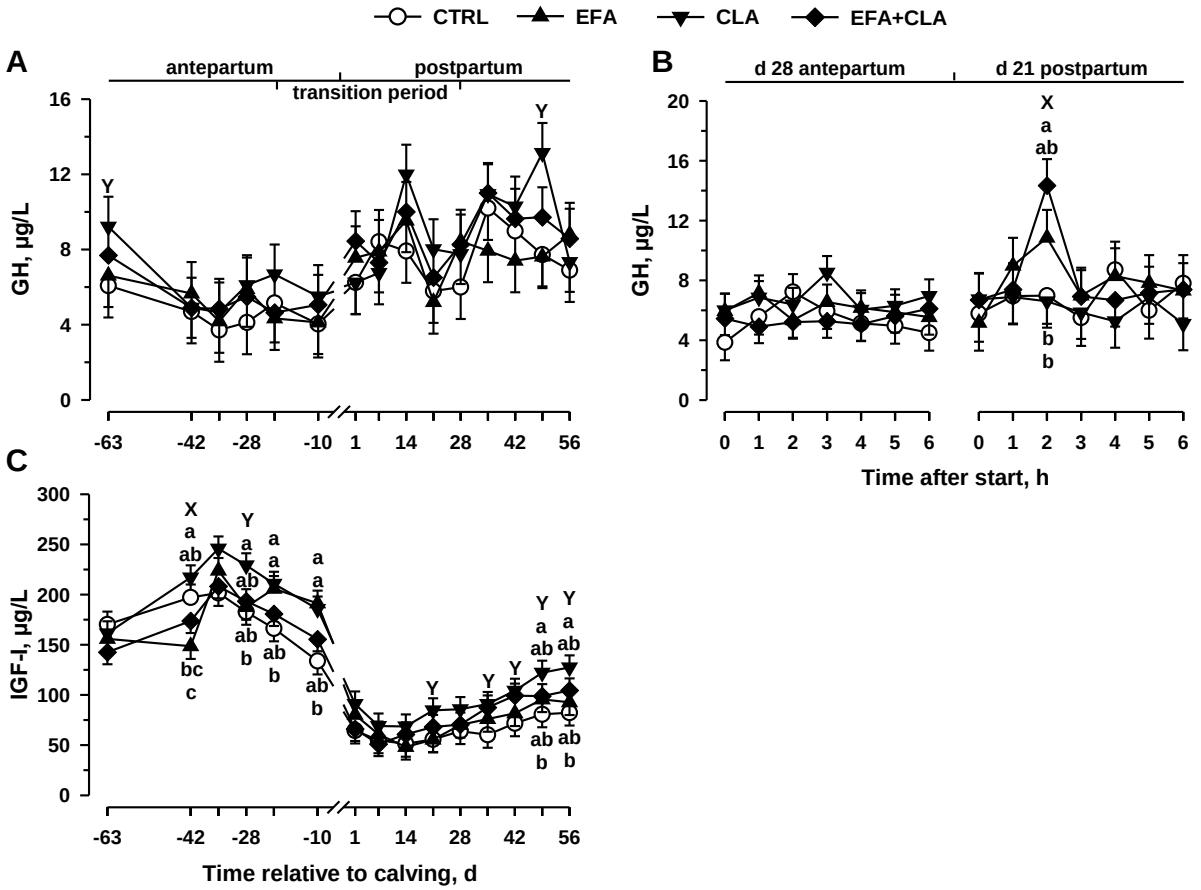

Figure 4.3 Concentrations of plasma of growth hormone (GH; A) and IGF-I (C) during the entire study as well as GH (B) during 6-h metabolic profiling with feed withdrawal on d 28 antepartum and d 21 postpartum in cows supplemented daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany; n = 10), and EFA+CLA (◆; n = 10) abomasally from d 63 antepartum until d 56 postpartum. Data are presented as LSM ± SE, LSM with different superscripts (a, b, c) differ ($P < 0.05$) at the respective time point. X: EFA effect at the respective time point. Y: CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects for the concentration of plasma GH during the antepartum (time), transition (time), and postpartum (time) periods, during the entire study (time), and during profiling (day, EFA × day). Statistically significant ($P < 0.05$) effects for the concentration of plasma IGF-I during the antepartum (time; EFA × time; EFA × CLA × time), transition (time; EFA × CLA; EFA × CLA × time), and postpartum (time; CLA) periods and during the entire study (time; EFA × time; EFA × CLA × time).

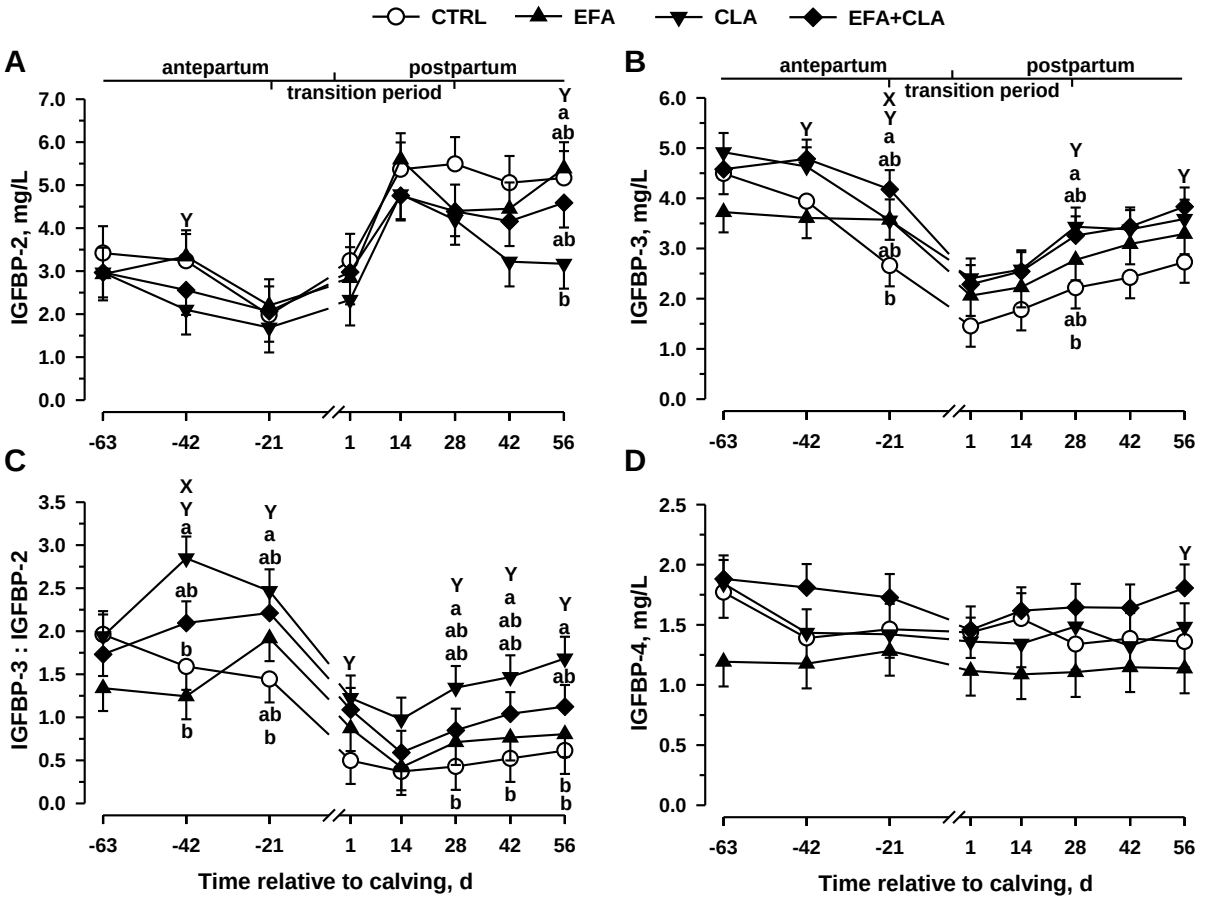

Figure 4.4 Concentrations of plasma IGF-binding protein 2 (IGFBP-2; A), IGFBP-3 (B), the calculated ratio (IGFBP-3: IGFBP-2; C) and IGFBP-4 (D) in cows supplemented daily with coconut oil (○ CTRL; n = 9), linseed and safflower oil (▲ EFA; n = 9), Lutalin (▼ CLA; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany; n = 10), and EFA+CLA (◆; n = 10) abomasally from d 63 antepartum until d 56 postpartum.
Data are presented as LSM ± SE, LSM with different superscripts (a, b) differ ($P < 0.05$) at the respective time point. X: EFA effect at the respective time point. Y: CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects for the concentration of plasma IGFBP-2 during the antepartum (time), transition (time), and postpartum (time) periods, and during the entire study (time). Statistically significant ($P < 0.05$) effects for the concentration of plasma IGFBP-3 during the antepartum (time; EFA × time), transition (time; CLA), and postpartum (time; CLA) periods and during the entire study (time; CLA; EFA × time). Statistically significant ($P < 0.05$) effects for the IGFBP-3/-2 ratio during the antepartum (CLA; CLA × time), transition (time; CLA), and postpartum (time; CLA) periods and during the entire study (time; CLA). Statistically significant ($P < 0.05$) effects for the concentration of plasma IGFBP-4 during the antepartum period (time; EFA × time) and during the entire study (time).

4.4.3 Liver Glycogen Concentration and Gene Expression Involved in Glucose Metabolism and the Somatotropic Axis

One cow of the CLA group was not included in the analyses due to failure to obtain liver samples by biopsies. The hepatic glycogen content decreased at calving ($P < 0.001$) by 58% and was 16% higher ($P < 0.05$) in CLA than in non-CLA treated cows on d 28 PP (**Figure 4.5A**). The abundance of *PC* mRNA increased ($P < 0.001$) 3.7-fold on d 1 PP and was increased up to 100% ($P < 0.05$) in CTRL on d 1, indicating a decreasing effect ($P < 0.05$) of EFA and CLA treatment (**Figure 4.5B**). The *PCK1* mRNA abundance was lower ($P < 0.05$) AP than PP and

increased 3-fold with ongoing lactation ($P < 0.001$), with 42% lower expression ($P < 0.05$) being observed in EFA- than non-EFA-treated cows on d 28 PP (**Figure 4.5C**). The abundance of *PCK2* mRNA increased at calving ($P < 0.001$) by 32%, was lower in EFA+CLA than in CTRL ($P < 0.05$) on d 1 PP, and was highest ($P < 0.05$) in CLA on d 28 PP (**Figure 4.5D**). The abundance of *PCK2* mRNA indicated a decreasing effect of EFA treatment ($P < 0.05$) on d 1 and 28 PP by 37 and 42%, respectively. The abundance of *G6PC* and *PCCA* mRNA increased ($P < 0.01$) after d 1 PP by 100 and 133%, respectively (**Figure 4.5E** and **4.5F**). On d 28 PP, the abundance of *PCCA* and *G6PC* was 58 and 43% lower ($P < 0.01$) in EFA than in the non-EFA groups, with higher expression ($P < 0.05$) being observed in CLA than in EFA+CLA (*G6PC*) or EFA (*PCCA*). The mRNA abundance of *HMGCS2* increased 2-fold after calving ($P < 0.001$), was 2-fold higher ($P < 0.05$) on d 28 PP in CLA than in EFA+CLA, and was decreased 53% ($P < 0.05$) by EFA treatment (**Figure 4.5G**).

The abundance of *GHR1A* and *IGF1* was lowest ($P < 0.05$) on d 1 PP and increased 3-fold up to d 63 PP, respectively (**Figure 4.6A** and **4.6B**). The abundance of *GHR1A* showed an increasing tendency ($P < 0.1$) by 75% on d 63 PP in EFA+CLA than in CTRL. In addition, *GHR1A* mRNA showed a tendency to be stimulated 78% ($P < 0.1$) by CLA treatment on d 28 PP and 36% by EFA on d 63 PP. The abundance of *IGFBP2* increased 2.5-fold ($P < 0.001$) from AP to the end of the study, with lower expression being observed in EFA on d 28 PP ($P < 0.01$; by 60%) and in CLA ($P < 0.001$) on d 1 and d 63 PP by 56 and 47% (**Figure 4.6C**). The *IGFBP2* mRNA abundance was higher ($P < 0.05$) on d 28 PP in CLA than in EFA+CLA and was higher ($P < 0.05$) on d 63 PP in EFA than in CLA and EFA+CLA. The abundance of *IGFBP3* was highest ($P < 0.001$) on d 63 PP and was decreased 49% by EFA treatment on d -21 AP (**Figure 4.6D**). The abundance of *INSR* mRNA slightly increased ($P < 0.001$) throughout the experimental period and was 47 and 63% lower ($P < 0.05$) in EFA than in non-EFA groups on d 1 and 28 PP (**Figure 4.6E**). On d 28 PP *INSR* mRNA abundance was higher ($P < 0.05$) in CLA than in EFA and EFA+CLA. The *INSR* mRNA abundance across all time points was 52% higher in the CLA cows ($P < 0.05$) and showed a tendency to be 44% higher in CTRL ($P = 0.07$) than in EFA.

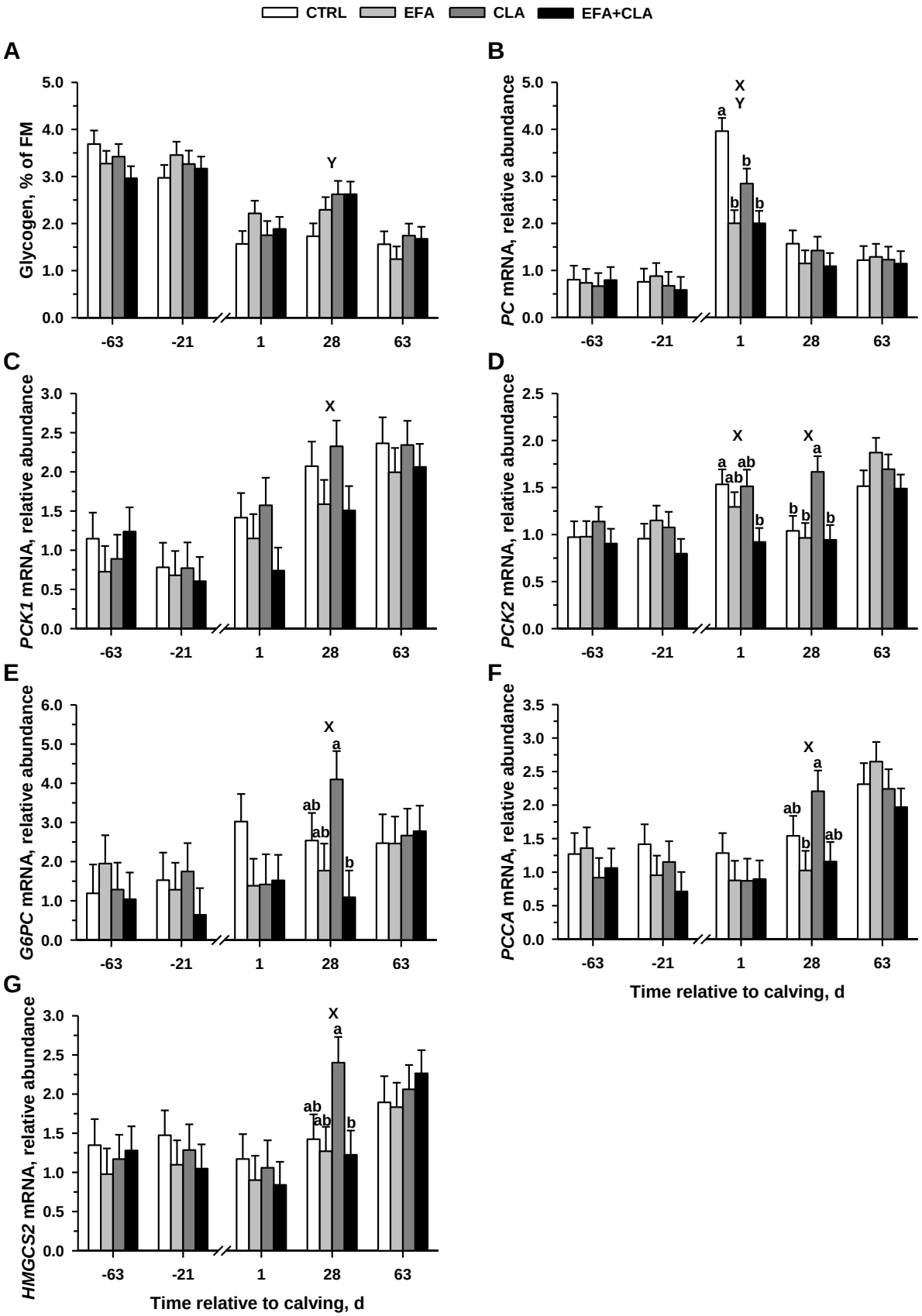
CTRL
EFA
CLA
EFA+CLA
A
Glycogen, % of FM
B
PC mRNA, relative abundance
C
PCK1 mRNA, relative abundance
D
PCK2 mRNA, relative abundance
E
G6PC mRNA, relative abundance
F
PCCA mRNA, relative abundance
G
HMGCS2 mRNA, relative abundance
-63
-21
1
28
63
Time relative to calving, d

Figure 4.5 Liver glycogen concentration (A) and relative hepatic mRNA expression of pyruvate carboxylase (*PC*; B), cytosolic phosphoenolpyruvate carboxykinase (*PCK1*; C), mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*; D), glucose-6-phosphatase (*G6PC*; E), mitochondrial propionyl-CoA carboxylase alpha chain (*PCCA*; F) and hydroxyl-methyl-glutaryl-CoA-synthase 2 (*HMGCS2*; G) in cows supplemented daily with coconut oil (CTRL; white bars; n = 9), linseed and safflower oil (EFA; light gray bars; n = 9), Lutalin (CLA; dark gray bars; *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; n = 9), and EFA+CLA (black bars; n = 10) abomasally from d 63 antepartum until slaughter on d 63 postpartum.
Data are presented as LSM ± SE, LSM with different superscripts (a, b) differ ($P < 0.05$) at the respective time point. X: EFA effect at the respective time point. Y: CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects on the liver glycogen concentration during the entire study (time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *PC* during the entire study (time; EFA; EFA × time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *PCK1* during the entire study (time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *PCK2* during the entire study (EFA × CLA; time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *G6PC* during the entire study (time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *PCCA* during the entire study (time). Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *HMGCS2* during the entire study (time).

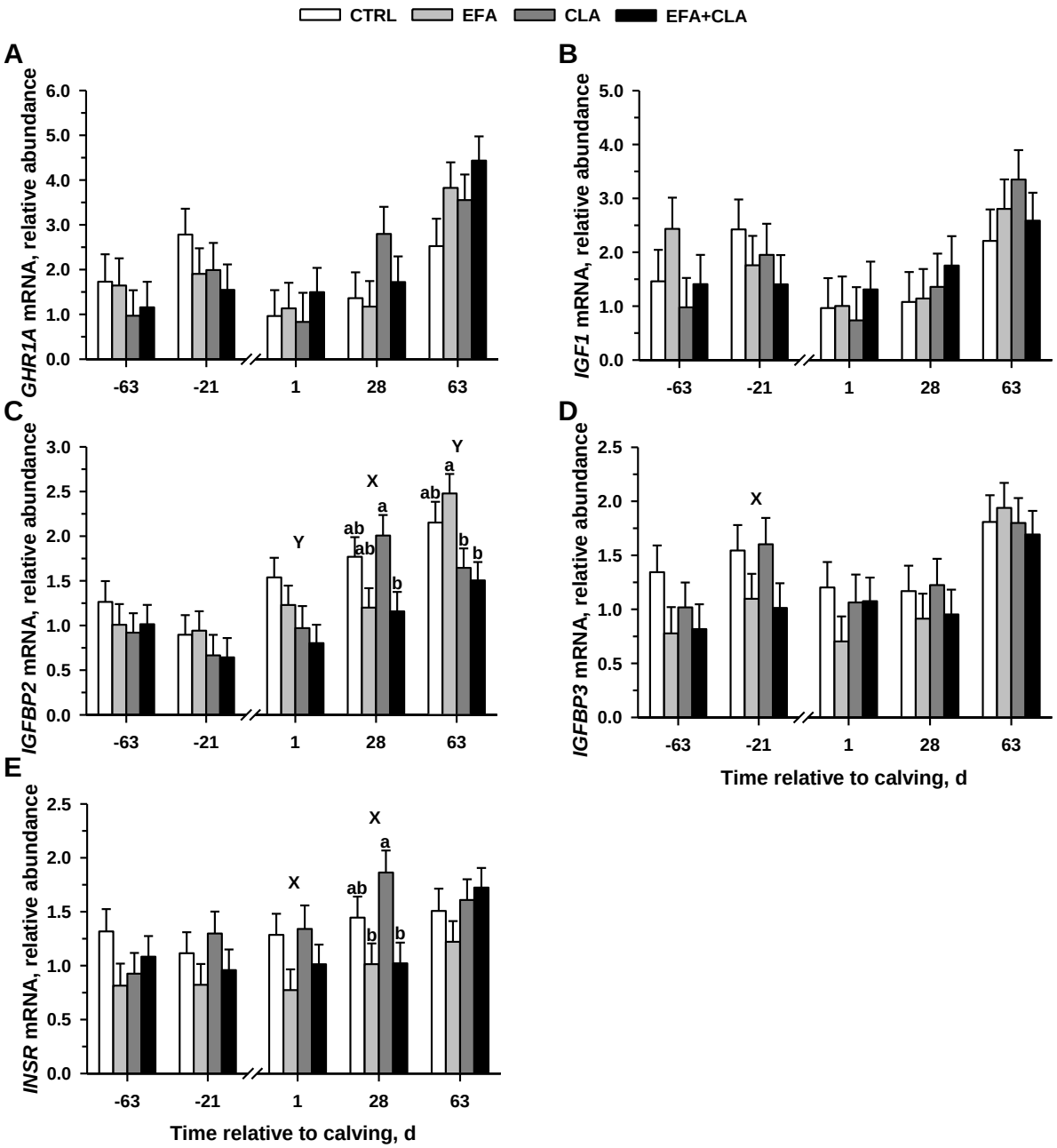

Figure 4.6 Relative hepatic mRNA expression of growth hormone receptor 1A (*GHR1A*; A), IGF-I (*IGF1*; B), IGF binding protein 2 (*IGFBP2*; C), IGF binding protein 3 (*IGFBP3*; D) and insulin receptor (*INSR*; E) of cows supplemented abomasally daily with coconut oil (CTRL; white bars; n = 9), linseed and safflower oil (EFA; light gray bars; n = 9), Lutalin (CLA; dark gray bars; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; BASF SE, Ludwigshafen, Germany; n = 9), and EFA+CLA (black bars; n = 10) from d 63 antepartum until slaughter on d 63 postpartum.
Data are presented as LSM ± SE, LSM with different superscripts (a, b) differ ($P < 0.05$) at the respective time point. X: EFA effect at respective time point. Y: CLA effect at respective time point. Statistically significant ($P < 0.05$) effects for relative hepatic mRNA expression of *GHR1A* during the entire study (time). Statistically significant ($P < 0.05$) effects for relative hepatic mRNA expression of *IGF1* during the entire study (time). Statistically significant ($P < 0.05$) effects for relative hepatic mRNA expression of *IGFBP2* during the entire study (time; CLA). Statistically significant ($P < 0.05$) effects for relative hepatic mRNA expression of *IGFBP3* during the entire study (time). Statistically significant ($P < 0.05$) effects for relative hepatic mRNA expression of *INSR* during the entire study (time; EFA).

4.5 Discussion

4.5.1 Glucose Metabolism, Endocrine Regulation, and Hepatic mRNA Abundance

The metabolic changes in terms of decreased glucose and increased BHB concentrations in dairy cows during transition followed the expectations resulting from previously described findings (Hammon et al. 2009, Gross et al. 2011a, Weber et al. 2013b). The rates of eGP and GOx measured in this study were consistent with recently presented data from our group (Hammon et al. 2008; Hötger et al. 2013; Weber et al. 2016). The increase in eGP on d 21 PP compared with d 28 AP ensured an adequate glucose supply to the mammary gland for milk production (Aschenbach et al. 2010). In addition, whole-body GOx and the ratio of GOx to eGP decreased with the onset of lactation, which increased the availability of glucose for milk production (Drackley et al. 2001).

We observed only minor differences in the basal plasma glucose concentration because of EFA supplementation, which was consistent with previous studies (Zachut et al. 2010, Mach et al. 2013; do Prado et al. 2016). Interestingly, the present study revealed an elevated concentration of plasma BHB due to EFA treatment on d 21 PP in basal blood samples and during hourly measurements. Previous investigations of the effect of n-3 FA supplementation in dairy cows on the plasma BHB concentration showed no changes or even decreased plasma BHB in early lactation (Mach et al. 2013; do Prado et al. 2016). On d 21 PP, the basal plasma glucose concentration and the concentration during profiling were lowest in EFA cows. A shortage of glucose availability for milk production is associated with elevated plasma NEFA and BHB concentrations during early lactation (Drackley et al. 2001), and the plasma NEFA concentration on d 21 PP was high in EFA cows in the present study (Vogel et al. 2020). Therefore, the low plasma glucose concentration may partly explain the elevated BHB concentration observed on d 21 PP. However, eGP on d 21 PP was highest in the EFA cows, indicating counterregulation to maintain plasma glucose concentration in EFA cows. Interestingly, a stimulatory effect of α-linolenic acid on eGP, which was the leading FA in the EFA treatment, was observed in bovine hepatocytes (Mashek and Grummer 2003).

Plasma BHB was not noticeably elevated in the CTRL group at the time of calving and thereafter when compared with EFA and CLA groups. There is evidence in the literature that the medium chain FA that are enriched in coconut oil lead to faster oxidation and increased plasma ketone bodies such as BHB (Dayrit 2015), which is also seen in calves (Sato 1994). The dosage of coconut oil administered was probably too low to detect an effect of coconut oil on plasma ketone bodies in the present study. We found an elevated plasma BHB concentration but a decreased plasma NEFA concentration and an improved EB in EFA+CLA cows (Vogel

et al. 2020), which does not support the classical concept of an increase in the plasma BHB concentration associated with elevated plasma NEFA and an overloaded fat concentration in the liver during early lactation (Drackley et al. 2001). The elevated plasma BHB concentration in EFA+CLA was probably a consequence of milk fat depression caused by CLA (Bernal-Santos et al. 2003; Urrutia and Harvatine 2017; Vogel et al. 2020), and not increased BHB production in the liver. A decreased BHB production in liver is supported by the low hepatic mRNA abundance of *HMGCS2*, encoding a key enzyme in ketone body synthesis, in EFA+CLA cows on d 28 PP.

Despite the higher plasma glucose concentration observed on d 21 PP, eGP was decreased by CLA treatment. The inverse relationship between plasma glucose and eGP supported our previous finding of the effect of CLA treatment on plasma glucose and whole-body glucose metabolism (Grummer and Carroll 1991; Hötger et al. 2013). Recently published data of the present study indicated a strong milk fat depression during early lactation in CLA cows by 50% when compared with CTRL and EFA groups. (Vogel et al. 2020). The decrease in eGP due to CLA treatment indicated a decreased glucose demand for milk fat synthesis induced by *trans*-10, *cis*-12 CLA (Baumgard et al. 2000), but could also result from the more efficient use of metabolizable energy in CLA-treated cows (von Soosten et al. 2012; Hötger et al. 2013). In this context, it is noteworthy that cows supplemented only with CLA also showed a decrease in GOx and an elevated glucose/glucagon ratio on d 21 PP in the present study. This finding emphasizes less glucose utilization induced by CLA treatment.

Endocrine changes during the transition and early lactation periods supported the concept of alleviated glucose load by decreasing glucose utilization during the CLA treatment (Drackley et al. 2001; Reist et al. 2003; Weber et al. 2013b). An elevated insulin concentration in CLA-supplemented cows during the transition period was previously described (Saremi et al. 2014, Grossen-Rösti et al. 2018). The increased basal plasma insulin concentration and decreased glucagon to insulin and glucose to insulin ratios were consistent with the diminution of eGP after calving in CLA cows (De Koster and Opsomer 2013; Hammon et al. 2016). Plasma cortisol was decreased in CLA groups at the end of the profiling on d 21 PP. Cortisol may act as a gluconeogenic hormone in cattle (Brockman and Laarveld 1986) and evoke an insulin-resistant state in dairy cows (Kusenda et al. 2013; Hammon et al. 2016) and young calves (Scheuer et al. 2006). We therefore speculate that insulin sensitivity was increased in the CLA-treated groups due to decreased cortisol release in blood plasma. However, previous studies have not indicated increased insulin sensitivity under CLA treatment (Saremi et al. 2014). On the contrary, CLA treatment, especially *trans*-10, *cis*-12 CLA, caused an insulin resistant state in rodents, but dosages used in those studies were much higher than administered in the present study (Halade et al. 2010; Bezan et al. 2018). Further studies using insulin-dependent glucose clamps might be necessary to clarify this issue, but the fact that eGP as well as plasma

NEFA and hepatic triglycerides (Vogel et al. 2020) were decreased in CLA-treated cows may indicate no insulin resistant state because of the CLA treatment. The elevated glycogen concentrations in the liver confirmed the improved glucose and energy status of CLA groups (Vogel et al. 2020), because the hepatic glycogen concentration is positively associated with the EB after calving (Hammon et al. 2009; Weber et al. 2013b). The CLA supplementation did not affect the hepatic glycogen concentration during the transition period in previous studies, but the EB was also not affected by CLA treatment in these studies (Bernal-Santos et al. 2003, Hötger et al. 2013).

The temporal pattern of gluconeogenic enzyme mRNA abundance in the liver during the transition period was consistent with previously reviewed changes and was the consequence of an increased demand for glucose and a shift in gluconeogenic substrate availability after calving (Greenfield et al. 2000; Donkin 2016; Hammon et al. 2016). The higher abundance of *PC* mRNA as well *PCK2* mRNA observed at calving suggested an increased abundance of lactate available as a substrate for gluconeogenesis (Reynolds et al. 2003; Weber et al. 2013a; Hammon et al. 2016). Lactate originates from increased PDV release and enhanced endogenous lactate production by Cori cycling, and compensates for decreased availability of propionate because of insufficient DMI (Aschenbach et al. 2010; Weber et al. 2013a; Hammon et al. 2016). The *PCK1* mRNA expression was elevated after reaching maximal DMI and was shown to be responsive to rumen propionate production, indicating the feedforward control of gluconeogenesis (Weber et al. 2013a; Donkin 2016; Hammon et al. 2016). The mRNA abundance related to gluconeogenesis in the liver was less affected by CLA treatment, but decreased mRNA abundance was revealed in cows under EFA treatment during early lactation. These findings were not in accord with the elevated eGP production, increased plasma glucagon concentration and increased glucagon/insulin ratio observed in blood plasma during profiling, especially in cows treated only with EFA in early lactation, but they were associated with lower eGP production in EFA+CLA cows after calving. The reasons for these partially inconsistent findings between the observed gluconeogenic mRNA abundance in the liver and endocrine changes are presently not known.

Gluconeogenic enzymes are regulated at the transcriptional level by hormones such as glucagon and insulin but are also substrate regulated (Loor 2010; Donkin 2016; Hammon et al. 2016). Furthermore, the decreases in mRNA abundance in the liver caused by insulin differ among the gluconeogenic enzymes during the transition period in dairy cows (Weber et al. 2017). The decreased mRNA abundance of *PC*, *PCK1*, *PCK2*, *G6PC*, and *PCCA* during early lactation due to EFA treatment might be a consequence of improved insulin sensitivity. Previous studies in bulls and cows indicated enhanced insulin sensitivity when n-3 FA were supplied (Pires and Grummer 2008; Fortin et al. 2010; Hashemzadeh-Cigari et al. 2015). The association of hepatic gluconeogenic enzyme expression with the measurement of eGP and endocrine

changes showed the best correspondence under the EFA+CLA treatment. Cows treated only with EFA exhibited elevated eGP but low mRNA abundance of most of the measured enzymes on d 28 PP. In cows treated with CLA only, decreased eGP was associated with elevated mRNA abundance of *PCK1*, *PCK2*, *G6PC*, and *PCCA* during early lactation. The FA treatments applied in the present study clearly affected the regulation of gluconeogenic enzymes at the transcription level differentially.

4.5.2 Somatotropic Axis and Hepatic mRNA Abundance of the GH-IGF System

The changes in GH, IGF-I, and IGFBP-2 and IGFBP-3 in blood plasma around the time of calving and during early lactation corresponded to the changes in the EB in these cows (Vogel et al. 2020). The negative EB around the time of calving and during early lactation is associated with increasing concentrations of plasma GH and IGFBP-2 but decreasing plasma IGF-I and IGFBP-3 concentrations (Reist et al. 2003; Gross et al. 2011b; Kessler et al. 2013). In general, an insufficient energy status or undernutrition are connected with an uncoupling of the somatotropic axis, indicating increasing GH and decreasing IGF-I concentrations as well as a lower IGFBP-3 to IGFBP-2 ratio in blood plasma (Etherton and Bauman 1998; Renaville et al. 2002; Lucy 2004). Because the liver significantly contributes to the systemic somatotropic axis, the negative EB during the transition period leads to corresponding changes in key factors in the somatotropic axis in the liver. Thus, the mRNA abundance of *GHR1A*, *IGF1*, and *IGFBP3* decreased, but the *IGFB2* mRNA abundance increased (Kobayashi et al. 1999; Fenwick et al. 2008; Gross et al. 2011b), and the *INSR* mRNA abundance did not change at calving (Gross et al. 2011b; Weber et al. 2017). Similar responses regarding the abundance of these mRNA were determined in the present study, and the findings in blood plasma and the liver were consistent with the overall concept of nutrition repartitioning at the beginning of lactation (Bauman 2000; Lucy 2004; Gross and Bruckmaier 2019).

Cows treated with CLA exhibit an increased plasma IGF-I concentration during early lactation (Castañeda-Gutiérrez et al. 2007; Csillik et al. 2017), which was also found in the present study. The improved energy status in CLA cows (Vogel et al. 2020) was closely related to the increasing IGFBP-3 to IGFBP-2 ratio in blood plasma. IGFBP-3 binds most of the IGF-I present in blood plasma, whereas IGFBP-2 may support the transport of IGF-I from blood plasma into tissue (Jones and Clemmons 1995). The stimulation of the somatotropic axis, i.e., elevated IGF-I by decreased GH in blood plasma, takes place when plasma glucose and insulin concentrations are elevated in dairy cows during the transition period (Butler et al. 2003; Rhoads et al. 2004). Because the improved energy status in CLA cows was associated with an improved glucose and insulin status, the stimulation of the somatotropic axis in the present study was closely related to enhanced glucose and insulin availability in these cows (McGuire et al.

1995; Brameld et al. 1999; Clemmons 2018). On the other hand, the elevated plasma IGFBP-4 concentration observed at the end of the study in CLA-treated cows might counteract the increased plasma IGF-I concentration because IGFBP-4 has mainly inhibitory effects on IGF-I action (Jones and Clemmons 1995; Clemmons 2018). Plasma GH was less affected by CLA treatment, even though previous findings indicated a stimulatory effect of CLA on plasma GH (Qin et al. 2018).

The CLA treatment showed only minor effects on stimulating the parameters of the somatotropic axis in the liver. The most obvious finding was the inhibition of *IGFBP2* by CLA treatment during early lactation, which was consistent with the lower plasma IGFBP-2 concentration observed in CLA-treated cows at the end of the study. In addition, there were some minor stimulatory effects on *GHR1A* mRNA but not on *IGF1* mRNA. Although the liver is involved in the release of components of the somatotropic axis to the blood plasma, the liver is not the only organ that contributes to the systemic somatotropic axis, and regulation of the hepatic IGF system might occur beyond the transcription level (Thissen et al. 1994; Le Roith et al. 2001). Changes in plasma concentrations related to the somatotropic axis were less affected by EFA treatment. These findings corresponded well with the lack of an effect of EFA treatment on the EB of these cows during the transition period (Vogel et al. 2020). Therefore, our results differ from earlier studies reporting a stimulatory effect of n-3 FA treatment on the somatotropic axis in cows (Carriquiry et al. 2009a; Dirandeh et al. 2016; Doyle et al. 2019). In the liver, there was also no stimulatory effect of EFA treatment on mRNA abundance related to the somatotropic axis, which again contrasted with the findings of Dirandeh et al. (2016). Interestingly, n-3 FA supplementation did not affect the stimulation of the hepatic somatotropic axis by GH treatment (Carriquiry et al. 2009b). In contrast, some inhibitory effects of EFA treatment on the mRNA abundance of *IGFBP2*, *IGFBP3*, and *INSR* have been observed, but a direct inhibitory effect of n-3 FA on gene expression related to the somatotropic axis in the liver of cows has yet to be demonstrated.

4.6 Conclusions

Our results indicated an improved glucose and insulin status along with the stimulation of the somatotropic axis in dairy cows treated with CLA, which corresponded well with the improved EB during late and early lactation in CLA cows (Vogel et al. 2020). In contrast, EFA treatment had hardly any influence on the endocrine regulation of nutrient partitioning during the investigated experimental period, but resulted in highest eGP PP in cows treated exclusively with EFA and, on the contrary, showed decreased hepatic mRNA abundance of genes related to gluconeogenesis. The combined EFA+CLA treatment showed very similar results to the CLA treatment concerning the blood data related to the insulin response and the somatotropic axis, but the effects on gene expression in the liver regarding gluconeogenesis were more consistent to the effects of the EFA treatment only. No additive stimulation of the somatotropic axis by the combined EFA and CLA treatment was found in the present study.

4.7 Acknowledgements

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4.8 Supplementary Material

Table 4.3 Characteristics of primers and real-time PCR conditions

Primer[1]	Sequence (5´-3´)	Accession number[2]	Amplicon size bp	Mean Cq[1]	Annealing °C	Efficiency
Reference genes						
LRP10		BC149232	139	23.93	60	1.85
Forward	CCAGAGGATGAGGACGATGT					
Reverse	ATAGGGTTGCTGTCCCTGTG					
POLR2A		NM_001206313	130	26.31	60	1.86
Forward	GTCCGGATGAACTGAAGCGA					
Reverse	CGACCCGTCCTCTCAATCAC					
Genes related to glucose metabolism						
PC		NM_177946	353	24.26	60	1.83
Forward	ACACCAACTACCCCGACAATG					
Reverse	CAGCGGGAGGTCAGGGAAG					
PCK1		NM_174737	367	20.66	60	1.83
Forward	ATGACAACTGCTGGTTGGCT					
Reverse	TGGAGGCACTTGACGAACTC					
PCK2		NM_001205594	181	22.63	60	1.85
Forward	GCCGTAGACCCAAAGGAGTC					
Reverse	TCAAGGTAGCGCCCAAAGTT					
G6PC		BC114011	275	20.97	60	1.86
Forward	ATGTTGTGGTTGGGATTCTGG					
Reverse	CAGCGGGAGGTCAGGGAAG					

Table 4.3 Continuation

Primer[1]	Sequence (5´-3´)	Accession number[2]	Amplicon size bp	Mean Cq[1]	Annealing °C	Efficiency
PCCA		NM_001083509	189	21.54	53	1.85
Forward	AACGTTTGGCAGCAGAAGAT					
Reverse	TGACAGGGTAGCCAATTTCC					
HMGCS2		NM_001045883	126	20.25	60	1.88
Forward	TCTGGCCCATCACTCTGCC					
Reverse	AGTGGGGAGCCTGGAGAAGC					
Genes related to the somatotropic axis						
GHR1A		XM_019982775	86	23.07	60	1.87
Forward	CCAGCCTCTGTTTCAGGAGTGT					
Reverse	TGCCACTGCGAAGGTCAAC					
IGF1		NM_001077828	215	25.22	56	1.85
Forward	ATAGAGCCTGCGCAATGGAA					
Reverse	GGCATCTTCACCTGCTTCAAGA					
IGFBP2		NM_174555	136	19.36	60	1.82
Forward	CACCGGCAGATGGGCAA					
Reverse	GAAGGCGCATGGTGGAGAT					
IGFBP3		NM_174556	139	22.57	56	1.86
Forward	AAAGGTCATGCCAAGGACAG					
Reverse	GGTTCAGCGTGTCTTCCATT					
INSR		XM_002688832	163	25.05	62	1.85
Forward	TCCTCAAGGAGCTGGAGGAGT					
Reverse	GCTGCTGTCACATTCCCCA					

[1]*LRP10*, low-density lipoprotein 10; *POLR2A*, RNA polymerase II; *PC*, pyruvate carboxylase; *PCK1*, phosphoenolpyruvate carboxykinase (cytosolic); *PCK2*, phosphoenolpyruvate carboxykinase (mitochondrial); *G6PC*, glucose-6-phosphatase; *PCCA*, propionyl-CoA carboxylase alpha chain (mitochondrial); *HMGCS2*, hydroxyl-methyl-glutaryl-CoA-synthase 2; *GHR1A*, growth hormone receptor 1A; *IGF1*, insulin-like growth factor I; *IGFBP2*, insulin-like growth factor binding protein 2; *IGFBP3*, insulin-like growth factor binding protein 3; *INSR*, insulin receptor
[2]Quantification cycle
[3]Database used: National Center for Biotechnology Information (NCBI) Entrez Nucleotide (http://www.ncbi.nlm.nih.gov/nucleotide)

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5. GENERAL DISCUSSION

For dairy cows, lactation performance and nutrition partitioning significantly determines the energy metabolism during early lactation and thus affect health status and productivity of the animal. By adding EFA or CLA to the diet, productivity is improved, as these bioactive FA are known to possess various beneficial effects and functional properties that promote whole-body energy metabolism (Moallem 2018; Shokryazdan et al. 2017). As these FA interact with each other, the main objective of this thesis is to investigate the effects of single and combined EFA and CLA supplementations on performance and energy metabolism in cows during the transition period. In order to investigate said effects on performance and energy metabolism accurately, it is of major importance to eliminate ruminal biohydrogenation as a factor, and instead identify them as results of respective FA administrations.

5.1 Application Methodology

Over the last decade, various studies have been conducted to evaluate the metabolic and health-promoting properties of EFA and CLA. Among studies, the effects of EFA and CLA supplementation on dairy cows' performance and metabolism were dependent on the method, dose and duration of FA supplementation, as well as on the stage of lactation.

In the present study, post-ruminal infusions of EFA and CLA were used to ensure that effects of supplemental FA on ruminal microbes or microbial hydrogenation of infused FA can be safely excluded, so that the investigated impacts can be directly linked to the supplemented FA. Feeding trials are often contaminated by the influence of the gastro-intestinal compartment (e.g., by ruminal biohydrogenation), which impedes evaluation of individual FA treatments. Duodenal cannulas proved to be a valuable component for research on cattle nutrition but were deselected due to frequent criticism concerning animal welfare. Therefore, FA supplements were infused directly into the abomasum to bypass ruminal biohydrogenation. This technique comprised of inserting an infusion line through the rumen cannula and the sulcus omasi to finally end up in the abomasum had already been used before (Spires et al. 1975; Drackley et al. 1992; Benson et al. 2001; Brickner et al. 2009). The functionality of the abomasal administration could be confirmed by the accumulation of the infused FA pattern in the milk of the respective animals. Accurate and secure placement of abomasal infusion lines was essential for the success of this research study. Therefore, the correctness of the position of the abomasal line placement was checked and confirmed weekly.

The doses of the supplied EFA (linseed and safflower oil in a ratio of 19.5:1; providing an n-6/n-3 FA ratio of 1:3 in the supplement mixture) and CLA were recently evaluated in a companion study on dose-response in mid-lactating dairy cows (Haubold et al. 2020a, b). The treatments focused on the supply of FA that provide a reduced n-6/n-3 EFA ratio, in particular an increase in ALA supply, and CLA. Such a dietary FA composition usually occurs in cows fed fresh grass or cows that are on pasture (Kelly et al. 1998; Ferlay et al. 2006). Cows were supplied with ALA (from linseed oil), CLA (*cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA in equal amounts), or a combination of both in increasing amounts and comparable with a pasture-based feeding system (Kelly et al. 1998; Ferlay et al. 2006). The dose-response relationship of combined EFA and CLA supplementation by abomasal infusion on basic metabolic functions and changes in FA composition of milk and blood in dairy cows were investigated in a 4 x 4 Latin square study. Cows were fed with the same corn silage-based diet as used in the present study. The magnitude of response of the metabolism was studied to determine adverse and beneficial dosage levels of supplemented FA. The used dosage for this follow-up study was verified as suitable due to a lack of DMI depressive effects despite a direct abomasal infusion of the FA.

Nearly all post-ruminal FA-infusion studies were conducted in cows in established or late lactation and were short-term evaluations (Chouinard et al. 1999; Baumgard et al. 2001; Benson et al. 2001; Baumgard et al. 2002; Mackle et al. 2003; Kazama et al. 2010; Khas-Erdene et al. 2010). Similar amounts of respective FA administration did not necessarily always lead to equal responses because of different lactation stages and varying durations of supplementation. In dairy cows, the time around calving is the most critical physiological stage, which consists of challenging metabolic and endocrine changes that are unique for this lactation period. Therefore, to improve our understanding of metabolic changes with EFA and CLA supplementation, post-ruminal trials also had to be conducted in early lactation and extended for a longer period of time, starting in late lactation. To the best of our knowledge, this study is the first conducted survey of abomasal long-term EFA and CLA supplementation from late gestation to early lactation.

5.2 Discovering Relationships of Essential Fatty Acids with Conjugated Linoleic Acid Supplementation

5.2.1 Production Performance

In the present thesis, the hypotheses were tested that in cows combined CLA and EFA treatment supports production performance, altering milk composition and FA pattern, and further improve energy utilization around the time of calving in an additive manner.

Study results show that the known hypophagic effect of PUFA was probably not recorded because of the moderate doses of linseed oil supplied to the cows (Drackley et al. 1992; Bremmer et al., 1998). In the companion dose-response study in mid-lactating dairy cows, a DMI depressive effect did not occur with increasing administration of EFA, but the combination of EFA and CLA treatment at the highest dosages lowered DMI in a synergistic manner (Haubold et al. 2020a). A hypophagic effect of CLA was detected at the end of the trial in the mentioned study. An important reason for the decrease in DMI and energy intake can be attributed to the reduction of the energy requirement due to lower milk fat production and ECM in the CLA-treated cows (Baumgard et al. 2000; Harvatine et al. 2009; Moallem et al. 2010; Schäfers et al. 2017). Results of this study indicate a reduced milk fat content after CLA, with or without EFA supplementation, during late and early lactation (Baumgard et al., 2000). However, the lack of changes in milk fat in EFA in the study was consistent with findings in mid-lactating cows supplied with the same EFA dose (Haubold et al. 2020a). Milk fat reduction with CLA supplementation was particularly obtained thanks to a reduced *de novo* FA synthesis in the mammary gland, as indicated by the proportional decrease in <C16 FA and a resulting shift to longer-chain FA (Chouinard et al. 1999; Perfield II et al. 2002). Milk citrate is indirectly associated with FA synthesis in the mammary gland of ruminants. Elevated citrate concentrations in milk of the CLA cows point at a reduced NADPH production for FA synthesis and represent a decline in *de novo* FA synthesis (Linzell et al. 1976; Mackle et al. 2003; Garnsworthy et al. 2006; Haubold et al. 2020a). Milk citrate was less affected by combined EFA and CLA treatment, indicating that EFA supplementation may influence changes achieved by CLA in the mammary gland's FA metabolism.

An elevated milk protein content after CLA supplementation was observed only in late lactation. Reduced milk protein and urea concentrations in early lactation after CLA administration are probably caused by a higher body protein accretion and nitrogen retention after CLA supplementation (Moallem et al. 2010; von Soosten et al. 2011 and 2012). The different results of CLA supplementation on the milk protein concentration in late and early lactation might be a consequence of the lactation stage. The protein balance was positive during late lactation but turned to negative results during early lactation, which could have affected CLA responses to

milk protein content. Supplementation of EFA led to a higher content of n-3 FA and a shift in the n-6/n-3 ratio in the milk FA profile compared with CTRL and CLA. The differences in the proportions of n-3 and n-6 FA in milk fat reflected the composition of the infused FA in EFA and EFA+CLA (Petit 2002; Kazama et al. 2010; Moallem et al. 2012; Moallem 2018). The accumulation of ALA and LA was higher in EFA+CLA than in EFA due to the lower milk fat content and reduction of *de novo* FA synthesis in the mammary gland following CLA supplementation. Lower transfer efficiencies of ALA and the infused CLA isomers after parturition could be a consequence of the enrichment of these FA in colostrum during the dry period, which probably reached a plateau at the end of colostrogenesis. With ongoing milk production in early lactation, transfer efficiency rates of the infused FA increased anew.

In the current study, the reduced milk fat content in the CLA and EFA+CLA groups leads to a significantly improved EB in these cows, which additionally resulted in less BW reduction PP and more body and omental fat in CLA-supplemented cows at the end of the study. Therefore, the huge milk fat depression and reduction in body fat mobilization due to CLA treatment support enhanced accretion of body fat in these cows. In both CLA-supplemented groups, the energy status of the cows was improved. The EFA treatment may have affected the relative degree of fat mobilization in different fat depots but not the overall degree of body fat mobilization.

To summarize the results, very few effects of the EFA treatment alone were evident with regard to production performance from late gestation to early lactation, indicating low importance of an enhanced EFA supply in overcoming the critical metabolic situation of the negative EB after calving. Our data confirmed the reduced milk fat content, improved energy status, and enhanced accretion of body fat in cows treated with CLA but not with exclusive EFA supplementation during late and early lactation. Supplementation with CLA but not EFA affects milk protein, urea, and citrate content, whereas EFA supplementation influences changes in mammary gland FA metabolism achieved by CLA. The milk FA pattern changed according to the respective supplemented FA in EFA and CLA. The observed different degrees of FA's effects on milk performance during late and early lactation were probably not only a consequence of the different lactation stage but also the duration of treatment because cows in early lactation received the FA for a much longer time.

5.2.2 Metabolic and Endocrine Changes

In the present thesis, a hypothesis was tested that in cows, combined CLA and EFA treatment alters lipid and glucose metabolism around the time of calving and affects the partitioning of nutrients by endocrine changes in an additive manner.

Results of this study show that in the CLA groups, reduced severity of negative EB lowered the mobilization of adipose tissue and in turn led to a lower increase of circulating NEFA concentration around calving (Bauman and Currie 1980; Kay et al. 2006; Odens et al. 2007; Weber et al. 2013; Galamb et al. 2017). Additionally, PP liver TG was reduced in the CLA groups, as liver TG is associated with elevated lipomobilization and plasma NEFA levels (Bobe et al. 2004; Overton and Waldron 2004; Weber et al. 2013). Processed FA from the liver can be delivered as lipoprotein-associated cholesterol fractions in plasma. Higher plasma LDL cholesterol concentration after EFA+CLA supplementation indicated a lower mammary uptake of cholesterol and not an enhanced export rate of cholesterol from the liver, particularly because liver TG was diminished in the CLA groups. Supplementation of EFA minorly affected metabolites in blood plasma related to lipid metabolism.

We observed only slight differences in the basal plasma glucose concentration because of EFA supplementation. During early lactation in EFA cows, the low plasma glucose concentration may partly explain the elevated NEFA and BHB concentration observed on d 21 PP (Drackley et al., 2001). However, eGP on d 21 PP was highest in the EFA cows, indicating counter-regulation to maintain plasma glucose concentration in EFA cows. However, in the liver, decreased mRNA abundance of genes related to gluconeogenesis (*PC*, *PCK1*, *PCK2*, *G6PC*, and *PCCA*) was observed in EFA treatment during early lactation and might be a consequence of improved insulin sensitivity (Pires and Grummer 2008; Fortin et al. 2010; Hashemzadeh-Cigari et al. 2015; Weber et al. 2017). However, these findings were not in accord with the results revealing elevated eGP production in cows treated only with EFA in early lactation. The inverse relationship between higher plasma glucose concentration and decreased eGP observed on d 21 PP by CLA treatment supported previous findings of the effect of CLA treatment on plasma glucose and whole-body glucose metabolism (Hötger et al., 2013). This decrease in eGP indicated a decreased glucose demand for milk fat synthesis induced by *trans*-10,*cis*-12 CLA but could also result from the more efficient use of metabolizable energy in CLA-treated cows (Baumgard et al. 2000; von Soosten et al. 2012; Hötger et al. 2013). In cows treated with CLA only, decreased eGP was associated with elevated mRNA abundance of *PCK1*, *PCK2*, *G6PC*, and *PCCA* during early lactation. The FA treatments applied in the present study clearly affected the regulation of gluconeogenic enzymes at the transcription level differentially. In this context, it is noteworthy that cows supplemented only with CLA also showed a decrease in GOx and an elevated glucose/glucagon ratio on d 21 PP in the present study. The elevated glycogen concentrations in the liver confirmed the improved glucose and energy status of CLA groups because the hepatic glycogen concentration is positively associated with the EB after calving (Hammon et al. 2009; Weber et al. 2013). These findings indicate less glucose utilization induced by CLA treatment.

An elevated plasma BHB concentration in EFA+CLA was probably a consequence of milk fat depression and reduced utilization of acetyl-CoA caused by CLA, and not increased BHB production in the liver (Bernal-Santos et al. 2003; Urrutia and Harvatine 2017). A decreased BHB production in the liver is possible thanks to the low hepatic mRNA abundance of *HMGCS2*, encoding a key enzyme in ketone body synthesis, in EFA+CLA cows on d 28 P.

Endocrine changes during the transition and early lactation period supported the concept of alleviated glucose load caused by a decreased glucose utilization during the CLA treatment (Drackley et al., 2001; Reist et al., 2003; Weber et al., 2013b). The increased basal plasma insulin concentration as well as a decreased glucagon to insulin ratio and glucose to insulin ratio were consistent with the diminution of eGP after calving in CLA cows and indicate an increased insulin sensitivity under CLA treatment (De Koster and Opsomer, 2013; Hammon et al., 2016). To clarify the effects of CLA on insulin resistance in dairy cows, further studies using insulin-dependent glucose clamps might be necessary but decreased eGP, plasma NEFA, and liver TG in CLA-treated cows indicate no insulin-resistant state under the CLA treatment.

Cows supplemented with CLA exhibit an increased plasma IGF-I concentration during early lactation and a greater IGFBP-3 to IGFBP-2 ratio than CTRL in blood plasma (Castañeda-Gutiérrez et al. 2007; Csillik et al. 2017). Our data confirmed a greater stimulation of the somatotropic axis in CLA cows with an improved energy status that was related to enhanced glucose and insulin availability in these cows during the transition period (McGuire et al. 1995; Brameld et al. 1999; Clemmons 2018). The alterations in hepatic gene related to the somatotropic axis were seen as inhibition of *IGFBP2* by CLA treatment during early lactation, which proved consistent with the lower plasma IGFBP-2 concentration observed in CLA-treated cows at the end of the study. In addition, there were some minor stimulatory effects of CLA treatment on *GHR1A* mRNA but not on *IGF1* mRNA, whereby regulation of the hepatic IGF system might occur beyond the transcription level (Thissen et al. 1994; Le Roith et al. 2001). On the other hand, EFA supplementation had minor effects on the systemic somatotropic axis. This finding corresponds well with the lack of any effect of EFA treatment on the EB of these cows during the transition period. No additive stimulation of the somatotropic axis by the combined EFA and CLA treatment was found in the present study.

In summary, the results of the study indicate that supplementation with CLA and the combination of CLA with EFA resulted in reduced plasma NEFA and liver TG after calving. The decreased eGP and increased liver glycogen content PP indicated a glucose-sparing effect in CLA and EFA+CLA cows to retain glucose homeostasis due to less milk fat synthesis by CLA treatment. Results showed elevated concentrations of plasma insulin along with the stimulation of the somatotropic axis in cows treated with CLA, which corresponded well with the improved EB during late and early lactation in CLA cows. The EFA treatment enhances glucose produc-

tion but inhibits hepatic mRNA abundance related to gluconeogenesis PP, pointing at a variable influence of EFA on hepatic glucogenic enzyme gene expression in dairy cows. To retain glucose homeostasis in PP cows, the decreased glucose utilization induced by reduced milk fat synthesis with CLA treatment is counterbalanced by reduced eGP in CLA cows. In EFA cows, glucose homeostasis is achieved by an up-regulation of liver glucose production.

5.2.3 Principal Component Approach

A Principal Component Analysis (PCA; **Figure 5.1**) was conducted to explore the most important results more fully out of Manuscript 1 and Manuscript 2 and aims to display the maximum amount of variation in a data profile within a few principal components. That type of analysis is a technique used to increase the interpretability of major findings, put them into perspective by reducing the dimensionality, and at the same time minimizing information loss of the dataset. The PCA analysis was carried out by the frequency time (levels: day or week relative to calving) to visualize the relationships between 55 variables of performance and milk composition data, metabolites and hormones in blood plasma related to the lipid and glucose metabolism, and hepatic gene expression data related to gluconeogenesis and the somatotropic axis. For a better interpretation of the data, a Pearson correlation was done between all 55 variables. The aim of this analysis was to explore nutritional and metabolic interrelationships between EFA and CLA supplementation. As such, it was examined whether individual and/or synergistic effects of EFA and CLA exist at the levels that refer to the supply of EFA and related rumen and tissue CLA production in dairy cows receiving fresh grass or cows on pasture.

The analysis revealed that the first two principal components account for approximately 29.1% of the total variance of the dataset. The Loadings Plot (**Figure 5.1A**) graphs the unrotated loading matrix between the variables and the components showing the correlations between the original variables and the first two principal components. Loadings with orientation or direction close to -1 or 1 indicate that the variable strongly influences the component. Loadings close to 0 indicate that the variable has a weak influence on the component. The length of a vector in the space shows the changeability of this variable by the displayed component, so a closer localization to the center of the cross indicates low correlations to the considered components. The angles between vectors of different variables show their correlation in this space: small angles represent high positive correlation, right angles represent lack of correlation, opposite angles represent high negative correlation.

The Score Plot (**Figure 5.1B**) graphs each component's calculated values in relation to the other. The projection of cases demonstrates the individual cows based on the 55 variables and shows the differences between the treatment groups, especially between EFA+CLA and CTRL.

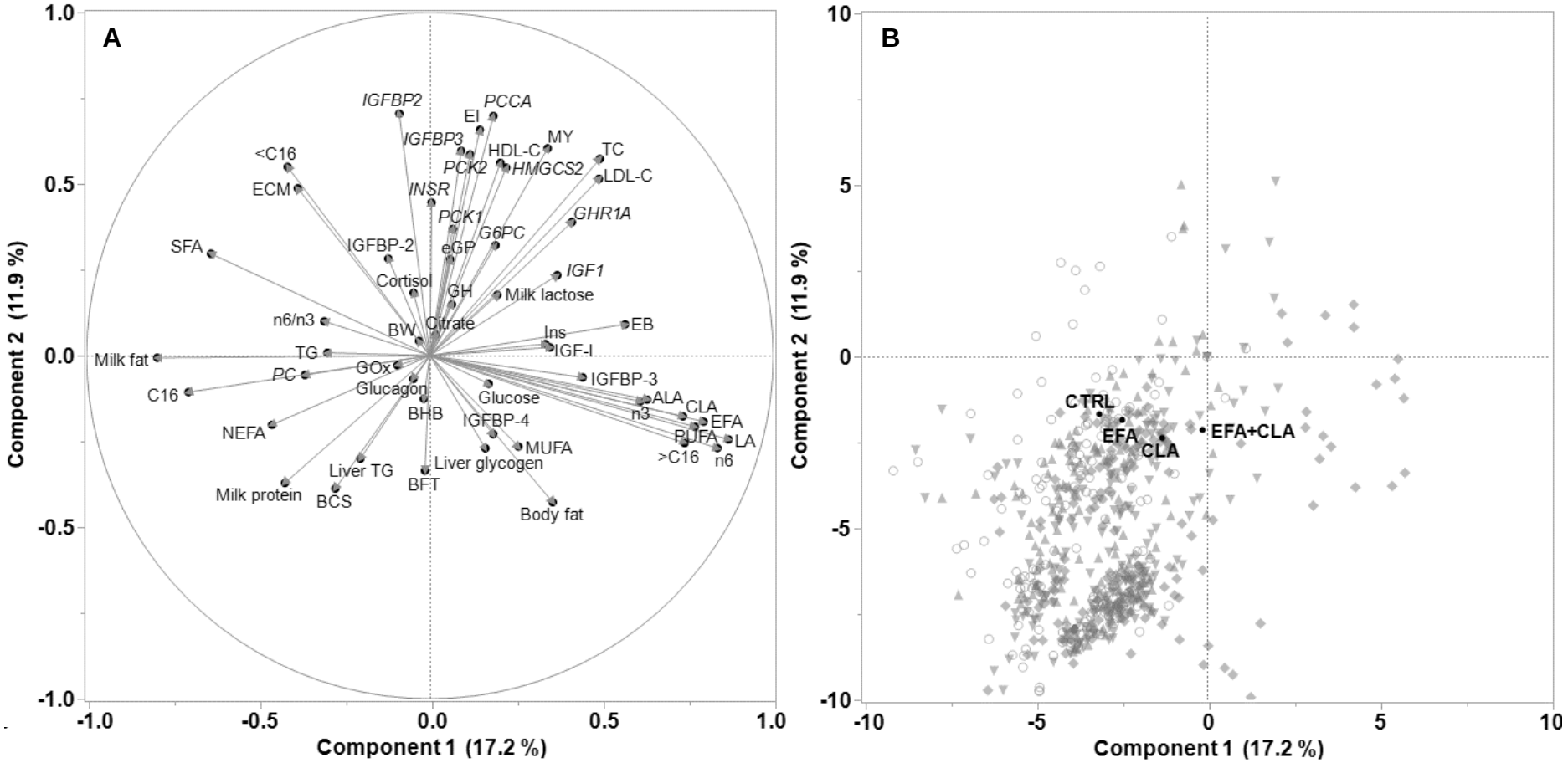

Figure 5.1 Visualization of relationships between performance and milk composition data, metabolites related to the lipid metabolism, metabolites, hormones and gene expression data related to the glucose metabolism, as well as hormones and gene expression data of the somatotropic axis by Principal Component Analysis (A projection of variables; B projection of cases) of cows supplemented daily with coconut oil (CTRL;○; n = 9), linseed and safflower oil (EFA;▲; n = 9), Lutalin® (CLA;▼; *cis*-9,*trans*-11 and *trans*-10,*cis*-12; BASF SE, Ludwigshafen, Germany; n = 10), and EFA+CLA (◆; n = 10) abomasally from d 63 antepartum until slaughter on d 63 postpartum.

Variables of the analysis: <C16, sum of fatty acids <C16 in milk fat; >C16, sum of fatty acids >C16 in milk fat; ALA, α-linolenic acid in milk fat; BCS, body condition score; BFT, back fat thickness; BHB, concentration of plasma β-hydroxybutyrate; Body fat, sum of subcutaneous, retroperitoneal, omental, and mesenteric fat at slaughter; BW, body weight; C16, C16 fatty acids in milk fat; Citrate, citrate in milk; CLA, conjugated linoleic acid in milk fat; Cortisol, concentration of plasma cortisol; EB, energy balance; ECM, energy corrected milk; EFA, essential fatty acids in milk fat; eGP, endogenous glucose production;

EI, NE_L intake; *G6PC*, relative hepatic mRNA expression of glucose-6-phosphatase; GH, concentration of plasma growth hormone; *GHR1A*, relative hepatic mRNA expression of growth hormone receptor 1A; Glucagon, concentration of plasma glucagon; Glucose, concentration of plasma glucose; GOx, glucose oxidation; HDL-C, concentration of plasma high-density lipoprotein cholesterol; *HMGCS2*, relative hepatic mRNA expression of and hydroxyl-methyl-glutaryl-CoA-synthase 2; ; *IGF1*, relative hepatic mRNA expression of IGF-I; IGFBP-2, concentration of plasma IGF-binding protein 2; *IGFBP2*, relative hepatic mRNA expression of IGF-binding protein 2; IGFBP-3, concentration of plasma IGF-binding protein 3; *IGFBP3*, relative hepatic mRNA expression of IGF-binding protein 3; IGFBP-4, concentration of plasma IGF-binding protein 4; IGF-I, concentration of plasma IGF-I; Ins, concentration of plasma insulin; *INSR*, relative hepatic mRNA expression of insulin receptor; LA, linoleic acid in milk fat; LDL-C, concentration of plasma low-density lipoprotein cholesterol; Liver Glycogen, liver glycogen; Liver TG, liver triglyceride; Milk fat, milk fat concentration; Milk lactose, milk lactose concentration; Milk protein, milk protein concentration; MUFA monounsaturated fatty acids in milk fat; MY, milk yield; n3, n-3 fatty acids in milk fat; n6, n-6 fatty acids in milk fat; n6/n3, ratio of n-6/n-3 fatty acids in milk fat; NEFA, concentration of plasma non-esterified fatty acids; *PC*, relative hepatic mRNA expression of pyruvate carboxylase; *PCCA*, relative hepatic mRNA expression of mitochondrial propionyl-CoA carboxylase alpha chain; *PCK1*, relative hepatic mRNA expression of cytosolic phosphoenolpyruvate carboxykinase; *PCK2*, relative hepatic mRNA expression of mitochondrial phosphoenolpyruvate carboxykinase; PUFA polyunsaturated fatty acids in milk fat; SFA, saturated fatty acids in milk fat; TC, concentration of plasma total cholesterol; TG, concentration of plasma triglyceride

Figure 5.1A shows that the variability of component 1 is strongly influenced by the milk FA composition. These variables relate to the long-chain unsaturated FA, such as LA, n-6 FA, EFA, PUFA, >C16, CLA, ALA, and n-3 FA in the respective order, and cluster positive in component 1 with less impact on component 2. ALA relates to the n-3 family of PUFA and is known to be an EFA. It enhances ALA concentration in milk fat, increases n-3 FA (r = 1.00, $P < 0.001$), PUFA (r = 0.94, $P < 0.001$), EFA (r = 0.94, $P < 0.001$), and decreases the n6/n3 ratio (r = -0.84, $P < 0.001$) in milk fat. An elevated concentration of >C16 FA has a negative relation with SFA (r = -0.92, $P < 0.001$), C16 FA (r = -0.81, $P < 0.001$), and <C16 FA (r = -0.72, $P < 0.001$), but a positive relation with MUFA (r = 0.76, $P < 0.001$) in milk fat. The results of the present study intensively illustrate how EFA and CLA supplementation are transferred into milk and change milk composition, including FA pattern (Manuscript 1). The pattern and magnitude of changes in milk FA in response to EFA and CLA was based on previous research (Chouinard et al. 1999: Baumgard et al. 2001; Bernal-Santos et al. 2003; Petit et al. 2002; Kazama et al. 2010; Moallem et al. 2012; Moallem 2018). The altered milk FA composition of EFA and CLA supplementation was characterized by a shift to lower *de novo* synthesized FA (<C16) and a greater content of longer-chain FA (Bauman and Griinari 2001; Khas-Erdene et al. 2010; Côrtes et al. 2011). All of that can be considered a proof of concept that the FA composition of the supplements is reflected by an increase of the respective FA in milk. Furthermore, the correlation matrix emphasizes changes in milk FA composition as a causative factor for the variation in the data profile. This proves that modifications of the metabolism can be related to the corresponding supplementation of EFA or CLA.

Milk fat is pointed in opposite direction to the cluster of milk long-chain unsaturated FA of component 1 with the highest negative correlation to CLA (r = -0.84, $P < 0.001$), EB (r = -0.72, $P < 0.001$) and a positive correlation to ECM (r = 0.63, $P < 0.001$). This confirms, through results of our (Manuscript 1) and other numerous studies, that a reduced milk fat concentration is related to a higher CLA content in milk (de Veth et al. 2004; Bauman et al. 2008). The CLA-induced variation in milk fat content occurs in a time- and dose-dependent manner, whereby post-ruminal supplementation of 10 g/d *trans*-10,*cis*-12 CLA result in the highest milk fat depression (Baumgard et al. 2001). The study results of Manuscript 1 demonstrate how CLA-induced milk fat depression contributes to an improved EB in the CLA-treated cows, which is additionally indicated by a positive correlation of EB and milk CLA content (r = 0.59, $P < 0.001$). Several studies have repeatedly shown that the development of energy metabolism alterations is related to the severity of milk fat depression resulting from CLA supplementation (Bernal-Santos et al. 2003; Moallem et al. 2010; Schäfers et al. 2017; Grossen-Rösti et al. 2018).

Overall, the PCA show that supplementation of EFA or CLA becomes apparent through changes in milk composition and FA pattern, which might cause metabolic modifications. Component 2 of the PCA seemed to be driven by the variables such as *PCCA*, *IGFBP2*, NE_L intake,

milk yield, *IGFBP3*, *PCK2*, TC, <C16, HDL-C, *HMGCS2*; LDL-C, and reveals a relationship between these variables by clustering of NE_L intake, milk yield, and cholesterol metabolites together with gene expression data. There exist a moderate amount of positive correlations between the mentioned genes of the cluster, but no considerable relationship to performance and milk composition data, metabolites, or hormones. The milk yield correlates positively with eGP ($r = 0.72$, $P < 0.001$), NE_L intake ($r = 0.64$, $P < 0.001$), TC ($r = 0.65$, $P < 0.001$), and LDL-C ($r = 0.52$). These results confirm the general concept of energy requirements for milk production that include increased level of NE_L intake, eGP, and cholesterol as a means to ensure nutrient supply to the mammary gland (Aschenbach et al. 2010; Kessler et al. 2014; Bauman and Currie 1980; Drackley et al. 2001; Baumgard et al. 2017). Interestingly, out of Manuscript 1 and Manuscript 2, whole-body GOx is correlated negatively with milk lactose concentration ($r = -0.54$, $P < 0.001$) and affirm the idea of reduced glucose uptake and its usage as an energy source by peripheral tissues, such as adipose tissue and skeletal muscle, in early lactation to allow partitioning of a greater percentage of glucose to the mammary gland for lactose synthesis. In Manuscript 1, higher concentration of plasma NEFA also increased plasma TG ($r = 0.52$, $P < 0.001$) and correlate at a moderately positive level to liver TG ($r = 0.32$, $P < 0.001$). It is well known, that in the liver of early lactating dairy cows, the resulting plasma NEFA from adipose tissue mobilization compensates for the energy deficit in order to meet nutritional demands of milk synthesis, and will be esterified into TG and stored if uptake exceeds hepatic oxidation capacity and liver's ability to synthesize and secrete lipoproteins (Bobe et al. 2004; Weber et al. 2013). The review from Bobe et al. (2004) indicated a strong association between elevated liver TG content and plasma BHB concentration, as high concentrations of BHB decrease oxidation rates in hepatocytes. Results from Manuscript 1 and Manuscript 2 suggest that liver TG content seems to be also associated with BHB ($r = 0.34$, $P < 0.001$) and becomes detectable in enhanced MUFA concentration in milk fat ($r = 0.42$, $P < 0.001$). An increased MUFA concentration in milk fat points to an enhanced mobilization of oleic acid. Studies have shown that oleic acid is the predominant MUFA in ruminant adipose tissue and that MUFA in milk fat is elevated during increased plasma NEFA or BHB concentrations, which indicates a negative EB in dairy cows (Reus and Mansfeld 2020).

Interestingly, only milk CLA content, but not ALA, show relationships with important metabolic parameters and have an impact on lipid and glucose metabolism in transitioned dairy cows. As mentioned before, the results of the first study (Manuscript 1) clearly show that supplementation of CLA increased the concentration of CLA in milk and reduced milk fat content. Accordingly, the decrease in milk fat content of cows supplemented with CLA was associated with a reduction in energy required for milk production, which changed the EB equation on the energy expenditure side. Thus, due to less energy expenditure for milk synthesis, the elevated milk

CLA content was accompanied by an improved EB (r = 0.59, $P < 0.001$) and reduced concentration of plasma NEFA (r = -0.37, $P < 0.001$) and TG (r = -0.33, $P < 0.001$). Reduced severity of EB should accordingly reduce the requirement of mobilizing adipose tissue reserves because changes in lipolysis rates are reflected by plasma NEFA (Bauman and Currie 1980). Lipomobilization and circulating plasma NEFA levels are associated with liver TG (Bobe et al. 2004; Overton and Waldron 2004; Weber et al. 2013). Thus, increased concentration of CLA in milk is accordingly related to reduced liver TG content (r = -0.15, $P = 0.001$). Moreover, the study also demonstrated an enhanced body fat accretion due to CLA-induced energy saving from milk fat depression, as indicated by a positive correlation of body fat to milk CLA content (r = 0.26, $P < 0.001$). Body fat is also positive correlated to BFT (r = 0.75, $P < 0.001$), BCS (r = 0.61, $P < 0.001$), and BW (r = 0.51, $P < 0.001$), but the relatively moderate correlation of BFT and BCS with BW makes it difficult to associate changes in energy partitioning with body weight loss.

In early lactation of dairy cows, endocrine-related factors, such as insulin and hormones of the somatotropic axis, including GH, IGF-I, and their binding proteins, control homeorhetic processes by directing nutrient flow toward the mammary gland and inhibiting nutrient uptake by other peripheral tissues (Bauman and Currie 1980; Etherton and Bauman 1998; Kessler et al. 2013). As mentioned before, the homeorhetic mechanism of nutrient partitioning towards the mammary gland is characterized by mobilization of substrates, like NEFA and TG, from adipose body depots, and elevated circulating concentration of plasma NEFA (Drackley 1999; Renaville et al. 2002). Results of both studies (Manuscript 1 and Manuscript 2) indicate correlations of EB and IGF-I (r = 0.53, $P < 0.001$) as well as NEFA (r = -0.66, $P < 0.001$). Furthermore, a negative relation exists between concentration of plasma IGF-I and NEFA (r = -0.47, $P < 0.001$). These results are in line with the hypothesis stating that in a situation of high nutrient demand, such as during negative EB, the GH-IGF axis uncouples in the liver and is associated with a reduction in total circulating IGF-I (Fernwick et al. 2008). Body fat is positively correlated with plasma IGF-I (r = 0.59, $P < 0.001$) and insulin concentration (r = 0.56, $P < 0.001$). These correlations point at IGF-I and insulin as potential markers for improved metabolic conditions. The hormone IGF-I is positively correlated with the expression of the corresponding hepatic genes *IGF1* (r = 0.62, $P < 0.001$) and *GHR1A* (r = 0.54, $P < 0.001$). This leads to the general concept of GH-induced stimulation, mainly through GHR1A action, of IGF-I secretion (Le Roith et al. 2001; Renaville et al. 2002). In the case of the uncoupled somatotropic axis in periparturient cows, the decrease in GHR1A action leads to a decrease in hepatic *IGF1* mRNA and a decrease in plasma IGF-I concentrations (Radcliff et al. 2003; Manuscript 2). The IGF-I bioavailability is regulated through its binding to IGFBP. These circulating IGFBP act as carrier proteins, transporting IGF-I out of the circulation to the target tissues and prolonging the half-life of the IGF-I by protecting them from proteolytic degradation (Le Roith et al.

2001). In component 2, *IGFBP2* correlates positively with its protein IGFBP-2 (r = 0.50, $P < 0.001$), and negatively with body fat (r = -0.58, $P < 0.001$). A negative relationship of *IGFBP2* mRNA expression and body fat provided evidence for the IGFBP-2's role in decreasing bioavailability of IGF-I for peripheral tissues as a means to restrict the insulin-like activity during catabolic states (McGuire et al. 1995; Fenwick et al. 2008; Gross et al. 2011; Laeger et al. 2014; Thissen et al. 1994; Breier 1999). Our results demonstrate higher hepatic *IGFBP2* mRNA abundance and synthesis of IGFBP-2, which are triggered by negative EB and low insulin concentrations in early lactation (Manuscript 1 and Manuscript 2).

From the results of Manuscript 2, the milk CLA content reveals positive correlations with insulin (r = 0.24, $P < 0.001$), factors of the somatotropic axis, like IGF-I (r = 0.39, $P < 0.001$) and IGFBP-3 (r = 0.32, $P < 0.001$), and liver glycogen content (r = 0.18, $P < 0.001$). These results may contribute to the theory of reduced nutrient uptake by the mammary gland due to spared energy after CLA-induced milk fat depression and stimulation of the somatotropic axis. Furthermore, the CLA-induced alterations of the metabolism of dairy cows affect the partitioning of nutrients by endocrine changes, indicating alleviation of the metabolic load during early lactation.

In conclusion, this study demonstrated that a FA profile high in ALA and CLA, as in pasture feeding, is beneficial to ensure a transition cow's metabolic health during high production response in early lactation. The outcomes of the study, as well as possible impacts of the FA based on the findings, were associated with a significant influence on performance and energy metabolism during late gestation and early lactation by supply of CLA instead of ALA or provision of a modified n-6/n-3 ratio. The EFA administration does minorly interfere with the performance and lipid or glucose metabolism of transitioned cows and alters hepatic gene expression. However, this effect alone seems to be insufficient to compensate for energy deficiency in early lactation. The EFA treatment had hardly any influence on the endocrine regulation of nutrient partitioning during the investigated experimental period but resulted in the highest eGP PP as a result of the organism's effort to retain glucose homeostasis, which might both improve and stabilize the cow's overall metabolism.

As assumed, the combined EFA and CLA intake increases performance and reduces energy expenditure for milk production during the transition period, but similar improvements were also seen with CLA supplementation alone. The CLA-induced reduction of milk fat improved the EB, and the apparent decrease in BW loss suggests that energy spared from the reduction in milk fat yield may have been retained in body tissues. Therefore, this study provides proof of principle that a drastic reduction in milk fat content by CLA supplementation can be used as a means to provoke alterations in energy requirements for milk synthesis, modify systemic energy utilization, and further increase a cow's performance during late gestation and early

lactation. Additionally, supplementation of CLA was shown to alleviate the critical metabolic situation of negative EB after calving. The study confirms an improved metabolic status with CLA, but not with exclusively EFA supplementation during early lactation. In contrast, EFA treatment had hardly any influence on the endocrine regulation of nutrient partitioning during the investigated experimental period. Results indicate low importance of an enhanced EFA supply for metabolic-modulating properties in dairy cows. Minor synergistic effects of EFA and CLA supplementation were observed. The systemic effects of the combined EFA+CLA treatment, which resulted in eGP, NEFA, liver TG, liver glycogen, insulin response, and the somatotropic axis, were very similar to those of CLA treatment, but the changes in hepatic gene expression regarding gluconeogenesis partially corresponded to those of EFA treatment.

In this respect, to prevent metabolic diseases and excessive body condition loss in early lactation by mitigating the natural homeorhetic partitioning of nutrients from body reserves to milk, an improved CLA- instead of EFA-status in cows proves more beneficial. Metabolic and endocrine changes in blood plasma support the improved energy status in CLA-supplemented cows. On the other hand, additive EFA treatment, as in pasture feeding, does not improve the results in the present study. Keeping dairy cows on pasture as a strategy to promote metabolic health and animal welfare provides cows with EFA and CLA, as was demonstrated in the present study by the supplementation of EFA+CLA. This improves the energy status of dairy cows thanks to the CLA supplementation and supposedly optimizes cows' periparturient metabolism in the form of changes in hepatic glucose metabolism thanks to the EFA supplementation.

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Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96: 165-180

SUMMARY

Common dairy cow nutrition changed from pasture-based feeding to barn systems with the incorporation of preserved feed with corn silage as the main component in the diet. Cows on pasture take up high amounts of essential fatty acids, especially α-linolenic acid. Corn silage, on the other hand, is rich in linoleic acid but contains low levels of fat and α-linolenic acid. The unsaturated α-linolenic and linoleic acid are classified as essential fatty acids because of the inability of mammals, including ruminants, to synthesize them endogenously *de novo* and must be obtained by feed, particularly in the form of fresh grass. Conjugated linoleic acid is a bioactive compound formed either in the rumen, by biohydrogenation from essential fatty acids, or is synthesized in mammary gland tissue. Therefore, feeding regime and forage type strongly affect the essential fatty acid status and n-6/n-3 fatty acid ratio as well as the conjugated linoleic acid status of dairy cows. The essential fatty acid and conjugated linoleic acid isomers might have distinct metabolic modulating characteristics and functions in dairy cows and conjugated linoleic acid effects can be partly independent of or synergistic to the effects of essential fatty acids. An insufficient essential fatty acid and conjugated linoleic acid supply might lead to impaired metabolic functions and additional supplementation with these fatty acids could potentially be useful in stabilizing the metabolism of a dairy cow, independently or in combination with each other, by compensating for an insufficient energy intake during the transition period, which could be utilized as a strategy to promote animal health and welfare.

Therefore, a study with high-yielding dairy cows was conducted that aimed to assess the scope of impact of a combined essential fatty acid and conjugated linoleic acid supplementation on performance and energy utilization during the transition period and to inspect changes in milk composition with a reflecting milk fatty acid pattern resulting from pasture-based dairy nutrition. In the present study, the impact on lipid and glucose metabolism and the regulation of nutrient partitioning through the somatotropic axis resulting from the abomasal infusion of essential fatty acids, mainly α-linolenic acid, together with conjugated linoleic acid during late and early lactation was evaluated.

High-yielding rumen cannulated German Holstein cows in their second lactation (n = 40) were set up in 5 blocks of 8 cows from wk 9 antepartum to wk 9 PP respectively, and dried off wk 6 before calving. The cows were fed with a corn-silage-based total mixed ration *ad libitum* to provide low amounts of essential fatty acids, especially α-linolenic acid, and conjugated linoleic acid. Cows were assigned to one of 4 treatment groups and abomasally supplemented with either coconut oil, linseed and safflower oil, conjugated linoleic acid, or a combination of the last two. Milk composition was then analyzed weekly, and blood samples were taken several

times before and after parturition to determine plasma concentrations of metabolites and hormones related to lipid and glucose metabolism and somatotropic axis. Liver samples were obtained by biopsy on d 63 and 21 antepartum and on d 1, 28, and 63 postpartum to measure triglyceride and glycogen concentration and mRNA abundance of genes related to gluconeogenesis and the somatotropic axis. On d 28 antepartum and 21 postpartum, endogenous glucose production and glucose oxidation were measured via tracer technique. Body composition was determined after slaughter.

Supplementation with conjugated linoleic acid was found to improve the energy status thanks to reduced milk fat concentration, increased body fat mass, and improved energy balance in late and early lactation. Furthermore, after calving, conjugated linoleic acid additives reduced non-esterified fatty acid concentration in plasma, lowered triglyceride and raised glycogen content in the liver, decreased endogenous glucose production, and stimulated the somatotropic axis. Thus, the critical metabolic situation of the negative energy balance after calving was shown to be alleviated by supplementation of conjugated linoleic acid. The different degrees of effects during late and early lactation were most likely not only a consequence of a different lactation stage but also due to the fact that cows in early lactation received the treatments for a much longer time. The essential fatty acid administration minorly interferes with the performance and lipid or glucose metabolism of transitioned cows. Treatment with exclusively essential fatty acids had hardly any influence on the endocrine regulation of nutrient partitioning during the investigated experimental period but resulted in the highest endogenous glucose production postpartum to retain glucose homeostasis, which might improve as well as stabilize a cow's overall metabolism. Our results indicate that supplementing essential fatty acid in addition to conjugated linoleic acid may have influenced changes in mammary gland fatty metabolism achieved by conjugated linoleic acid and had a variable influence on hepatic mRNA expression. The combined treatment showed particularly similar results as the conjugated linoleic acid treatment and improved both the energy status of dairy cows (due to conjugated linoleic acid supplementation) and supposedly optimized cows' periparturient metabolism (due to changes in hepatic glucose metabolism due to essential fatty acid supplementation).

In summary, outcomes and possible impacts based on the findings were connected to a significant influence on performance and energy metabolism during late gestation and early lactation by supply of conjugated linoleic but not of α-linolenic acid or provision of a modified n-6/n-3 ratio. Metabolic and endocrine changes in blood plasma support the improved energy status in cows supplemented with conjugated linoleic acid, but additive essential fatty acid treatment, as in pasture feeding, does not improve the results. This study demonstrated that a fatty acid profile high in conjugated linoleic acid instead of α-linolenic is more crucial to ensure transition cow metabolic health during high production response in early lactation.

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ZUSAMMENFASSUNG

Einfluss von essenziellen Fettsäuren und konjugierter Linolsäure auf die Leistung und den Energiestoffwechsel von Milchkühen von der Spätträchtigkeit bis zur Frühlaktation

Die übliche Fütterung von Milchkühen hat sich von einer weidebasierten Fütterung zu einer Stallfütterung unter Verwendung von konserviertem Futter wie Maissilage als Hauptbestandteil der Ration verändert. Kühe nehmen auf der Weide große Mengen an essenziellen Fettsäuren, insbesondere α-Linolensäure, auf. Demgegenüber ist Maissilage reich an Linolsäure, enthält aber wenig Fett und α-Linolensäure. Die α-Linolen- und Linolsäure werden als essenzielle Fettsäuren eingestuft, da Säugetiere, einschließlich Wiederkäuer, nicht in der Lage sind diese Fettsäuren endogen neu zu synthetisieren und müssen über das Futter, insbesondere durch frisches Gras, aufgenommen werden. Die konjugierte Linolsäure ist eine bioaktive Verbindung, die entweder im Pansen durch Biohydrierung aus essenziellen Fettsäuren gebildet oder im Eutergewebe synthetisiert wird. Daher beeinflussen besonders das Fütterungsregime und der Grundfuttertyp den essenziellen Fettsäurestatus und das n-6/n-3-Fettsäureverhältnis, sowie den Status an konjugierten Linolsäuren von Milchkühen. Essenzielle Fettsäuren und ihre Metaboliten, sowie verschiedene Isomere der konjugierten Linolsäure können bei Milchkühen unterschiedliche metabolisch modulierende Eigenschaften und Funktionen aufweisen, und die Effekte der konjugierten Linolsäure können teilweise unabhängig von den Wirkungen der essenziellen Fettsäuren sein oder mit diesen synergistisch interagieren. Eine unzureichende Versorgung mit essenziellen Fettsäuren und konjugierter Linolsäure könnte zu einer Beeinträchtigung der Stoffwechselfunktionen führen. Demgegenüber könnte eine zusätzliche Ergänzung mit diesen Fettsäuren nützlich sein, um den Stoffwechsel während der Transitphase zu stabilisieren und damit die Gesundheit und das Wohlergehen der Milchkühe zu fördern.

Ziel der Studie war es, bei Hochleistungsmilchkühen mit einer kombinierten Ergänzung von essenziellen Fettsäuren und konjugierter Linolsäure eine fettarmen Grundration auszugleichen und ein Fettsäuremuster in der Milch zu etablieren, welches den auf der Weide gehaltenen Milchkühen ähnelt. Bei diesen Kühen sollten die Auswirkungen einer abomasalen Infusion von essenziellen Fettsäuren, hauptsächlich α-Linolensäure, zusammen mit konjugierter Linolsäure, während der späten und frühen Laktation auf den Lipid- und Glucosestoffwechsel und die Regulierung der Nährstoffverteilung durch die somatotrope Achse untersucht werden.

Hochleistende pansenfistulierte Deutsche Holstein-Kühe (n = 40) wurden in ihrer zweiten Laktation in 5 Blöcken zu je 8 Kühen von Woche 9 vor bis Woche 9 nach der Abkalbung untersucht und 6 Wochen vor der Kalbung trockengestellt. Die Kühe wurden mit einer auf Maissilage basierenden Gesamtmischration *ad libitum* gefüttert, um geringe Mengen an essenziellen Fettsäuren, insbesondere α-Linolensäure und konjugierter Linolsäure, bereitzustellen. Die Kühe wurden einer von 4 Behandlungsgruppen zugeordnet und mit Kokosnussöl, Lein- und Distelöl, konjugierter Linolsäure oder einer Kombination der letzteren in den Labmagen infundiert. Die Milchzusammensetzung wurde wöchentlich analysiert, und mehrmals vor und nach der Kalbung wurden Blutproben entnommen, um die Plasmakonzentrationen von Metaboliten und Hormonen im Zusammenhang mit dem Lipid- und Glucosestoffwechsel und der somatotropen Achse zu bestimmen. Durch Leberbiopsien wurden am Tag 63 und 21 vor sowie am Tag 1, 28 und 63 nach der Abkalbung Gewebeproben entnommen, um die Triglycerid- und Glykogenkonzentration sowie die mRNA Anreicherung von Genen im Zusammenhang mit der Glukoneogenese und der somatotropen Achse zu messen. Am Tag 28 antepartum und 21 postpartum wurden die endogene Glukoseproduktion und die Glukoseoxidation mittels Tracer-Technik gemessen. Die Körperzusammensetzung wurde nach dem Schlachten bestimmt.
Die Ergänzung mit konjugierter Linolsäure verbesserte den Energiestatus durch eine verringerte Milchfettkonzentration, eine erhöhte Körperfettmasse und eine verbesserte Energiebilanz in der späten und frühen Laktation. Weiterhin bewirkte der Zusatz von konjugierten Linolsäure nach dem Abkalben eine Reduktion der Konzentration an nicht veresterten Fettsäuren im Plasma, senkte den Triglycerid- und erhöhte den Glykogengehalt in der Leber, verringerte die Glukoseproduktion und stimulierte die somatotrope Achse. Es konnte gezeigt werden, dass die Ergänzung von konjugierter Linolsäure die kritische Stoffwechselsituation der negativen Energiebilanz nach dem Abkalben abschwächt. Die unterschiedlichen Effekte, die sich während der späten und frühen Laktation zeigten, waren wahrscheinlich nicht nur eine Folge des unterschiedlichen Laktationsstadiums, sondern auch auf die Tatsache zurückzuführen, dass Kühe in der frühen Laktation die Supplementationen viel länger erhielten als in der Spätlaktation. Die Verabreichung von essenziellen Fettsäuren beeinträchtigt die Leistung und den Lipid- oder Glucosestoffwechsel von Transitkühen geringfügig. Die Behandlung mit essenziellen Fettsäuren hatte während des untersuchten Versuchszeitraums kaum Einfluss auf die endokrine Regulation der Nährstoffverteilung, führte jedoch zur höchsten endogenen Glukoseproduktion nach der Kalbung, um die Glukose-Homöostase aufrechtzuerhalten, was den Gesamtstoffwechsel der Kuh verbessern und stabilisieren könnte. Unsere Ergebnisse deuten darauf hin, dass die zusätzliche Supplementierung mit essenziellen Fettsäuren zur konjugierten Linolsäure möglicherweise die Veränderungen des Fettstoffwechsels durch die konjugierte Linolsäure im Eutergewebe beeinflusste und variablen Einfluss auf die Expression von Genen

in der Leber hat. Die kombinierte Behandlung zeigte sehr ähnliche Ergebnisse wie die Behandlung mit konjugierter Linolsäure allein und verbessert sowohl den Energiestatus der Milchkuh aufgrund der Supplementierung der konjugierten Linolsäure als auch eine vermeintliche Optimierung des periparturienten Metabolismus der Kühe durch Veränderungen des hepatischen Glucosestoffwechsels aufgrund der Ergänzung mit essenziellen Fettsäuren.

Zusammenfassend lässt sich sagen, dass die erzielte Entlastung des Energiestoffwechsels in der Spät- und Frühlaktation vor allem durch Zufuhr von konjugierter Linolsäure und weniger durch die Zufuhr von α-Linolensäure oder durch die Änderung des n-6/n-3-Verhältnisses hervorgerufen wurde. Stoffwechsel- und endokrine Veränderungen im Blutplasma weisen auf einen verbesserten Energiestatus bei den Kühen hin, denen konjugierte Linolsäure verabreicht wurde, aber eine zusätzliche Verabreichung mit essenziellen Fettsäuren wie bei der Weidefütterung beeinflusste die Ergebnisse in der vorliegenden Studie nicht. Diese Studie zeigte, dass ein Fettsäureprofil mit hohem Gehalt an konjugierter Linolsäure anstatt von α-Linolensäure bedeutender ist, um die metabolische Gesundheit der Transitkuh während einer hohen Produktionsleistung in der frühen Laktation sicherzustellen.

CURRICULUM VITAE

For reasons of data protection, the curriculum vitae is not published in the electronic version.

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DECLARATION

med. vet. Laura Vogel

Statutory Declaration

I herewith declare on oath that the submitted dissertation under the title
“Effects of Essential Fatty Acids and Conjugated Linoleic Acid on Performance and Energy Metabolism in Dairy Cows from Late Gestation to Early Lactation”
has been authored independently and without use of any other than the cited sources and aids. Sentences or parts of sentences quoted literally are marked as such; other references regarding the statement and scope are indicated by full details of the publications concerned. The thesis in the same or similar form has not been formerly submitted to another university department. Furthermore, I declare that the submitted written (bound) copies of the present thesis and the version submitted in electronic form are consistent with each other in contents.

Laura Vogel
Berlin, 20th October 2021

I herewith declare that I am not subject to any pending case of public prosecution.

Laura Vogel
Berlin, 20th October 2021

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