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Review: Effect of essential fatty acids and conjugated linoleic acid on the adaptive physiology of dairy cows during the transition period

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ABSTRACT

Cows fed total mixed rations (silage-based) may not receive as much essential fatty acids (**EFAs**) and conjugated linoleic acids (**CLAs**) as cows fed pasture-based rations (fresh grass) containing rich sources of polyunsaturated fatty acids. CLA-induced milk fat depression allows dairy cows to conserve more metabolisable energy, thereby shortening the state of negative energy balance and reducing excessive fat mobilisation at early lactation. EFAs, particularly α-linolenic acid, exert anti-inflammatory and antioxidative properties, thereby modulating immune functions. Thus, combined EFA and CLA supplementation seems to be an effective nutritional strategy to relieve energy metabolism and to improve immune response, which are often compromised during the transition from late pregnancy to lactation in high-yielding dairy cows. There has been extensive research on this idea over the last two decades, and despite promising results, several interfering factors have led to varying findings, making it difficult to conclude whether and under what conditions EFA and CLA supplementations are beneficial for dairy cows during the transition period. This article reviews the latest studies on the effects of EFA and CLA supplementation, alone or in combination, on dairy cow metabolism and health during various stages around parturition. Our review article summarises and provides novel insights into the mechanisms by which EFA and/or CLA influence markers of metabolism, energy homeostasis and partitioning, immunity, and inflammation revealed by a deep molecular phenotyping.

Implications

High-yielding dairy cows fed a total mixed ration (silage-based) fall into a low essential fatty acid supply, particularly α-linolenic acid, and conjugated linoleic acids when compared with fresh grass. Those fatty acid families modulate immunometabolism in transition dairy cows. To conserve metabolisable energy and to diminish immune systems and metabolic challenges during the transition period, supplementation with conjugated linoleic acids, either alone or in combination with essential fatty acids, appears to be an effective strategy during the transition period. The beneficial effects of conjugated linoleic acids and essential fatty acids on transition dairy cows, however, are time- and dose-dependent.

Introduction

Fat supplements are traditionally considered a source of energy to increase the energy density in dairy cows' diets and thus the net energy of lactation intake (Jenkins and McGuire, 2006). In recent years, dairy cow research has been more focused on delineating the specific roles of polyunsaturated fatty acids (**PUFAs**) since they are directly involved in physiological processes such as cellular membrane integrity, lipid metabolism, energy partitioning, hormonal pathways, oxidative stress and inflammatory pathways, and immune responses, which have been reviewed by Moallem (2018) and Bionaz et al. (2020). Among them, n-3 fatty acids, along with a cluster of fatty acids called the conjugated linoleic acid (**CLA**) family, have received specific attention as promising bioactive compounds with unique properties in dairy cows and human health and well-being, as reviewed by Shingfield et al. (2013).

In mammals, the precursors of the n-6 and n-3 fatty acid families, including linoleic acid (**LA**, C18:2n-6) and α-linolenic acid (**ALA**, C18:3n-3), have been described as essential fatty acids

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(**EFAs**) since they cannot be synthetised *de novo* and must be obtained from the diet (Palmquist, 2010). Desaturation and elongation are responsible for the endogenous processes of EFA, in which ALA is converted to eicosapentaenoic acid, a precursor of the anti-inflammatory 3-series of prostaglandins, whereas LA is converted to arachidonic acid, which is a precursor for the proinflammatory 2-series of prostaglandins (Palmquist, 2010).

CLAs occur naturally as stereo and positional isomers of forage-derived fatty acids, including oleic acid, LA, and ALA, with conjugated double bonds rearranged at positions 6,8-; 7,9-; 8,10-; 9,11-; 10,12-; 11,13-; 12,14-; and 13,15-, where it can occur in either *cis* or *trans* configurations (Benjamin et al., 2015). CLAs contain more than 56 isomers, with the most abundant and biologically active ones being *cis*-9, *trans*-11 CLA (ω-7 fatty acids), and *trans*-10, *cis*-12 CLA (ω-6 fatty acids) (Benjamin et al., 2015). It has been discovered that ruminants produce CLA from two different sources: endogenous (the primary source) and exogenous (inferior source). Endogenous synthesis is carried out through the action of Δ9-desaturase (also referred to as stearoyl-CoA desaturase), a tissue enzyme found in mammary glands, adipose cells, liver, and intestine, which is exclusively dependent upon the availability of *trans*-11 octadecenoic acid (vaccenic acid), another intermediate in ruminal biohydrogenation, as a precursor. The exogenous pathway involves rumen bacteria such as Butyrivibrio fibrisolvens, which isomerises *cis*-12 to *trans*-11 bonds, and Megasphaera elsdenii, which isomerises *cis*-9 to *trans*-10. The biosynthesis of CLA in ruminants was previously reviewed by Bauman et al. (2000) and Palmquist et al. (2005). *Cis*-9, *trans*-11 CLA or rumenic acid (**RA**) is the major CLA (70–90% of total CLA isomers) found in cow's milk, which together with n-3 fatty acids may exert anti-carcinogenic, anti-atherosclerotic, anti-diabetic, and anti-inflammatory properties (Dilzer and Park, 2012; Dipasquale et al., 2018; Moallem, 2018). On the other hand, the *trans*-10, *cis*-12 CLA isomer mainly functions to induce milk fat depression (**MFD**) in mammals and extensively so in dairy cows (reviewed by Bauman et al., 2008). Most of the recent studies in dairy cows have examined the effects of commercially available CLA supplements that contain a 50:50 mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers as synthetic synthesis leads to 1:1 production of those isomers (e.g., von Soosten et al., 2012; Qin et al., 2018; Rahbar et al., 2021; Vogel et al., 2020).

The initial purpose of EFA and CLA enrichment in dairy products was for human health and well-being as functional foods (reviewed by Dilzer and Park, 2012 and by Palmquist et al., 2005); however, dairy cows' diets supplemented with CLA alter body energy partitioning, regulate lipid metabolism, and modulate immune-inflammatory responses (e.g., Baumgard et al., 2001; von Soosten et al., 2012; Gnott et al., 2020; Vogel et al., 2021). Supplementing a dairy cow diet with EFA and CLA has been suggested to attenuate metabolic challenges during early lactation (Qin et al., 2018; Vogel et al., 2020). In this review, we provide an update to the recent investigation on the functional effect of CLA supplementation alone or in combination with EFA, particularly a low ratio of LA:ALA and n-6:n-3 fatty acids, in dairy cows during the transition period.

A majority of the mechanisms discussed in this review were already proposed/known from classical measurements of genes/metabolites in fatty acid-supplemented cows. In this review, various omics results were recruited to complement classical measurements for a better understanding of metabolic fingerprints, while on the other hand uncovering novel players and pathways that were not initially considered. The areas of focus addressed include, but are not limited to, EFA and CLA impacts on dairy cow performance, metabolic regulation and energy homeostasis, MFD, and markers of inflammation and immunity in multiple organs.

Essential fatty acids and conjugated linoleic acid digestibility and bioavailability

The rumen digestibility of EFA and CLA is been affected by several factors and has been previously reviewed by Gómez-Cortés et al. (2008). Ruminal biohydrogenation of PUFA proved to be highly extensive in ruminants (Huang et al., 2009; Pappritz et al., 2011a); therefore, various techniques have been developed to increase CLA influx into the intestines, such as encapsulation and calcium salt of fatty acids. For instance, in sheep, CLA supplemented as free fatty acids was biohydrogenated to approximately 97%, but feeding calcium salt of CLA reduced the biohydrogenation rate to 90% and provided approximately two times as much intestinal absorption as the free form (Huang et al., 2009). One of the frequently used techniques in dairy cow studies is direct abomasal infusion through the rumen cannula to entirely bypass ruminal biohydrogenation and ensure EFA as well as CLA cellular absorption (e.g., Saebø et al., 2005; Vogel et al., 2020).

In absence of CLA supplementation, supplementing diets with different sources of PUFA also affected CLA flow and bioavailability, and this is largely dependent on the basal diet and feeding system of cows. In this regard, there was a greater intestinal flow of *cis*-9, *trans*-11 CLA as well as *trans*-10, *cis*-12 CLA for cows (under a high concentrate:forage ratio diet) supplemented with sunflower oil (5.0 g/100 g DM) than those received fish oil (2.5 g/100 g DM) (Loor et al., 2005). In this study, the ruminal digestibility of *cis*-9, *trans*-11 CLA was greater in cows fed sunflower oil than in cows fed fish oil, while *trans*-10, *cis*-12 CLA digestibility was comparable (Loor et al., 2005). Regarding the basal diet and feeding system of cows, feeding dairy cows with concentrate-rich diets, especially when maize but not grass silage was provided, alters the ruminal biohydrogenation pattern of PUFA and increases *trans*-10, *cis*-12 and reduces *cis*-9, *trans*-11 CLA in the milk, which may result in a MFD (Nielsen et al., 2006). Moreover, supplementation of wheat starch plus corn oil to dairy cows' basal diet (grass hay and corn) increased trans-10, cis-12 CLA levels in milk (Fougère et al., 2018) and trans-9, trans-11 CLA levels in plasma triglyceride (Delavaud et al., 2022). There is also evidence that the ALA and CLA concentrations in blood plasma and milk were higher when dairy cows grazed on pasture rather than being fed a total mixed ration using alfalfa and corn silages as forage sources, as reviewed by Moscovici Joubran et al. (2021). Moreover, pasture-based milk had significantly lower saturated fatty acids and n-6:n-3 ratio as compared to total mixed ration (Moscovici Joubran et al., 2021). It appears that silage-based total mixed ration-fed cows slip into low n-3 and CLA supply and therefore benefit most from n-3 and CLA supplementations.

Effects of essential fatty acids and conjugated linoleic acid on production parameters, metabolites and hormones

The effect of n-3 fatty acids on dairy cow performance, milk fat composition, metabolites, and hormones has been outlined in recent reviews by Moallem (2018) and Bernard et al. (2018). In summary, ALA has been found to be beneficial to transition cow's metabolic health and fertility, even though there is little *in vivo* information about it as most research is oriented towards long-chain n-3 fatty acids. For instance, it has been reported that antepartum flaxseed supplementation (as a source of ALA) is an effective strategy to lower ketosis incidence and prevent fatty liver development in transition dairy cows by increasing liver glycogen and milk yield and decreasing liver triglyceride levels after calving (Petit et al., 2007, Moallem et al., 2020).

The major function of EFA and CLA supplementation in dairy cows during the transition period relates to their impacts on milk

fat synthesis and consequently energy metabolism and partitioning. It has been well documented that even small amounts of *trans*-10, *cis*-12 CLA dramatically diminish milk fat synthesis in dairy cows, as reviewed by Bernard et al. (2018), while milk yield and other milk components remained largely unaffected (reviewed by Bauman et al., 2008). However, there have been reports of both increased and decreased milk yield depending on the CLA dose. For instance, abomasal infusion of a very high dose of CLA (45 g daily dose of *trans*-10, *cis*-12 CLA) which affected characteristic of involution (i.e., apoptosis) caused a decrease in milk and lactose yields (Bell and Kennelly, 2003). On the other hand, rumen-protected CLA isomers (100 and 120 g/day from antepartum to postpartum) increased milk yield (Rahbar et al., 2021; Chandler et al., 2017). It is worth mentioning that CLA-induced MFD was not observed immediately at the onset of lactation, but after 2–4 weeks, even when supplementation was started during the antepartum period (Castañeda-Gutiérrez et al., 2005; von Soosten et al., 2011; Hötger et al., 2013; Schäfers et al., 2017). Though it is still unclear why CLA-induced MFD is delayed at the onset of lactation, a possible explanation might be due to intensive lipid mobilisation, which release a high amount of NEFA and competitively mask CLA's effect, as CLA mainly inhibits *de novo* fatty acid synthesis in the mammary gland. Interestingly, when CLA was directly infused into the abomasum, there was only a 2–5 days' lag to observe the significant MFD (Perfield et al., 2004; de Veth et al., 2005; Vogel et al., 2020). There is conflicting evidence regarding whether n-3 fatty acids also induce MFD, which has been reviewed extensively by Moallem (2018) and Bernard et al. (2018). However, the effects of EFA and n-3 fatty acids on MFD are still not certain. We have previously shown that abomasal infusion of EFA has no effect on milk yield and milk fat content, and only in combination with CLA induces MFD (Vogel et al., 2020), presumably because the ruminal conversion of EFA to CLA is limited. It should be noted that ruminants differ in their sensitivity to CLA-induced MFD since goats appear to be more resistant than cows or sheep (Zheng et al., 2020).

In general, the CLA effects on performance and zootechnical parameters were more relevant when it was fed at high dosages (defined as causing >20% MFD) and during the transition period in which energy homeostasis and metabolic adaptations were compromised. In some studies, however, deviations were reported between CLA and non-CLA groups in terms of performance, which seems to be more related to differences in experimental design, including differences in the administration of the CLA (feed supplementation or abomasal infusion), the duration of the experiment (long- and short-term), the stage of lactation (antepartum or postpartum), the fatty acid composition of the CLA mixture (alone or combined with EFA), the effects of CLA alone or in conjunction with other metabolites such as vitamins, and parity.

It has been clearly shown that low dosages (defined as causing <20% MFD) of rumen-protected CLA (50 g/d) from 14 days antepartum to 63 days postpartum had no effect on DM intake, BW, body condition score, back fat thickness, or energy-corrected milk, as well as milk protein and urea levels of dairy cows (Hötger et al., 2013). Similarly, higher dosages (60, 100, 150, 200, 400, and 600 g/d) of rumen-protected CLA supplements decreased the milk fat content and yield in a dose- and time-dependent manner without adversely affecting other production parameters (Moore et al., 2004; Qin et al., 2018; Kowalski et al., 2019).

However, the literature describes CLA's effects on DM intake as being dose- and time-dependent, with higher doses appearing to reduce DM intake (Pappritz et al., 2011b; von Soosten et al., 2012). According to von Soosten et al. (2012), CLA supplementation (100 g/d mixture of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA) protects against excessive body reserve (fat and protein) mobilisation during early lactation (between 1 and 42 days in milk), which in turn promotes the efficient utilisation of metabolisable energy and a higher daily retention of body energy and protein (during the postpartum period). However, no effect of CLA was observed on various fractions of the empty body mass, including the meat, bone, offal, hide, mammary gland, retroperitoneal fat, omental fat, mesenteric fat, and subcutaneous fat, as well as on heat production (calculated by subtracting milk and body mass energy changes from metabolisable energy intake). Chandler et al. (2017) reported that supplementation with CLA (100 g/d of lipid-encapsulated CLA) did not change BW, rumination minutes, and milk protein and fat yield during the transition period. However, in CLA-treated cows, the milk yield increased and milk fat content decreased. In other studies, CLA supplementation at early lactation induced less BW loss and lowered blood non-esterified fatty acids (**NEFAs**) and β-hydroxybutyric acid concentrations (Hutchinson et al., 2011; Qin et al., 2018). According to Qin et al. (2018), supplementing dairy cows with high doses (100–150 g/d) of lipid-encapsulated CLA decreased the plasma concentrations of NEFA, β-hydroxybutyric acid, ceruloplasmin, and bilirubin, while glucose, triglyceride, and leptin increased in a time-dependent manner.

The effects of long-term (63 d antepartum to 63 d postpartum) abomasal infusions of EFA and CLA, alone or in combination, on the performance and plasma metabolites of transition dairy cows were shown to be fatty acids and time-dependent (Vogel et al., 2020; 2021). For instance, abomasal infusion of CLA alone or with EFA did not affect DM intake, milk yield, feed efficiency for milk production, BW, body condition score, back fat thickness, cortisol, glucagon, or growth hormone in dairy cows during the transition to the lactation period (Vogel et al., 2020; 2021). Moreover, carcass weight and various organ weights after slaughter were not affected by CLA treatment, except for omental fat (proportion of BW), which was higher in CLA-supplemented cows (Vogel et al., 2020). However, it was found that cows receiving CLA alone had an elevated milk citrate concentration early in lactation (indicator of decreased de novo fatty acid synthesis), whereas cows receiving the combination of EFA and CLA had an increased milk acetone level (indicator of increased ketogenesis). In the CLA-treated groups, energy-corrected milk was lower and energy balance was higher during the postpartum period compared to the CTRL and EFA groups. After calving, the CLA-treated cows had a slower increase in NEFA in plasma and lower hepatic and plasma triglyceride concentrations than the non-CLA-treated cows. The total cholesterol concentration in blood plasma was not affected by EFA, CLA or the combination of the two. However, a significant increase (from d 42) in low-density lipoprotein was observed with the combination of EFA and CLA, and a significant decrease (from d 42 postpartum) in high-density lipoprotein was found in the CLA group. Similarly, there is other evidence in dairy cows indicating that CLA supplementation during the transition period reduced the severity of NEB, decreased adipose tissue mobilisation, diminished hepatic triglyceride overload, and reduced circulating NEFA concentrations mainly by inducing MFD (Kay et al., 2006; Galamb et al., 2017). It is important to note that during early lactation, lipid metabolism and transport mechanisms are diminished, resulting in lower plasma levels of lipoproteins (Vogel et al., 2020) and their constituent apolipoproteins (**APOs**) (Veshkini et al., 2022b). In this regard, hepatic and plasma proteomics results provided evidence that CLA plays a pivotal role in regulating cytochrome P450 and lipoprotein metabolism (Veshkini et al., 2022a; Veshkini et al., 2022b). In particular, CLA combined with EFA increased CYP4F2 protein abundance and decreased CYP1A1 at d 28 postpartum, which are two cytochromes involved in cholesterol biosynthesis pathways (Veshkini et al., 2022a). In addition, abomasal infusion of combined EFA and CLA stimulates the abundance of APO, including APOC3, APOA1, APOA4, and APOC4, in dairy cows, which are required for lipoprotein assembly (Veshkini et al., 2022b). Consid-

ering that cholesterol and APO are the main components of lipoproteins, modifying them with combined EFA and CLA would support the cholesterol biosynthesis pathways, allowing hepatic triglycerides to be transported more efficiently. EFA and CLA supplementation could therefore lead to reduced hepatic triglyceride accumulation. However, reduced triglyceride accumulation in the liver by CLA is also a result of less body fat mobilisation, as shown by the reduced plasma NEFA concentration (Vogel et al., 2020). In accordance with liver results, phosphoproteomics analysis also revealed that CLA supplementation increased lipid turnover in adipose tissue of transition cow through phosphorylation of proteins involved in lipolysis and lipogenesis (Daddam et al., 2021). Improved milk performance at early lactation solely by CLA was reported in another study, in which rumen-protected CLA isomers (120 g/d from 21 d antepartum to 60 d postpartum) were found to increase milk yield, milk protein yield, and milk lactose yield and to reduce milk fat and milk fat yield compared with Ca salts of PUFA (110 g/day) (Rahbar et al., 2021). However, BW, body condition score, DM intake, net energy balance, cholesterol, triglyceride, blood urea nitrogen, high-density lipoprotein, low-density lipoprotein, β-hydroxybutyric acid, glucose, NEFA, progesterone, oestradiol, insulin, and IGF-1 were not affected by CLA treatment either antepartum or postpartum (Rahbar et al., 2021).

Despite the lack of direct evidence in other tissues than adipose tissue, supplementation of CLA and the combination of EFA and CLA seem to increase lipid turnover throughout the whole body. According to this hypothesis, MFD provided more energy for maintaining the pathways that were supposed to downregulate or dysregulate during early lactation (e.g. lipid metabolism; Veshkini et al., 2022b), but also there is evidence that lipid transport pathways were induced in the liver of CLA-supplemented cows (Veshkini et al., 2022a). Therefore, combined EFA and CLA supplementation probably helps dairy cows experience less metabolic pressure at parturition by restoring metabolic homeostasis faster during the early postpartum. Fig. 1 summarises major regulatory mechanisms that are affected by EFA and CLA supplementation, including accelerated lipid turnover in critical tissues such as liver, adipose tissue, and mammary glands, which is in part due to increased energy balance and also through higher affinity for ligand activation of nuclear transcription factors.

Molecular mechanisms related to the changes in energy partitioning by milk fat depression

Increasing doses of *trans*-10 and *cis*-12 CLA lead to a curvilinear (dose-dependent) reduction in milk fat yield, but there is a linear relationship between the levels of *trans*-10, *cis*-12 CLA absorbed into milk fat (de Veth et al., 2004), indicating that the mechanisms promoting MFD go beyond just CLA uptake by the mammary gland but are more likely to be at the molecular regulatory level. Milk fat synthesis is controlled by numerous transcriptional and posttranscriptional regulation factors, including sterol response element binding proteins (**SREBPs**) and peroxisome proliferator-activated receptors (**PPARs**), and their downstream genes, such as acyl-coenzyme A synthetase short-chain family member 2 (***ACSS2***), fatty acid synthesis (acetyl-CoA carboxylase (***ACACA***) and fatty acid synthase (***FASN***)), and stearoyl-CoA desaturase (***SCD***) (Mu et al.,

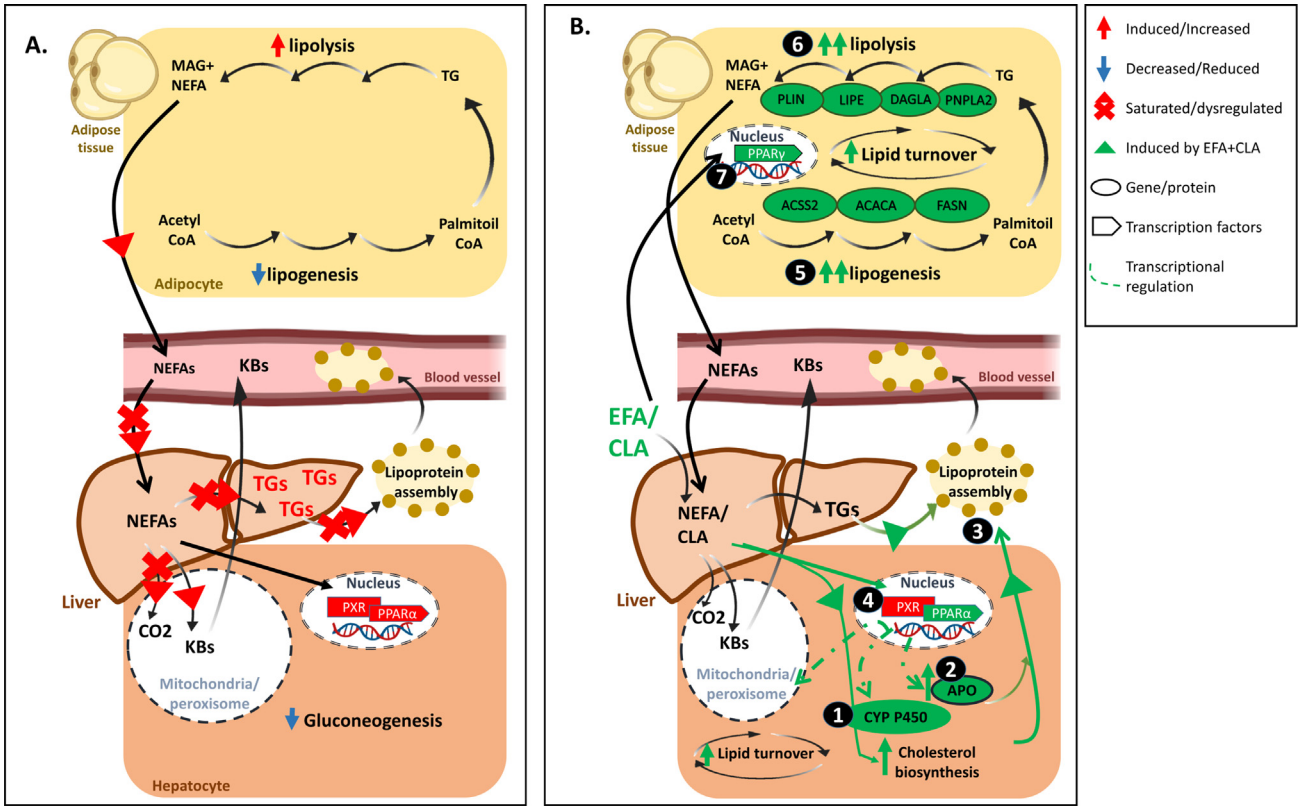

Fig. 1. (A) Schematic representation of metabolic regulatory mechanisms at early lactation in liver and adipose tissue of dairy cows without essential fatty acids (EFAs)/conjugated linoleic acids (CLAs), (B) with EFA/CLA. Combined EFA and CLA supplementation stimulates metabolic regulation mechanisms and accelerates homeostatic metabolic adaptations through 1. Induced hepatic lipid turnover by inducing cholesterol and TG metabolism, 2. Induction of apolipoprotein synthesis 3. Increased lipoprotein metabolism, 4. Ligand activation of PPARα, 5. Induced adipose tissue lipid turnover by induction of both lipogenesis and 6. Lipolysis, through 7. Ligand activation of PPARγ. ACACA = acetyl-coa carboxylase-1, ACSS2 = acetyl-coenzyme A synthetase, APO = apolipoprotein, CYP P450 = cytochrome P450, DAGLA = diacylglycerol lipase alpha. FASN = fatty acid synthase, KBs = ketone bodies, LIPE = hormone-sensitive lipase, MAG = monoacylglycerol, NEFAs = non-esterified fatty acids. PLIN = perilipin, PNPLA2 = adipose triglyceride lipase, PPARs = peroxisome proliferator-activated receptors, PXR = pregnane × receptor, TGs = triglycerides.

2021). This process is initiated by converting acetic acid (acetate) and β-hydroxybutyric acid to acetyl-CoA by ACSS2, second to malonyl-CoA by ACACA, and finally to medium-chain fatty acids and to some saturated long-chain fatty acids under the action of FASN (reviewed by Mu et al., 2021). In addition, SREBP-1 is a master regulator of lipid synthesis and belongs to a family of nuclear transcription factors controlling genes involved in *de novo* fatty acid synthesis, fatty acid desaturation, long-chain fatty acid uptake, and triglyceride esterification (Li et al., 2014).

CLA induces MFD via transcriptional and posttranscriptional mechanisms, in which SREBF1 and thyroid hormone responsive spot 14 (**S14**, encoded by the *THRSP* gene) are considered primary regulators (Harvatine et al., 2018). In particular, CLA triggered a coordinated downregulation of key lipogenic enzymes in the mammary gland of dairy cows through inhibition of the proteolytic activation of SREBP-1, which resulted in a decrease in SREBP-1 expression (reviewed by Bernard et al., 2018). Moreover, as *de novo* fatty acid synthesis requires reducing equivalents (NADPH), which can be provided by the citrate-isocitrate or pentose phosphate pathways, increased milk citrate concentrations in CLA-treated cows indicate reduced *de novo* fatty acid synthesis in the mammary gland (Vogel et al., 2020). It is interesting to note that milk citrate induction was limited to CLA and was not observed by the combination of EFA and CLA (Vogel et al., 2020).

Recently, the application of Omics technology has opened up new insight into the molecular regulation of CLA-induced MFD. For instance, differential expression analysis revealed 1,256 upregulated and 268 downregulated genes associated with *trans*-10, *cis*-12 CLA-induced MFD in the ewe milk somatic cell transcriptome (Suárez-Vega et al., 2019). The downregulated genes induced by CLA, including previously described enzymes involved in *de novo* milk fatty acid synthesis, were annotated to biological processes including cofactor binding, fatty acid catabolic process, fatty-acyl-CoA binding, citrate metabolic process, and mitochondrial matrix pathway. In this regard, a number of downregulated genes are related to acetyl-CoA metabolism, which feeds the fatty acids synthesis process with building blocks, including activation of acetoacetate to acetoacetyl-CoA (*AACS*), activation of fatty acids with CoA (*ACSS2, ACSS3*), and desaturation of fatty acids (*FADS2*). In addition, CLA reduced the expression of several mitochondrial genes, which may be involved in mitochondrial FA synthesis. In ruminants, mitochondrial FA synthesis is the second major system for producing FA, but it is poorly characterised (Nowinski et al., 2020). There are several downregulated genes in mitochondrial FA synthesis, including hydroxyacyl-CoA dehydrogenase and glutaryl-CoA dehydrogenase, isovaleryl-CoA dehydrogenase, NADH:ubiquinone oxidoreductase subunit AB1, propionyl-CoA carboxylase beta chain, and carbonyl reductase family member 4, which require further investigation (Suárez-Vega et al., 2019). Surprisingly, the majority of differentially expressed genes associated with MFD were upregulated and annotated to a broad range of biological processes, including the mitogen-activated protein kinase signalling pathway, NF-kappa B (**NF-κB**) signalling pathway, tumour necrosis factor (**TNF**) signalling pathway, toll-like receptor (**TLR**) signalling pathway, T-cell receptor signalling pathway, Rap1 signalling pathway, Ras signalling pathway, adipocytokine signalling pathway, sphingolipid signalling pathway, cAMP signalling pathway, phosphatidylinositol signalling system, as well as pathways related to adipocyte differentiation, lipoprotein metabolism, and ATP-binding cassette (**ABC**) transporters (Suárez-Vega et al., 2019). Some of the remarkable genes within these pathways are *TNF*, NF-κB (*NFKB1* and *NFKBIA*), *MAP3K8*, interleukin 1 beta (***IL1b***), *ABCA1*, *ABCG1*, *TLR2* and *6*, prostaglandin-endoperoxide synthase 2 (***PTGS2***), *CD14*, APO b receptor (***APOBR***), and lipolysis-stimulated lipoprotein receptor (***LSR***) (Suárez-Vega et al., 2019).

A recent multiomics study examined the transcriptome and metabolome profile of rumen and milk in goats fed high-rumen degradable starch to mimic CLA-mediated MFD (Zheng et al., 2020). High-starch feeding decreased the rumen microbial populations of B. fibrisolvens and Pseudobutyrivibrio and increased the milk CLA content. Myristic acid, arachidic acid, stearic acid, and nervonic acid levels in mammary vein plasma were significantly lower after feeding starch. Moreover, there was a significant downregulation of lipogenesis genes mediated by CLA, including those related to the SREBP pathway, such as insulin-induced gene 1 (***INSIG1***), *ACSS2*, mevalonate diphosphate decarboxylase (***MVD***), alkylglycerone phosphate synthase (***AGPS***), SCD5, fatty acid desaturase 2 (***FADS2***), cerebral endothelial cell adhesion molecule (***CERCAM***), hydroxysteroid 17-beta dehydrogenase 7 (***HSD17B7***), *HSD17B12*, and growth differentiation factor 1 (***GDF1***) (Zheng et al., 2020).

The incorporation of EFA and/or CLA into energy metabolism and partitioning makes it an intriguing potential functional agent during transition periods, when most dairy cows suffer from severe energy deficits, as mentioned above. As reviewed by Renaville et al. (2002), a complex feedback and control mechanism regulates energy partitioning and homeostasis through the somatotropic axis, which consists of growth hormone, IGF-I and their related carrier proteins and receptors as well as their coordinating regulators, such as insulin, leptin, glucocorticoids or thyroid hormones. Studies evaluating EFA, particularly n-3 fatty acids, on energy metabolism and partitioning in transition dairy cows reported that they are not or are only partially effective mediators of energy markers. For instance, abomasal infusion of EFA in transition cows resulted in no effect on plasma glucose, insulin, glucagon, cortisol, IGF-1, IGFBP-2, or IGFBP-3 concentrations or on markers of glucose metabolism, including endogenous glucose production and glucose oxidation (Vogel et al., 2021). However, hepatic mRNA expression of enzymes involved in glucose metabolism and the somatotropic axis, such as cytosolic and mitochondrial phosphoenolpyruvate carboxykinase (cytosolic *PCK1*; mitochondrial *PCK2*), glucose-6-phosphatase (***G6PC***), propionyl-CoA-carboxylase α (***PCCA***), 3-hydroxy-3-methyl-glutaryl-CoA synthase 2 (***HMGCS2***), insulin receptor (***INSR***), and IGFBP-2, was decreased by EFA in a time-dependent manner (Vogel et al., 2021). Another study showed that encapsulated flaxseed oil, a rich source of ALA, supplementation during a transition period had no effect on EB and plasma concentrations of glucose, NEFA, β-hydroxybutyric acid, and cortisol, although it partially affected the liver and adipose tissue endocannabinoid system, which regulates energy metabolism (Kra et al., 2022). However, the authors reported that these partial effects are due to lower feed intake caused by oil supplementation.

It has been suggested that CLA may affect ruminant energy mobilisation and repartitioning through the somatotropic axis and insulin sensitivity. There is no clear understanding of the molecular mechanisms responsible for CLA-induced insulin resistance (**IR**), but there are several mechanisms already suggested, including (1) fatty acids oxidation and downstream metabolic pathways, such as TCA; (2) inflammatory cytokines that cause endoplasmic reticulum stress and mitochondrial dysfunction; and (3) bioactive lipid production. In this regard, cows receiving CLA supplementation experienced a lower EB, while the plasma glucose concentration was higher immediately after calving (Hötger et al., 2013). The plasma concentrations of insulin, IGF-I, and HP were not affected by CLA supplementation (Qin et al., 2018). Furthermore, CLA did not affect plasma glucose and insulin concentrations during the glucose tolerance test but reduced endogenous glucose production in week 3 after parturition. The authors discussed that elevated plasma glucose concentrations, yet reduced endogenous glucose production, in CLA-fed cows, are not the result of IR, but the underlying mechanism is unclear

(Hötger et al., 2013). In another study, CLA-treated cows showed a decrease in systemic insulin sensitivity (**RQUICKI**), despite increased insulin secretion (Saremi et al., 2014).

We have previously shown that abomasal infusion of CLA or combined EFA and CLA influenced markers of the somatotropic axis in dairy cows. Cows from both CLA-treated groups had higher plasma glucose concentrations than non-CLA-treated cows. At various timepoints during the transition period, IGF-I, IGFBP-3 and IGFBP-3/-2 levels were higher in the CLA and combined EFA and CLA groups, and IGFBP-2 was lower in the CLA group. Plasma concentrations of β-hydroxybutyric acid (except for the higher levels when EFA and CLA were combined on d 28), glucagon, and cortisol were not affected by the CLA infusion. However, higher glucose (after calving) and insulin (before and after calving) plasma concentrations were observed in CLA-supplemented cows, and lower glucagon/insulin and glucose/insulin ratios could be observed close to calving. The plasma growth hormone concentration was higher in the CLA group and in the combination of EFA and CLA group on d 49 postpartum. The endogenous glucose production tended to be lower, and glucose oxidation was lower in the CLA group on d 21 postpartum. A decrease in endogenous glucose production was associated with an increase in hepatic mRNA expression of PCK1, PCK2, G6PC, and PCCA in cows treated with CLA. As a result of these findings, CLA-treated cows use metabolisable energy more efficiently and have a lower glucose utilisation rate (Hötger et al., 2013; Vogel et al., 2021).

Hepatic glycogen levels were higher in CLA-treated cows at 28 days in milk (Vogel et al., 2021), which confirmed the improved glucose and energy status in the CLA groups in view of the positive association between hepatic glycogen and EB after calving (Hammon et al., 2009; Weber et al., 2013). Most differences in hepatic mRNA abundance were observed in early postpartum, when *PC* decreased in the CLA and in the combination of EFA and CLA group and *PCK2* decreased in the combined EFA and CLA group. In addition, significant differences were observed at the hepatic level between the CLA group and the combination of EFA and CLA group, in which *HMGCS2*, *G6PC*, *INSR*, and *IGFBP-2* were all higher in the CLA group than the combined EFA and CLA group on d 28 postpartum (Vogel et al., 2021). However, other studies reported no consistent changes in the relative abundance of candidate genes associated with energy metabolism and inflammation in the liver, mammary glands, and subcutaneous adipose tissue depots. The gene list consists of adiponectin (***ADIPOQ***), ADIPOQ receptor 1 (***ADIPOR1***), leptin (***LEP***), haptoglobin (***HP***), and interleukin-6 (***IL-6***) (Saremi et al., 2012; Saremi et al., 2014). The relative expression of *PPARγ2* mRNA decreased in the mammary gland (42 days in milk) and tended to decrease in the liver and adipose tissue of CLA-treated cows. During early lactation, hepatic mRNA expression of leptin receptor (**LEPR**) isoform b (***LEPRB***) and tumour necrosis factor-α (***TNF-α***) tended to increase, while *PPARγ1* tended to decrease in liver and adipose tissue (Saremi et al., 2012; 2014).

Later, Qin et al. (2018) provided more evidence regarding CLA supplementation affecting adipose tissue metabolism, IR, and ceramide metabolism. They found that supplementing transition dairy cows with rumen-protected CLA shifted energy partitioning towards adipose tissue instead of the mammary gland. In particular, CLA-treated dairy cows had decreased body fat mobilisation, increased glucose concentration, and reduced insulin sensitivity, which might be signs of IR. However, it was found that the CLA diet resulted in less variation in insulin-related gene expression from weeks 3 to 15, suggesting smoother and less severe physiological adaptation at early lactation (Qin et al., 2018). In adipose tissue, CLA altered the expression of candidate genes related to insulin signalling, inflammation, and sphingolipid metabolism. In this regard, adipocyte fatty acid binding protein (***FABP***) 4, *PPARG*, and hormone-sensitive lipase (***LIPE***) were downregulated, while perilipin 2 (***PLIN2***) was upregulated in adipose tissue as a result of CLA supplementation. Moreover, the mRNA expression of *NFKB1*, protein kinase B2 (***AKT2***), N-acylsphingosine amidohydrolase 1 (***ASAH1***), and serine palmitoyltransferase long-chain base subunit 1 (***SPTLC1***) were all time-dependently induced by CLA in adipose tissue. CLA supplementation affects the PI3K insulin signalling pathway by increasing *AKT2* mRNA expression (Qin et al., 2018). However, *IRS1*, the upstream gene in that pathway was not affected, which makes it hard to conclude that IR has been reduced by CLA. It is possible that the CLA diet affects IR by inducing higher ceramide synthesis, as CLA induced time-dependent expression of SPTLC1, which encodes the long-chain subunits of serine palmitoyltransferase, a rate-limiting enzyme in ceramide synthesis. In contrast, CLA upregulated *ASAH1*, a gene encoding acid ceramidase (greater ceramide hydrolysis), in adipose tissue. The results suggest that CLA supplementation increases ceramide metabolism, but IR has yet to be confirmed.

In a recent study, Yang et al. (2021) indicated that supplementing postpartum dairy cows with a mixture of CLA isomers affected the plasma and muscle tissue metabolome, mainly related to glycerophospholipids and sphingolipids, which led to IR in muscle. In particular, CLA-treated cows had higher concentrations of dopamine, alanine, and hexoses in their skeletal muscles at 21 days in milk. In addition, 23 serum metabolites mainly related to long-chain (>C24) diacyl phosphatidylcholine PC (**PC-aa**, C24:0, C40:1, C40:2, C42:0, C42:1, C42:2, and C42:4) and acyl-alkyl phosphatidylcholine (**PC-ae**, C38:1, C38:2, C40:1, C40:3, C40:4, C42:0, C44:6, C42:1, C42:2, C42:3, C42:4, C42:5, C44:3, C44:4, and C44:5), along with lysophosphatidylcholine acyl (**lysoPC-a**) C26:1, decreased with CLA supplementation. Serum and muscle acylcarnitine profiles as well as the mRNA expression of the carnitine acyltransferases *CPT1B* and *CPT2* were not affected by CLA treatment. In the author's argument, higher dopamine levels indicated IR in the skeletal muscles of CLA-supplemented cows and probably caused higher plasma insulin concentrations. Higher dopamine increases cAMP concentrations in muscles by interacting with the β-adrenergic receptor, thereby leading to decreased glycogen synthase activity, increased glucose-6-phosphate synthesis, and consequently decreased insulin-stimulated glucose uptake. However, these results were affected by a slight difference in DM intake. On day 21, CLA supplementation did not affect serum and muscle amino acid concentrations in dairy cows but increased the mammalian target of rapamycin (***mTOR***, limited to mRNA expression but not protein abundance), F-box only protein 32 (***FBXO32***), α and β polypeptide of branched-chain α-keto acid dehydrogenase ***(BCKDHA & B)*** and ribosomal protein S6 kinase (**S6K1**) protein abundance, which may reflect higher protein turnover (anabolic and catabolic pathways) (Yang et al., 2020).

These results suggest that CLA supplements are an effective strategy for regulating elevated energy demands at the onset of lactation. CLA reduces milk fat synthesis by regulating transcriptional and posttranscriptional mechanisms, and the somatotropin axis facilitates energy partitioning throughout the body, although its effects are time, tissue, and isomer dependent. In the author's opinion, CLA-induced IR might also be mediated by fatty acid accumulation in the liver and skeletal muscles, which triggers lipotoxicity and tissue unresponsiveness and further causes systemic IR. Consequently, IR appears to interact with the affected markers of energy and the somatotropic axis to restore homeostasis. IR can also be both caused by or the cause of inflammation (Vinuesa et al., 2021), which is not yet clearly understood. Nevertheless, a system biology approach that integrate the results of Omics studies at different level and tissues at the same time to provide a better understanding of regulatory mechanisms is still lacking. Fig. 2 summarises the most recent information regarding the molecular

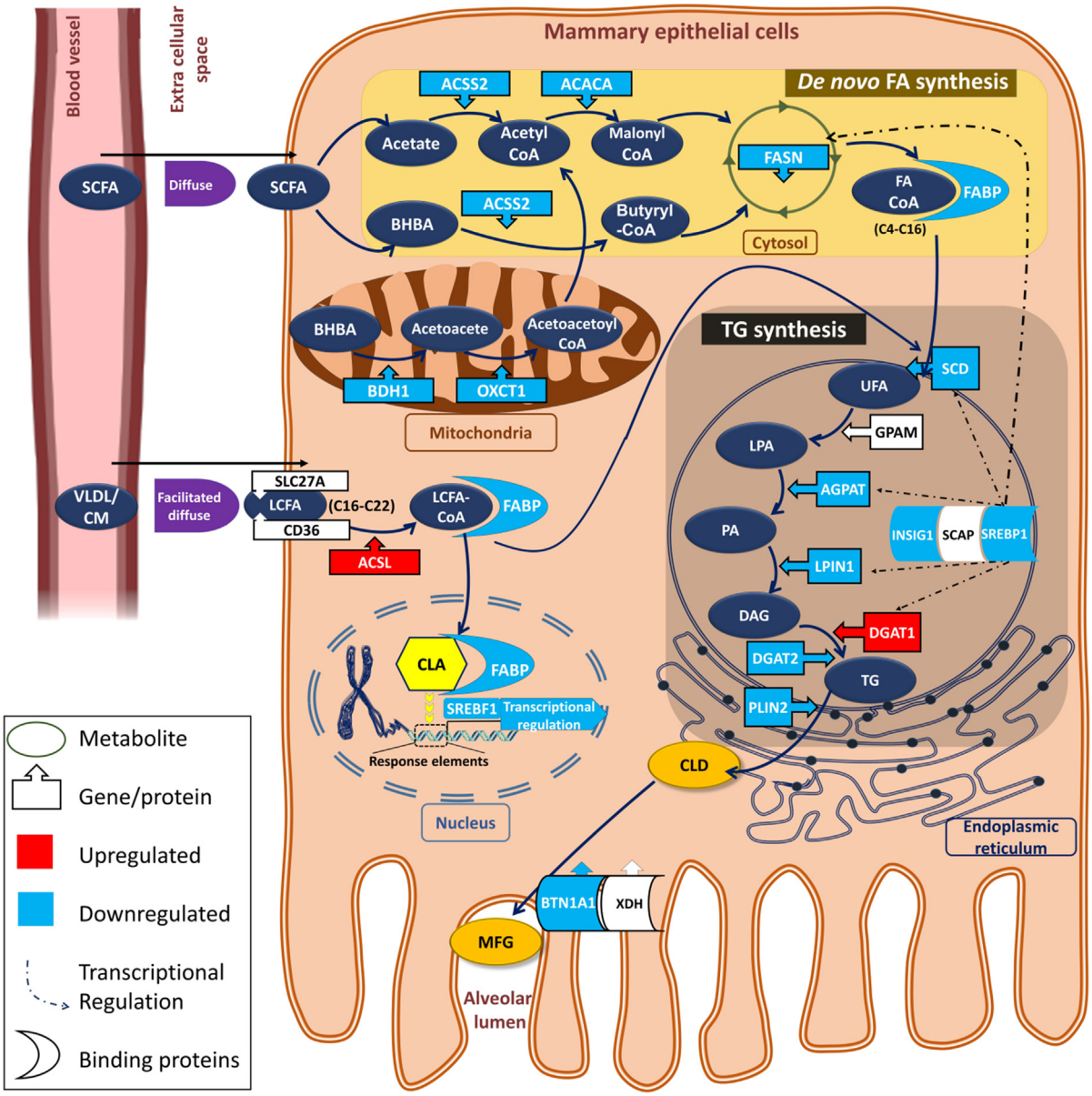

Fig. 2. Highlights of genes and proteins involved in conjugated linoleic acid (CLA)-induced milk fat depression in ruminants. ACACA = Acetyl-CoA carboxylase alpha, ACSS2 = acyl-CoA synthetase short-chain family member 2, AGPAT = 1-acylglycerol-3-phosphate o-acyltransferase, BDH1 = 3-hydroxybutyrate dehydrogenase 1, BHBA = β-hydroxybutyric acid, BTN1A1 = butyrophilin subfamily 1 member a1, CD36 = cluster of differentiation 36, CLDs = cytoplasmic lipid droplets, CM = chylomicrons, DAG = diacylglycerol, DGAT = diacylglycerol O-acyltransferase, FABPs = fatty acid binding proteins, FASN = fatty acid synthase, GPAM = glycerol-3-phosphate acyltransferase, mitochondrial, LCFAs = Long-chain fatty acids, LPA = lysophosphatidic acid, LPIN1 = lipin 1, MFG = Milk Fat Globules, OXCT1 = 3-oxoacid CoA-transferase 1, PA = phosphaditic acid, PLIN2 = perilipin 2, SCD = Stearoyl-CoA Desaturase, SCFAs = Short-chain fatty acids, SLC27 = fatty acid transport proteins, SREBF = sterol regulatory element binding transcription factor, TG = triglyceride, UFAs = unsaturated fatty acids, VLDL = very-low-density lipoprotein, XDH = xanthine dehydrogenase.

regulatory network by which CLA induces milk fat depression obtained from Omics studies in ruminants.

Impact of essential fatty acids and/or conjugated linoleic acid supplementation on inflammation, oxidative stress, and immune function

The involvement of PUFA in immune and inflammatory responses was previously reviewed by Sordillo (2016), Moallem (2018), and Veshkini (2022). EFA and CLA may influence immune function in dairy cows through several mechanisms that are mostly interlinked and might regulate each other through positive and negative feedback loops, although the full extent of these interactions has yet to be elucidated.

Most of the knowledge in the field relies on *in vitro* identification of appropriate dosages (ranges) of fatty acids alone that are not lethal to immune cells and determination of their effects on specific markers under normal or stress-induced physiological conditions. Although this information provides valuable insights into the related mechanisms, with regard to the practical supplementation of dairy cows, there are several known and unknown factors that could interfere with what we expect from the *in vitro* results. It is possible that interfering factors affect the final concentration of desired fatty acid reached at the cell surface, thereby causing ineffectiveness or toxicity. Additionally, it is possible that reverse fates may be induced by the interaction between different fatty acids *in vivo*, namely, n-6 and n-3 fatty acids, which compete to activate or inhibit critical pathways. *In vivo* studies investigating

the synergistic effect of fatty acids in dairy cows are rare. Our aim, in this section, was to provide an update on the recent development of EFA and/or CLA supplementation on dairy cows' immune function.

Dairy cows are exposed to a number of challenges during lactation and pregnancy that may negatively affect the immune system function. The transition from pregnancy to lactation is a typical example, where high-yielding dairy cows naturally experience an integral state of systemic inflammation and immune system dysfunction (reviewed by Horst et al., 2021; Bradford et al., 2015). It has been demonstrated that systemic inflammation during early lactation triggers peripheral IR (and decreased insulin sensitivity) by promoting ceramide synthesis, which consequently inhibits insulin signalling via the phosphoinositide 3-kinase (**PI3K**) pathway (Rico et al., 2017).

With regard to PUFA, longer-chain n-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are more effective modulators of immune functions than ALA and have been shown to have anti-inflammatory and antioxidant properties, as reviewed by Sordillo (2016) and Moallem (2018). Very recently, n-3 fatty acids (fish oil rich in eicosapentaenoic acid and docosahexaenoic acid) were administered to transition dairy cows through venal infusion to avoid rumen biohydrogenation (Mezzetti et al., 2022). Reactive oxygen metabolites in the plasma tended to be lower after venal infusion of n-3 fatty acids during the first three weeks of lactation, but the plasma concentrations of thiol groups, β-carotene, and tocopherol were not affected. Despite the fact that venal infusion of long-chain n-3 fatty acids marginally affected oxidant species release, with regard to ALA, the results were quite different. We have previously shown that abomasal infusions of EFA in transition cows affected inflammation markers (Gnott et al., 2020) but not markers of oxidative stress (Veshkini et al., 2023). EFA-treated cows had lower plasma HP in antepartum and bilirubin at calving. Moreover, a higher concentration of IL-1β and a trend towards higher paraoxonase were observed in the plasma of cows treated with EFA compared to non-EFA-treated cows. However, there was no effect of EFA supplementation on the relative expression of inflammatory genes, including *TNF*, *IL1A*, *IL1B*, *HP*, serum amyloid A (***SAA2***), fibrinogen (***FGA***), C-reactive protein (***CRP***), *TLR4*, *PON1*, cyclooxygenases (*COX1* and *COX2*), in the liver (Gnott et al., 2020).

In contrast to EFA, CLA appears to be more functional in immune functions during the transition period (Veshkini et al., 2023). The primary function of CLA supplementation appears to be the increase in the feasible energy source for the adequate function of immune cells. Elevated glucose and depressed β-hydroxybutyric acid concentrations were observed in an experiment investigating the response of CLA-treated dairy cows during a bacterial lipopolysaccharide inflammatory challenge (Gross et al., 2018). Glucose is a preferable source of energy for immune cells, and thus, elevated glucose means feasible fuel for immune cells, which consequently improves the immune response during an inflammatory process (Ingvartsen and Moyes, 2015). CLA may also increase glucose concentrations by repartitioning energy and stimulating immune cells to preferentially use β-hydroxybutyric acid as a source of energy (Gross et al., 2018). It is worth mentioning that our previous study showed that the higher plasma glucose concentration with CLA or the combination of EFA and CLA supplementation is a result of a CLA-induced decrease in glucose utilisation and not through increasing endogenous glucose production (Vogel et al., 2021). Moreover, both EFA and CLA influence immune cell function by altering the phospholipid fatty acid composition of the membrane and thus affecting the physical and signalling properties of these "lipid rafts" (Calder, 2020). We have previously reported that abomasal infusion of EFA and CLA in dairy cows increased both ALA and RA levels in the plasma-free fatty acids, cholesterol ester, phospholipids, triglyceride, and erythrocyte membranes (Gnott et al., 2020). Although the fatty acid composition of immune cells was not measured, erythrocyte measurements suggest a similar trend for peripheral mononuclear cells, which would also affect the physical properties of immune cells.

Regarding the role of CLA in modulating immune responses, few comprehensive studies have taken into account various aspects of signal transduction, immune cell migration into inflamed tissues (chemotaxis), the production of proinflammatory cytokines, and phagocytosis in dairy cows. The available results yield apparently contradictory results suggesting that CLA activity is isomer and tissue-dependent. There is evidence that CLA mixtures of isomers have greater immunomodulatory effects (than individual isomers) and interact synergistically to reduce apoptosis and increase inflammation-induced respiratory bursts in monocytes (Ávila et al., 2020). In this regard, a recent *in vitro* study found that the administration of 50 μM RA and *trans*-10, *cis*-12 CLA (50:50 CLA mixture) to bovine monocytes reduced apoptosis and increased the production of extracellular respiratory bursts (production of reactive oxygen species (**ROS**)) under inflammatory conditions (Ávila et al., 2020). However, CLA administration had no effect on bovine monocyte chemotaxis, phagocytosis, or killing ability (Ávila et al., 2020). RA and *trans*-10, *cis*-12 CLA (5–60 μM) had no effect on bovine aortic endothelial cell proliferation (Lai et al., 2005). Supporting these findings, the combination of CLA isomers was ineffective in inhibiting bovine peripheral mononuclear cell proliferation *in vitro* (Renner et al., 2012; Renner et al., 2013). In bovine peripheral mononuclear cells, RA administration had a marginal effect on the mRNA abundance of cytokines (*IL-4*, *IL-10*, and *TNF-α*) and *PPAR-γ* expression, which was not a true representative of anti-inflammatory or inflammatory effects (Renner et al., 2013).

Dairy cows are particularly susceptible to oxidative stress and immune disruption during the transition period due to excessive inflammatory and metabolic load that accentuates the production of reactive oxygen species (Abou-Rjeileh and Contreras, 2021). Excessive ROS production activates the NF-κB transcription factor, which in turn stimulates the expression of various proinflammatory mediators, including cytokines (Sordillo and Raphael, 2013). Generally, PUFAs are susceptible to non-enzymatic oxidation by ROS, forming α,β-polyunsaturated lipid aldehydes such as malondialdehyde (**MDA**), which play an important role in many cellular processes (Bochkov et al., 2017). These aldehydes are considered endogenous xenobiotic-like metabolites, which should be detoxified by phase I and phase II drug metabolism in cytochrome P450 pathways; otherwise, their accumulation would result in mitochondrial dysfunction and apoptosis (Rogero et al., 2020).

Another mechanism by which fatty acids influence oxidative stress is through the conversion of PUFA into pro- and anti-inflammatory oxylipids, such as prostaglandins, thromboxanes, leukotrienes, and lipoxins, through different enzymatic pathways led by cyclooxygenases, lipoxygenases, and cytochrome P450 (Sordillo, 2016), but less information is available on dairy cows in this area.

To date, only a few studies exist regarding the antioxidant capacity of CLA in dairy cows. In a recent study, it was shown that CLA isomers containing RA and *trans*-10, *cis*-12 reduced the production of ROS in BME-UV1 cells when compared with EFA (Dipasquale et al., 2018). Basirico et al. (2017) reported that treatment of BME-UV1 with EFA and CLA improved antioxidant capacity through the induction of higher intracellular glutathione (GSH) content, nicotinamide adenine dinucleotide phosphate (**NADPH**) concentration, and γ-glutamyl-cysteine ligase (**γGCL**) activity and reduced intracellular MDA levels (Basirico et al., 2017). The reduced concentration of MDA, as a biomarker of lipid peroxidation (Ayala et al., 2014), demonstrates the protective effect of CLA against lipid peroxidation (Basirico et al., 2017). It was also

suggested that replacing CLA with arachidonic acid reduced the amount of MDA produced by lipid peroxidation without reducing the relative lipid peroxidative activity (Livisay et al., 2000). A similar effect was reported in an *in vivo* study, showing that CLA-supplemented dairy cows exhibited a lower concentration of MDA, whereas the mean serum concentration of hydroperoxides, as an indicator of oxidative stress, remained unchanged (Hanschke et al., 2016). Basirico et al. (2015) reported the antioxidant properties of CLA, particularly the *trans*-10, *cis*-12 isomer, which generates a high redox status in BME-UV1 cells that protects against H_2O_2-induced oxidative stress. The author observed an induction of intracellular GSH, accompanied by high concentrations of NADPH, as well as increased γ-glutamyl-cysteine ligase, superoxide dismutase (**SOD**), glutathione peroxidase (**GPX**), and glutathione S-transferase (**GST**) activity, but no change in the gene expression of these antioxidant enzymes.

We have previously shown that CLA and the combination of EFA and CLA supplementation had no significant effect on haematological parameters, including the erythrocyte count, haematocrit, haemoglobin, red blood cell distribution width, and immunological parameters, including leucocytes, basophils, thrombocytes, and large immature cells, in the transition period (Veshkini et al., 2023). Additionally, plasma markers of oxidative stress, including reactive oxygen metabolites, biological antioxidant potential, oxidative stress index (reactive oxygen metabolites /biological antioxidant potential), thiol groups, GPX-1, ferric reducing antioxidant power, oxygen radical absorbance capacity and the concentrations of lipid-soluble antioxidants, including β-carotene, retinol, and α-tocopherol, were minimally affected by CLA and combined EFA and CLA supplementation during the whole pre- and postpartum period (Veshkini et al., 2023). Moreover, the same results were reflected in the erythrocyte markers of oxidative status, including reactive oxygen metabolites, GPX-1, SOD-1, and MDA, and none of them were influenced by CLA or the combination of EFA and CLA during the transition period or mid-lactation (Veshkini et al., 2023; Haubold et al., 2020). In the liver, the relative transcript abundance of oxidative markers, including *SOD-1*, *GPX-1*, and catalase (*CAT*), was barely changed by fatty acid treatment during the whole transition period, despite time-dependent differences (Veshkini et al., 2023; Haubold et al., 2020). Additionally, the relative protein abundances of GPX-1, GPX3, GSTM, GSTM3, GSTM4, GSTA1, GSTA2, GSTA4, GSTZ1, GSTP1, GSTK1, GSTT1, LANC like GST (**LANCL1**), microsomal GST (MGST1 and MGST3), glutathione synthetase (**GSS**), glutathione reductase (**GSR**), S-formylglutathione hydrolase, mitochondrial hydroxyacylglutathione hydrolase (**HAGH**), SOD2, SOD3, peroxiredoxins (PRDX1, PRDX2, PRDX3, and PRDX5) and CAT were not affected by supplementation with combined EFA and CLA at the hepatic level during the transition period (Veshkini et al., 2022a). In addition, the combination of EFA and CLA did not affect the plasma abundances of GPX3, CAT, and haemoglobin subunits (HBA and HBB) in transition dairy cows (Veshkini et al., 2022b). In contrast, Qin et al. (2018) found that CLA diets altered the local inflammatory response by upregulating TLR4 and its downstream gene *NFKB1* in adipose tissue, potentially increasing proinflammatory cytokine production and causing IR.

Another area linking CLA to inflammatory function that has remained largely unexplored is through modulation of lipid-derived immunomodulators which affects immunometabolism in transition dairy cows, as reviewed by Zachut et al. (2022). According to phosphoproteomics analysis, cows supplemented with CLA exhibited enhanced adipose tissue expression of the endocannabinoid system, which supports immune system homeostasis by suppressing inflammation (Daddam et al., 2021). In another study, n-3 fatty acids were also reported to modulate the endocannabinoid system components of antepartum dairy cows in the blood, adipose tissue, and liver (Kra et al., 2022). Oxylipids, the final products of omega oxidation of CLA are additional lipid-derived immunomodulator, which may affect inflammatory pathways (Zachut et al., 2022). It has been reported that n-6-fatty acid-derived oxylipids can be both pro- and antioxidative (Kuhn et al., 2021), but there is no report on CLA oxylipids in dairy cows. In the authors' opinion, there is a possibility that CLA-derived as well as n-3-fatty acid-derived oxylipids exert an anti-inflammatory effect. Nevertheless, the specific role of lipid-derived immunomodulators in immunometabolism of transition dairy cows should be investigated in deep in future studies.

Fig. 3 summarise the possible regulatory mechanisms by which EFA and CLA potentially impact immunometabolism. We also observed a trend towards a lower peak and shorter duration of oxidative stress markers and inflammation only with CLA compared to EFA (Veshkini et al., 2023). Despite its antioxidative potential, CLA should not be considered an anti-inflammatory and antioxidative supplement in transition dairy cows because its effectiveness can be affected by the systemic inflammation and tissue remodelling as continuous powerful inducers of

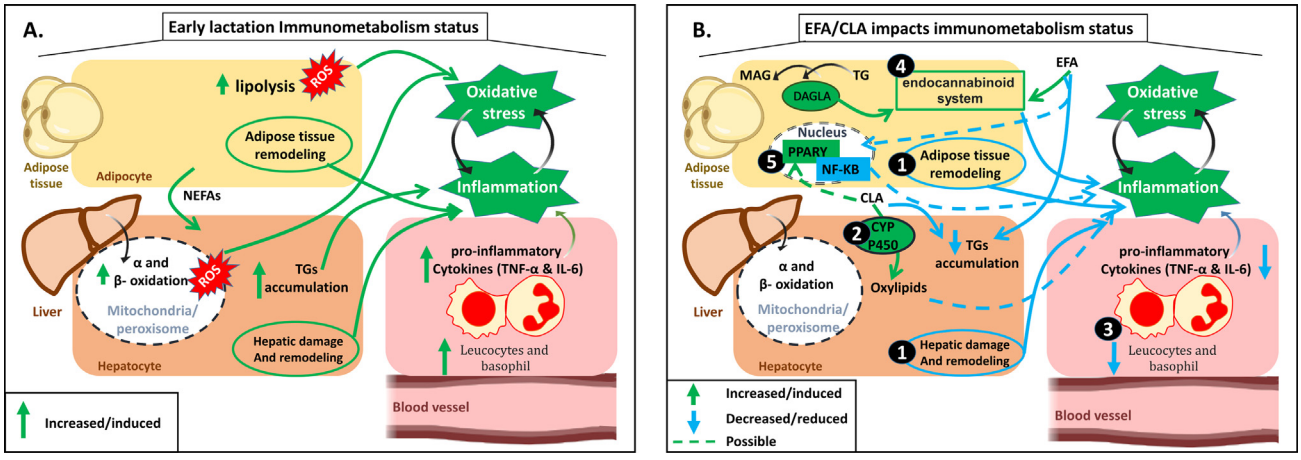

Fig. 3. Schematic representation of major mechanisms by which essential fatty acids (EFAs)/conjugated linoleic acids (CLAs) impact the immunometabolism of dairy cows during the transition period. A. Induction of systemic inflammation and oxidative stress at early lactation, B. EFA/CLA supplementation had minor effects on immunometabolism through the following pathways. (1) EFAs/CLAs decrease metabolic pressure on hepatic and adipose tissue, (2) CLAs induce oxylipid synthesis, (3) CLAs decrease immune cell count and cytokine production, (4) EFAs and CLAs impact the endocannabinoid system, and (5) EFAs/CLAs possibly reduce NF-κB activation. DAGLA = diacylglycerol Lipase Alpha, IL-6 = interleukin−6, NEFAs = non-esterified fatty acids, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, PPARγ = peroxisome proliferator-activated receptor gamma, ROS = reactive oxygen species, TGs = triglycerides, TNF-α = tumour necrosis factor-alpha.

oxidative agents. However, to understand how immunometabolism reacts to EFA and CLA supplementation, further *in vivo* studies are still required to investigate the specific markers on immune cells and, possibly, integrate them with markers of metabolism in other tissues.

Concluding remarks

This review based on the pivotal contribution of Omics techniques provides the most up-to-date mechanistic understanding of the effects of EFA and CLA supplementation on transition dairy cows' physiology, although several questions still remained to be elucidated in future studies. Both EFA and CLA appeared to positively regulate immunometabolism during the transition period. However, CLA and the combination of EFA and CLA were found to be more effective than sole EFA at restoring metabolic homeostasis in high-yielding dairy cows under a maize silage-based total mixed ration diet. During the transition period, CLA's main benefit is to conserve more metabolisable energy for dairy cows by causing MFD and accelerating cholesterol and triglyceride metabolism. There are also time- and dose-dependent effects of EFA and CLA on improving EB, reducing hepatic triglyceride accumulation, accelerating systemic lipid turnover in critical tissues, and attenuating markers of hepatic steatosis, which are associated with less metabolic stress. It should be noted that the accelerated systemic lipid turnover may only be advantageous during early lactation when a certain part of lipid metabolism such as lipogenesis and lipid transport pathways may be slowed or saturated as a part of metabolic adaptations. However, induced higher lipid turnover might have limited capacity, and therefore, benefits may only be attained with appropriate doses of fatty acids. Inflammatory and oxidative stress markers were marginally affected by EFA and CLA during the transition period, but the EFA effect was less. The underlying mechanisms, which have yet to be fully elucidated, involve providing higher metabolisable energy and complex feedback mechanisms via ligand activation with nuclear receptors as well as the contribution of lipid-derived immunomodulators. Supplementation with EFA (in particular ALA) has been shown to marginally affect the metabolites, hormones, and gene expression of some critical tissues towards improved metabolic health, but the *in vivo* research in this area is still limited. This could be because longer-chain n-3 fatty acid family members are more physiologically active, which attracted more attention. Even though CLA appeared to reduce NEB severity and improve hepatic metabolic markers, non-CLA-treated cows under maize silage-based total mixed ration showed no signs of metabolic disorders. Therefore, the decision on whether CLA supplementation should be considered in dairy cows largely depends on the individual farm situation. If a higher prevalence of metabolic disorders/diseases during the transition period at the herd level is expected, EFA and CLA supplementation may come in the front as a complementary rapid nutritional strategy along with other management strategies. Although in the authors' opinions, nutritional strategies should aim to increase natural CLA synthesis in the first place by considering pasture or fresh grass feeding as a partial replacement of maize silage.

Ethics approval

Not applicable.

Data and model availability statement

Data or models were not deposited in an official repository. No new datasets were created.

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Declaration of interest

None.

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