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Abomasal infusion of essential fatty acids and conjugated linoleic acid during late pregnancy and early lactation affects immunohematological and oxidative stress markers in dairy cows

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ABSTRACT

Oxidative stress and inflammation, as natural parts of metabolic adaptations during the transition from late gestation to early lactation, are critical indicators of dairy cows' metabolic health. This study was designed to investigate the effects of abomasal infusion of essential fatty acids (EFA), particularly α-linolenic acid, and conjugated linoleic acid (CLA) on plasma, erythrocyte, and liver markers of oxidative stress in dairy cows during the transition period. Rumen-cannulated German Holstein cows (n = 38) in their second lactation (11,101 ± 1,118 kg milk/305 d, mean ± standard deviation) were abomasally infused with one of the following treatments from d −63 antepartum until d 63 postpartum (PP): CTRL (n = 9; 76 g/d coconut oil); EFA (n = 9; 78 g/d linseed plus 4 g/d safflower oil); CLA (n = 10; isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; 38 g/d); and EFA+CLA (n = 10; 120 g/d). Hematological parameters as well as markers of oxidative status were measured in plasma, erythrocytes, and liver before and after calving. Immunohematological parameters, including erythrocyte number, hematocrit, hemoglobin, mean corpuscular hemoglobin, leukocytes, and basophils, were affected by time, and their peak levels were observed on the day after calving. The oxidative stress markers glutathione peroxidase 1 and reactive oxygen metabolites in plasma and erythrocytes were both affected by time, exhibiting the highest levels on d 1 PP, whereas β-carotene, retinol, and tocopherol were at their lowest levels at the same time. Immunohematological parameters were only marginally affected by fatty acid treatment in a time-dependent manner. As such, lymphocyte and atypical lymphocyte counts were both significantly highest in the groups that received EFA at d 1 PP. Moreover, EFA supplementation increased the mean corpuscular volume and showed a trend for induction of mean corpuscular hemoglobin compared with the CLA group during the transition period. The PP mean thrombocyte volume was higher in the EFA than in the CLA group (except for d 28) and both EFA and CLA reduced number of thrombocytes and thrombocrit at distinct time points. Hepatic mRNA abundance of markers related to oxidative status, including glutathione peroxidase (*GPX-1*) and catalase (*CAT*), was lower ($P < 0.05$) in EFA-treated than non-EFA-treated cows at d 28 PP. Dairy cows at the onset of lactation were characterized by induced markers of both oxidative stress and inflammation. Supplementing EFA and CLA had minor and time-dependent effects on markers of oxidative stress in plasma, erythrocytes, and liver. A comparison of EFA supplementation with CLA or CTRL showed higher immunohematological response at d 1 PP and lower hepatic antioxidant levels by d 28 PP. Supplementation with EFA+CLA had only a minor effect on oxidative markers, which were more similar to those with the EFA treatment. Altogether, despite the time-dependent differences, the current findings show only minor effects of EFA and CLA supplementation in the prevention of early lactation-induced oxidative stress.

Key words: antioxidant, erythrocyte, liver, blood immune cells

INTRODUCTION

Dairy cows fed with TMR for high milk production often experience a state of systemic inflammation, im-

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mune system malfunction, negative energy balance, and oxidative stress during the transition period, all of which are intrinsically linked with poor health and production outcomes (Drackley, 1999; Lopreiato et al., 2020). To compensate for negative energy balance, dairy cows mobilize their body reserves predominantly from adipose tissue in the form of nonesterified fatty acids, which increases the production of reactive oxygen species (**ROS**) and induces redox signaling pathways (Abuelo et al., 2019; Abou-Rjeileh and Contreras, 2021). Cellular oxidative stress, occurring as a result of excessive ROS production beyond the capacity of the antioxidative system, causes damage to DNA, proteins, and lipids (Birben et al., 2012). A positive feedback loop between oxidative stress and inflammatory mediators can occur under certain physiological conditions whereby a permanent pro-oxidative feature may induce a subclinical systemic proinflammatory state leading to chronic systemic damage, termed "OxInflammation" (Valacchi et al., 2018). Although dairy cows undergo a state of systemic inflammation during the transition period (Horst et al., 2021), there is no evidence that inflammation markers are linked to oxidative stress. Thus, it would be interesting to assess their relationship, particularly during the transition period, to determine whether OxInflammation can be used to monitor dairy cow metabolic health.

Fatty acids (**FA**), in particular n-3 FA and CLA, can exhibit antioxidative and anti-inflammatory properties depending on their chemical structure (number and localization of their double bonds) as well as their ratio in relation to other FA (n-6:n-3; Kaska et al., 2014). α-Linolenic acid (C18:3 n-3, **ALA**), as a precursor of the long chain n-3 FA, is an essential FA (**EFA**) for mammals that is highly concentrated in fresh grass and whole linseed (Elgersma, 2015). Supplementing ALA in mice with induced brain oxidative stress markedly reduced ROS and proinflammatory cytokine [interleukin-1b (IL-1β)] production (Alam et al., 2021). In another study, colostrum enriched with n-3 FA and α-tocopherol reduced indicators of oxidative stress (oxidant status index) in newborn calves (Opgenorth et al., 2020). Moreover, studies in dairy cows reported that n-3 FA supplements increased the levels of n-3 FA in both red blood cells (**RBC**) and white blood cells (Revskij et al., 2019; Gnott et al., 2020), which was associated with lower levels of inflammatory biomarkers such as soluble interleukin-6 (IL-6) and tumor necrosis factor receptor 2 (Fontes et al., 2015). The term CLA refers a group of octadecadienoic acids with 2 conjugated double bonds, primarily consisting of the 2 major isomers, *trans*-10,*cis*-12, and *cis*-9,*trans*-11 CLA, specifically formed by ruminal biohydrogenation of PUFA. Conjugated linoleic acid isomers have proven immune-modulating and antioxidative properties (for review, see Bionaz et al., 2020). For instance, in bovine mammary epithelial cells exposed to oxidative stress, CLA reduced ROS production by enhancing the expression of superoxide dismutase (**SOD-1**), glutathione peroxidase, and glutathione S-transferase and inhibiting the transcription of proinflammatory cytokines (Dipasquale et al., 2018; Ma et al., 2021).

Compared with TMR, pasture feeding systems resulted in higher levels of ALA and CLA in ruminant milk (reviewed by Moscovici Joubran et al., 2021), plasma (La Terra et al., 2010), muscle, and adipose tissue (Wang et al., 2019). As such, supplementing TMR diets with ALA and CLA is a strategy to not only reduce negative energy balance but also to increase antioxidative defense capacity during challenging time points. In this regard, we have shown previously that CLA supplementation in transition cows improved energy balance (Vogel et al., 2020) but exhibited proinflammatory effects, whereas EFA had anti-inflammatory properties, and the combination of EFA and CLA was less effective on markers of inflammation (Gnott et al., 2020). Additionally, EFA and CLA supplementation affected markers of oxidative stress (GPX-1 activity), but not inflammation, in mid-lactating dairy cows (Haubold et al., 2020). We concluded that FA supplementation has little effect on markers of oxidative and inflammatory status during mid-lactation when cows are not metabolically and immunologically challenged. Additionally, we have previously reported that maternal supplementation with EFA or CLA, or both, affected the inflammatory response and the oxidative and antioxidative status of their calves after birth, which includes lower plasma total bilirubin, cholesterol, IL-1β, and ferric reducing antioxidant power (**FRAP**; more pronounced with maternal EFA supplementation; Liermann et al., 2021).

In the present study, we aimed to investigate the hematological and immunological traits, as well as markers of oxidative stress, in blood plasma, erythrocytes, and hepatic samples from dairy cows supplemented with EFA or CLA, or both, during various time points around parturition. Dairy cows were fed corn silage TMR formulated to provide low amounts of ALA and CLA during late gestation and early lactation. Therefore, we hypothesized that abomasal infusion of ALA and CLA would improve the antioxidative capacity and alleviate markers of oxidative stress at challenging time points around parturition. This study under the same experimental design is complementary to our previously published work on the effects of EFA and CLA supplementation on inflammatory markers in transition dairy cows [haptoglobin (**HP**), fibrinogen, paraoxonase, bilirubin, IL-1β, and IL-6; Gnott et al.,

2020]. In addition, the correlation between oxidative stress and inflammatory markers was calculated to investigate their relationship.

MATERIALS AND METHODS

Animals, Housing, Feeding, and Supplementation

The experimental procedures were conducted under the previously described animal experiment by Vogel et al. (2020) and according to the animal welfare guidelines approved by the local authority of the State Mecklenburg-Western Pomerania, Germany (LALLF M-V/TSD/7221.3-1-038/15). Briefly, 38 German Holstein cows in their second lactation (expected milk yield ~11,000 kg/305 d, mean ± SD) from the experimental animal facility for cattle of the Research Institute for Farm Biology (FBN, Dummerstorf, Germany) were equipped with a rumen cannula (10-cm inner diameter cannula; Bar Diamond Inc.) and an abomasal infusion line. Animals were housed in freestall barns with ad libitum access to a corn silage-based TMR formulated according to recommendations provided by the Society for Nutrition Physiology (GFE, 2001, 2008, 2009) and the German Agricultural Society (Deutsche Landwirtschaftliche Gesellschaft; DLG, 2013), which was adapted for the dry period [wk −6 to 0 antepartum (**AP**), dry period] or lactation period [wk −10 to −7 AP and wk 1 to 8 postpartum (**PP**)]. The amounts of daily infused oil supplements, ingredients and chemical compositions of the experimental diets and a summary of the performance data were first published by Vogel et al. (2020) and are provided in Supplemental Tables S1, S2, and S3 (https://doi.org/10.5281/zenodo.7539752; Veshkini et al., 2023), respectively. Briefly, the energy content of the lactation diet was 7.1 MJ NEL/kg DM, and during the dry period, the energy content of the diet was 6.5 MJ NEL/kg DM. The chemical composition of the lactation diet consisted of 23 g/kg DM crude fat, 173 g/kg DM crude fiber and 146 g/kg DM crude protein (21, 219, and 141 g/kg DM in the dry period diet, respectively). Cows had free access to water and were milked twice per day at 0630 and 1800 h during late and early lactation. From d −63 AP to 63 PP, cows were infused into the abomasum with one of the following treatments: **CTRL** (control) group (n = 9), coconut oil (Bio-Kokosöl #665, Kräuterhaus Sanct Bernhard, KG; 76 g/d); EFA group (n = 9), a combination of linseed oil (DERBY Leinöl #4026921003087, DERBY Spezialfutter GmbH; 78 g/d) and safflower oil (GEFRO Distelöl, GEFRO Reformversand Frommlet KG; 4 g/d); conjugated linoleic acid (**CLA**) group (n = 10), Lutalin; *cis*-9,*trans*-11, 10 g/d *trans*-10,*cis*-12 CLA, BASF SE; 38 g/d); and **EFA+CLA** group (n = 10), a combination of linseed oil, safflower oil, and Lutalin in amounts reported for EFA and CLA, respectively. Dairy cows were assigned to groups according to their BW (662 ± 56 kg, mean ± SD) and milk yield (17.8 ± 4.0 kg/d, mean ± SD) on d −70 AP. Supplementation of daily dose was performed in equal portions twice per day, after morning milking (0700 h) and before the milking process in the afternoon (1630 h). During the dry period, each dose was halved (Vogel et al., 2020).

Blood Sampling, Immunohematological, and Antioxidative Status Analyses

Blood samples were taken on d 42, 35, 28, 21, and 10 before expected calving and on d 1, 7, 14, 21, 28, 35, 42, and 56 PP from the jugular vein into evacuated tubes (Vacuette, Greiner Bio-One International AG) containing either lithium-heparin (12–30 IU heparin per 10 mL) or K_3EDTA (1.8 g/L). Blood was collected in the morning after milking and before subsequent feeding. After collection, blood samples were immediately transferred to the laboratory on ice, and an aliquot of each was centrifuged for 20 min at 1,500 × g at 4°C. The plasma fraction was extracted and stored at −80°C until analysis.

Using an automatic hematology analyzer (ABX Pentra 60, Horiba ABX SAS), the hematocrit (**HCT**) and hemoglobin (**HGB**) concentrations in whole blood as well as specific blood cell parameters including the total counts of erythrocytes; red cell distribution width (**RDW**); mean corpuscular volume of erythrocytes (**MCV**); mean corpuscular hemoglobin of erythrocytes (**MCH**); mean corpuscular hemoglobin concentration of erythrocytes (**MCHC**); total counts of leukocytes, lymphocytes, atypical lymphocytes, basophilic granulocytes, and thrombocytes; mean thrombocyte volume; thrombocrit; and immature cells were determined in the blood of dairy cows on d −63, −42, and −21 AP, as well as at 1, 28, and 56 PP.

Markers of oxidative and antioxidative status were measured in plasma samples collected on d −42, 1, 28, and 56 relative to calving. Derivatives of reactive oxygen metabolites (**ROM**) in K3EDTA plasma were measured by the spectrophotometric method patented by Diacron (ROM test; Diacron; Cesarone et al., 1999; Bernabucci et al., 2002). The results of the analyses were expressed in Carratelli units (U.CARR). The value of 1 U.CARR corresponds to a concentration of 0.8 mg/L hydrogen peroxide. The biological antioxidant potential of plasma samples (**BAP**) was measured by colorimetric determination using a commercial kit (BAP test) based on the method described by Benzie and Strain (1996) using the ascorbic acid concentration as a calibrator. The results were expressed in μmol/L.

The plasma oxidative stress index was calculated as the ratio of ROM to BAP. According to Benzie and Strain (1996) and using Trolox (a water-soluble analog of vitamin E) as a calibrator, FRAP in plasma was determined with intra- and interassay coefficients of variability of 2.7 and 2.6%, respectively, and expressed as μmol/L. It is important to note that even though both the BAP and FRAP methods are based on ferric reduction to ferrous, their reagents and calibrators differ to cover both hydrophilic and hydrophobic antioxidant potential.

Plasma thiol groups (**SH**) were determined by titration with 5,5-dithiobis-2-nitrobenzoic acid by using a commercial kit (SHp test, Diacron; Bernabucci et al., 2005) and were expressed in μmol/L. Plasma GPX-1 activity was determined by a colorimetric commercial assay kit (EGPX-100 EnzyChrom Glutathione Peroxidase Assay Kit, BioAssay Systems) as previously described by Bernabucci et al. (2005). The GPX-1 activity is expressed in U/L, and one unit is the amount of enzyme that produces 1 micromole of oxidized glutathione per min at pH 7.6 and room temperature.

Plasma concentrations of β-carotene, α-tocopherol, and retinol were measured according to a previously described method (Liermann et al., 2021). Briefly, after extraction of vitamins from frozen aliquots with hexane, the concentrations of β-carotene, α-tocopherol, and retinol in lithium-heparin plasma were measured by reverse-phase high-performance liquid chromatography (HPLC, LC4000, JASCO Europe Ltd.) using a Zorbax Eclipse Plus C18 column, 3.5 μm, in a 150 × 4.6 mm column (Agilent Technologies), with a UV detector set at 290 nm (for α-tocopherol), 325 nm (for retinol), and 460 nm (for β-carotene), using methanol: tetrahydrofuran (80:20) as the mobile phase.

The method of Cao and Prior (1999) was used to measure the oxygen radical absorbance capacity (**ORAC**), which measures a fluorescent signal from a probe (fluorescein) that decreases in the presence of radical damage in lithium-heparin plasma (Haubold et al., 2020).

Markers of oxidative status [ROM, GPX-1, SOD-1, and malondialdehyde (MDA)] were measured in erythrocytes on d −42 AP and on d 1, 28, and 56 PP. The separation of erythrocytes from blood was performed following the method reported by Bernabucci et al. (2002, 2005). Briefly, erythrocytes were obtained by centrifuging 0.5 mL of blood at 2,200 × g at 4°C for 10 min. Erythrocytes were then washed 4 times with 3 mL of 0.9% NaCl solution by centrifuging for 10 min at 2,200 × g at 4°C. After the final wash, the RBC were lysed by hypotonic shock using 2.0 mL of cold redistilled water. The hemolysate was treated with 10 μL of butylhydroxytoluol (2%) as an antioxidant and stored at −80°C until analyses. The activity of SOD-1 (international units per milliliter) in erythrocytes was measured after diluting the lysates by 50-fold in 0.01 mmol/L phosphate buffer at pH 7.0 using a commercial kit (RANSOD, by Randox Laboratories). The methods for the determination of ROM and GXP-1 were as described above. The MDA concentration was measured by using a colorimetric kit (lipid peroxidation assay kit, ab118970, Abcam; Essid et al., 2012).

Hepatic Relative Transcript Abundance Analysis

Transcript abundance analysis was performed on liver biopsies collected on d −21, 1, 28, and 63 relative to calving using real-time PCR (LightCycler, Roche Molecular Biochemicals) and SYBR green I detection (Gnott et al., 2020). The procedures for sample collection, RNA extraction and measurement, primer product verification, real-time PCR, melting curve analysis, and reference mRNA identification were performed according to the MIQE guidelines (Bustin et al., 2009) and were previously described by Gnott et al. (2020). Briefly, RNA was extracted from homogenized liver tissue using an RNeasy Mini Kit (Qiagen GmbH) and checked for RNA yield quality (1.8–2) and the RIN factor (>6) using an optical density via a spectrophotometer (NanoPhotometer, Implen GmbH) and Agilent 2100 Bioanalyzer (Agilent Technologies). Reverse transcription was performed using 750 ng of RNA, 200 U RevertAid reverse transcriptase (Thermo Fisher Scientific), and 250 pmol random hexamer primers (Metabion International AG). The quality controls, normalization, and quantification of the target genes, including *GPX-1, SOD-1,* and catalase (*CAT*), and identified reference genes, including low-density lipoprotein receptor-related protein 10 (*LRP10*) and Hippocalcin-like 1 (*HPCAL1*), were performed in qBASE+ version 3.1 (Biogazelle; Supplemental Table S4, (https://doi.org/10.5281/zenodo.7539752; Veshkini et al., 2023). Gene expression results are presented as the ratio between the target gene copy number and the geometric mean of the reference genes.

Statistical Analysis

Statistical analyses were performed using SAS for Windows, release 9.4 (SAS Institute Inc.). Markers in whole blood, blood plasma, erythrocytes, and liver tissue were analyzed using the MIXED procedure by repeated-measures ANOVA containing EFA (level: yes, no), CLA (level: yes, no), time (levels: day relative to calving), block (levels: 1 to 5), and the respective interactions (EFA × CLA; EFA × time; CLA × time; and EFA × CLA × time) as fixed effects. The calving inter-

val and projected milk yield during the second lactation were used as covariates. In addition, measurements before the beginning of the FA supplementation were included as covariates when available (hematological measurements, vitamins, and FRAP and ORAC). Repeated measures on each cow were considered by using the repeated statement of the MIXED procedure with compound symmetry (timeline day or week) covariance structure. Gene expression results were normalized to reference genes and analyzed using the $2^{-\Delta\Delta CT}$ method. The least squares means (LSM) and their standard errors were computed for each fixed effect in the ANOVA model to display the results, and all group differences in the LSM were tested by the Tukey–Kramer procedure. The SLICE statement of the MIXED procedure was used to assess partitioned analyses of the LSM for interactions. Statistical significance was declared at $P < 0.05$, with trends applied to values at $P < 0.1$. The Pearson correlation between the obtained markers and our previously reported measurements (Gnott et al., 2020; Vogel et al., 2020) was calculated using the corrplot package in R (Version 4.0).

RESULTS

Hematological and Immunological Parameters in Transition Dairy Cows Supplemented with EFA and CLA

Hematological parameters for transition dairy cows under various FA treatments are illustrated in Figures 1 and 2. The erythrocyte cell count and HCT as well as MCV were affected by time ($P < 0.05$). They gradually increased AP, peaked at d 1 PP, and then gradually decreased PP (Figure 1A–C). On d −21 AP and d 28 and 56 PP, MCV was lower ($P < 0.05$) in CLA-treated cows than in non-CLA-treated cows, and on d 28 PP, MCV was lower ($P < 0.05$) in CLA-treated cows than in EFA-treated cows (Figure 1C). Moreover, RDW decreased over time ($P < 0.05$), but it was not affected by the treatment or interaction effects (Figure 1D). The concentration of HGB was only affected by time ($P < 0.05$) and increased during the AP period to its peak at parturition when it then gradually decreased afterward (Figure 2A). Both MCH and MCHC were affected by time ($P < 0.05$), being highest and lowest on the first day of PP, respectively (Figure 2B and C). There were also significant time-dependent differences in MCH and MCHC among the FA treatment groups. Dairy cows treated with CLA had lower ($P < 0.05$) MCH on d −21 AP and tended to have lower ($P < 0.1$) MCH over the entire experimental period than non-CLA-treated cows. On d 28 PP, MCHC was lower ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows and was higher ($P < 0.05$) in CLA-treated than in EFA+CLA-treated cows.

Figures 3 and 4 illustrate the immune cell counts and their related parameters. Leukocytes, lymphocytes, and atypical lymphocytes were affected by time ($P < 0.05$). These parameters increased until parturition and then gradually decreased (Figure 3A–C). Leukocytes showed an overall trend for a CLA effect ($P < 0.1$), and on d 56 PP, they were lower ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows and were lower ($P < 0.05$) in EFA+CLA-treated cows than in CTRL cows. Lymphocytes showed a trend ($P < 0.1$), while atypical lymphocytes had a significant EFA × time interaction, and both cell types were higher ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows on d 1 PP (Figure 3B and C). There were no significant time, treatment, or interaction effects on basophil counts (Figure 3D). Thrombocytes, thrombocrit, and mean platelet volume were affected by time ($P < 0.05$), as well as the interaction of time and EFA treatment ($P < 0.05$; Figure 4A–C). Thrombocytes (d −42 AP and d 1 and 56 PP) and thrombocrit (d 1 PP) were lower ($P < 0.05$), whereas mean platelet volume (all time points) was higher ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows. In addition, thrombocytes and thrombocrit were lower ($P < 0.05$) in CLA-treated cows than in non-CLA-treated cows on d 56 PP. Thrombocytes and thrombocrit were lower ($P < 0.05$) in EFA and CLA than in CTRL on d −42 AP and were lower ($P < 0.05$) in EFA+CLA-treated cows than in CTRL cows on d 56 PP. On d −21 AP, EFA treatment decreased ($P < 0.05$) the proportion of large immature cells, and the cell number was lower in the EFA and EFA+CLA groups than in the CTRL group (Figure 4D).

Plasma Markers of Oxidative Status in Transition Dairy Cows Supplemented with EFA and CLA

Figures 5, 6, and 7 illustrate the markers of oxidative status in plasma. The plasma concentration of ROM was affected by time ($P < 0.05$) but not by treatments or the interaction of time and treatments (Figure 5A). The highest plasma ROM concentration was observed at d 1 PP and then decreased ($P < 0.05$) during d 28 to 56 PP, except for the EFA+CLA group, where a numerical increase was seen from d 28 to d 56 PP. The plasma concentration of BAP decreased continuously during the transition period from d −42 to d 28 in all groups ($P < 0.001$; Figure 5B). There was an overall EFA treatment effect, and the concentration of BAP was lower ($P < 0.05$) in EFA than in non-EFA-

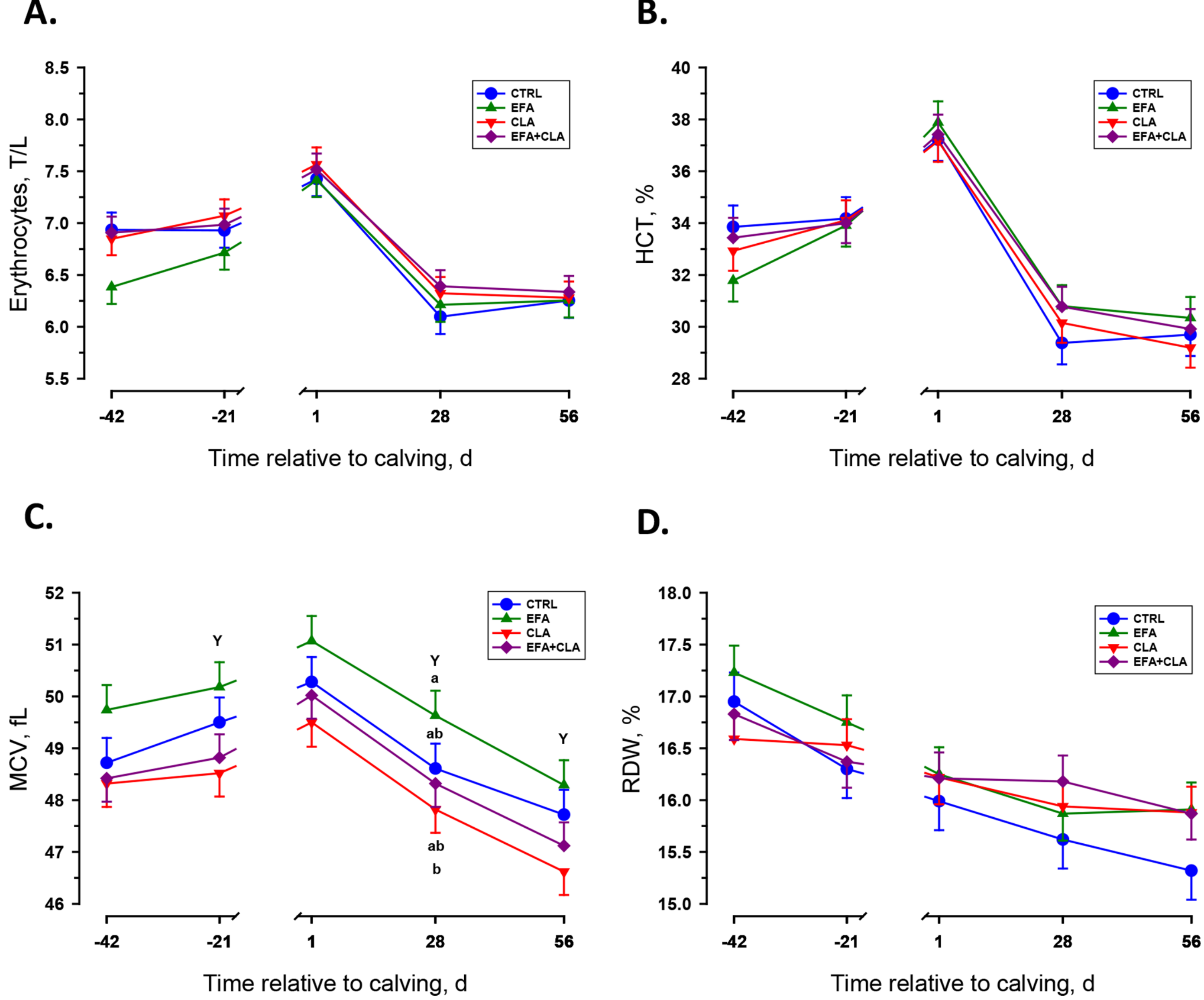

Figure 1. Blood hematological parameters including (A) erythrocyte count (T/L), (B) hematocrit (HCT, %), (C) mean corpuscular volume of erythrocytes (MCV, fL), and (D) red blood cell distribution width (RDW, %) in dairy cows that received fatty acids (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; LSM with different letters (a, b) differ ($P < 0.05$) at the respective time point. Y = CLA effect at the respective time point. Statistically significant ($P < 0.05$) effects for erythrocytes (time), HCT (time), MCV (time, CLA), and RDW (time). T/L: tera (10^{12} cells)/liter; fL = femtoliter.

treated cows on d 56 PP. Compared with CTRL, EFA treatment tended to reduce plasma BAP at d 28 PP ($P < 0.1$). The calculated plasma oxidative stress index (OSI = ROM/BAP) increased ($P < 0.05$) from AP to PP in all groups (Figure 5C) and was increased by EFA treatment when compared with non-EFA treatment on d 56 PP.

The concentration of plasma SH fluctuated over time ($P < 0.05$), initially increasing from d −42 AP to d 1 PP (Figure 6A). There was a trend for higher SH values in the EFA group compared with the CTRL group at d 56 PP ($P = 0.08$). Plasma GPX-1 was only affected by time ($P < 0.05$), whereas treatment and interaction effects were not significant (Figure 6B). Plasma GPX-1 was substantially increased and reached peak levels at d 1 PP ($P < 0.001$). The concentration of FRAP (Figure 6C) continuously decreased in all groups during AP and then peaked at parturition, with a sudden drop at d 14 and 28 PP, followed by a gradual increase on d 42 and d 56 ($P < 0.01$). In addition, ROM/FRAP has

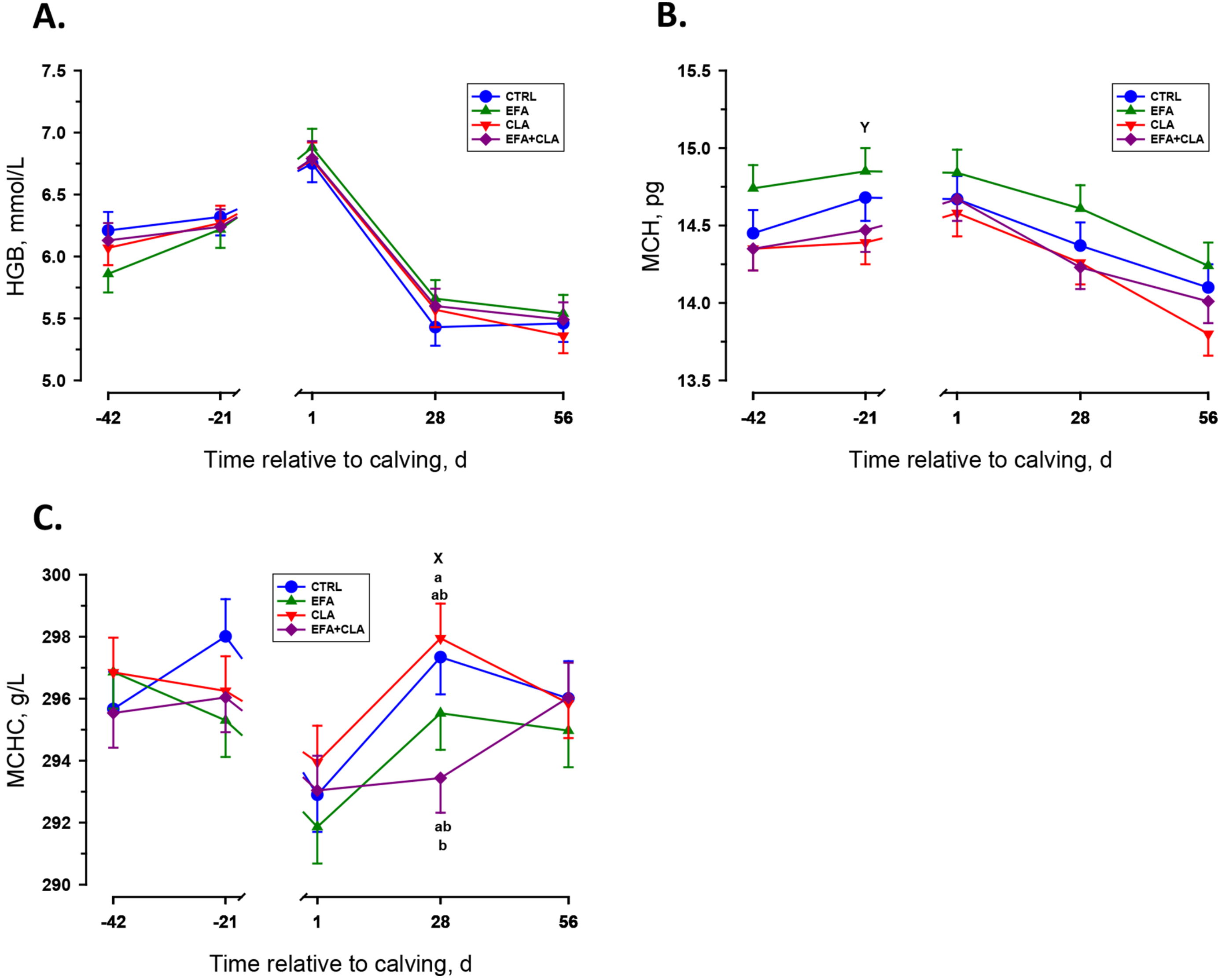

Figure 2. Blood hematological parameters including (A) hemoglobin (HGB, mmol/L), (B) mean corpuscular hemoglobin of erythrocytes (MCH, pg), and (C) mean corpuscular hemoglobin concentration of erythrocytes (MCHC, g/L) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; LSM with different letters (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 HGB, MCH, and MCHC (time).

also been calculated to better understand the oxidative stress status, but this ratio was not affected by time, treatment, or the interaction of the 2 (results not shown). The plasma ORAC concentration was affected by time ($P < 0.05$), with variable changes during late gestation and early lactation (Figure 6D). There were trends for EFA × CLA and EFA × time interactions ($P < 0.1$). Furthermore, the ORAC concentration was lower ($P < 0.05$) in the CLA group than in the CTRL group on d −21 AP and 42 PP and was lower ($P < 0.05$) in EFA+CLA-treated cows than in CTRL cows on d 42 PP. In addition, the plasma ORAC concentration was reduced by CLA treatment ($P < 0.05$) when compared with non-CLA treatment on d −21 AP and 42 PP.

Plasma concentrations of β-carotene, retinol, and tocopherol were affected by time, initially decreasing in all groups toward calving and then continuously increasing again during lactation ($P < 0.001$, Figure 7). The concentration of β-carotene was higher ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows at d −42 and −35 AP (Figure 7A). The plasma retinol concentration was higher ($P < 0.05$) in CLA-treated cows than in EFA-treated cows on d 21 and 56 PP,

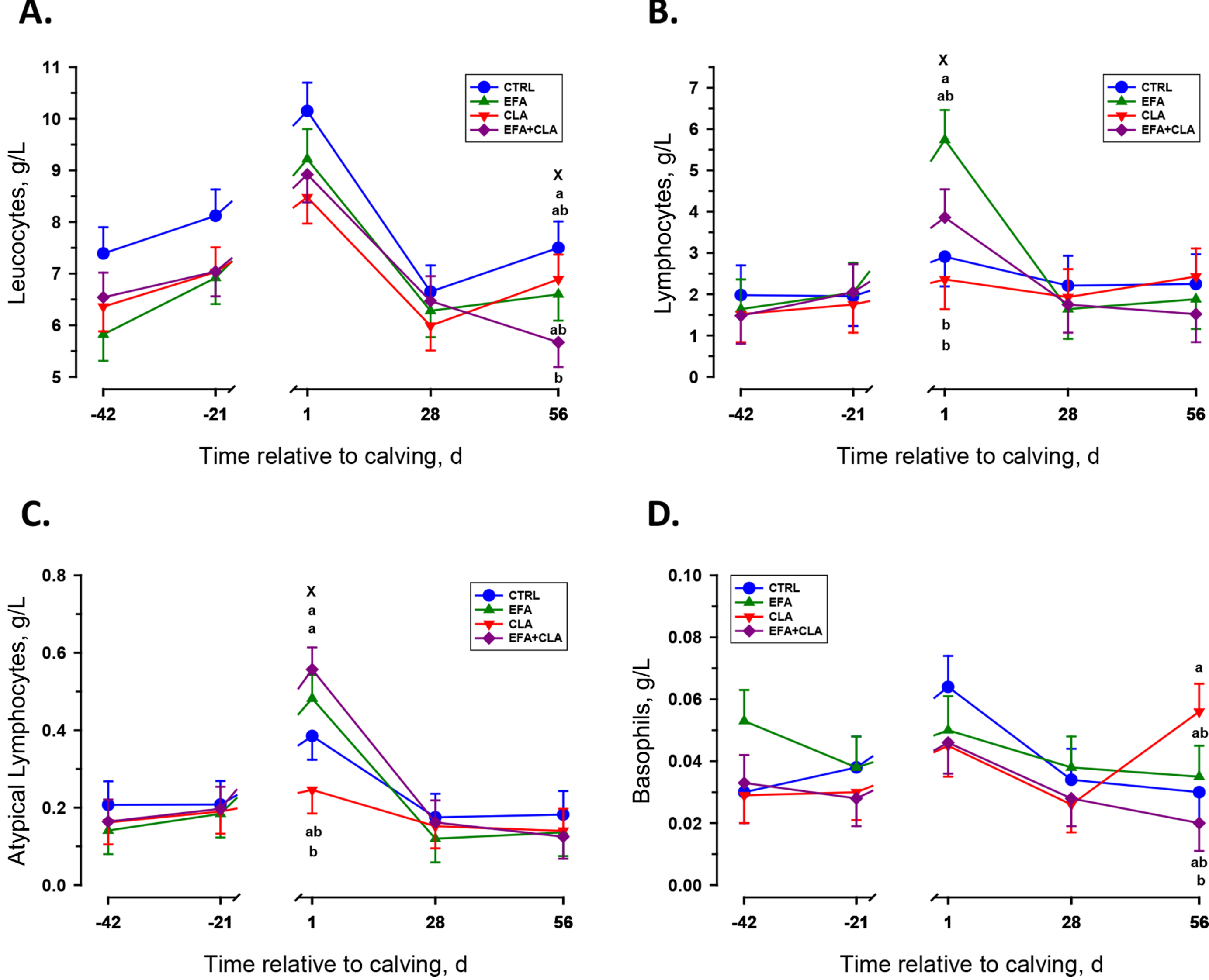

Figure 3. Blood immune cells including (A) leukocytes (g/L), (B) lymphocytes (g/L), (C) atypical lymphocytes (g/L), and (D) basophils (g/L) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; LSM with different letters (a, b) differ ($P < 0.05$) at the respective time point. X = EFA effect at the respective time point. Statistically significant ($P < 0.05$) effects for leukocytes (time), lymphocytes (time), and atypical lymphocytes (time, EFA × time).

was higher ($P < 0.05$) in CLA-treated than non-CLA-treated cows at d −28 and −21 PP, and was lower ($P < 0.05$) in EFA-treated than non-EFA-treated cows at d 21 and 28 PP (Figure 7B). The retinol/β-carotene ratio indicated a significant EFA × CLA interaction during the whole experiment but no time-specific treatment effect (Figure 7C). The retinol/β-carotene ratio tended to be higher ($P < 0.1$) in CLA-treated cows than in CTRL cows at all time points. The plasma tocopherol concentration was lower ($P < 0.05$) in EFA-treated cows than in CTRL cows at d 56 PP and was lower ($P < 0.05$) in CLA-treated than in non-CLA-treated cows at d 1 PP (Figure 7D).

Erythrocyte Markers of Oxidative Status in Transition Dairy Cows Supplemented with EFA and CLA

Figure 8 shows the erythrocyte markers of oxidative status in dairy cows treated with various FA supplements. The concentration of ROM increased ($P < 0.05$) in all groups (Figure 8A). The ROM concentration was

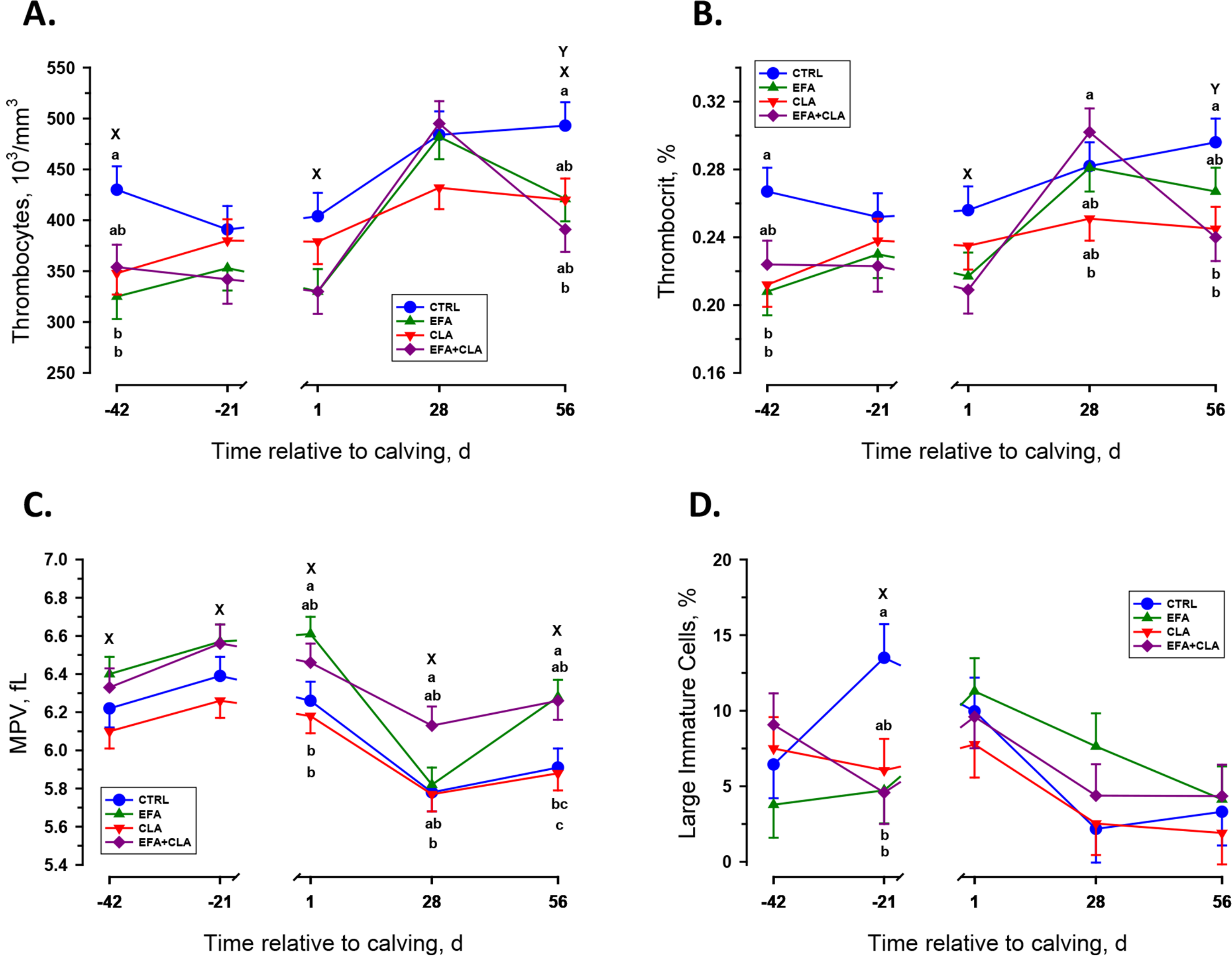

Figure 4. Blood cells and plasma markers including (A) thrombocytes (10^3/mm^3), (B) thrombocrit (%), (C) mean platelet volume (MPV, fL), and (D) large immature cells (%) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; LSM with different letters (a–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 thrombocytes (time, EFA × time), thrombocrit (time, EFA × time), MPV (time, EFA), and large immature cells (time).

not affected by EFA but tended to be reduced with CLA treatment ($P < 0.1$). The GPX-1 concentration tended to be affected by time ($P < 0.1$), with the lowest concentration observed before calving and by CLA treatment (Figure 8B). The activity of SOD-1 was not affected by time or by treatment (Figure 8C). The concentration of MDA was not affected by time but was affected by treatment (Figure 8D). There was a significant EFA × CLA interaction ($P < 0.01$) and a trend ($P < 0.1$) for a CLA effect throughout the experimental period for MDA.

Hepatic mRNA Abundance of Oxidative Status Markers in Transition Dairy Cows Supplemented with EFA and CLA

Figure 9 shows the hepatic relative expression of oxidative markers in response to supplemented FA. The relative expressions of *SOD-1*, *GPX-1*, and *CAT* were affected ($P < 0.05$) over time. On d 28 PP, the mRNA abundance of *GPX-1* and *CAT* was lower ($P < 0.05$) in EFA-treated cows than in non-EFA-treated cows (Figure 9B and C).

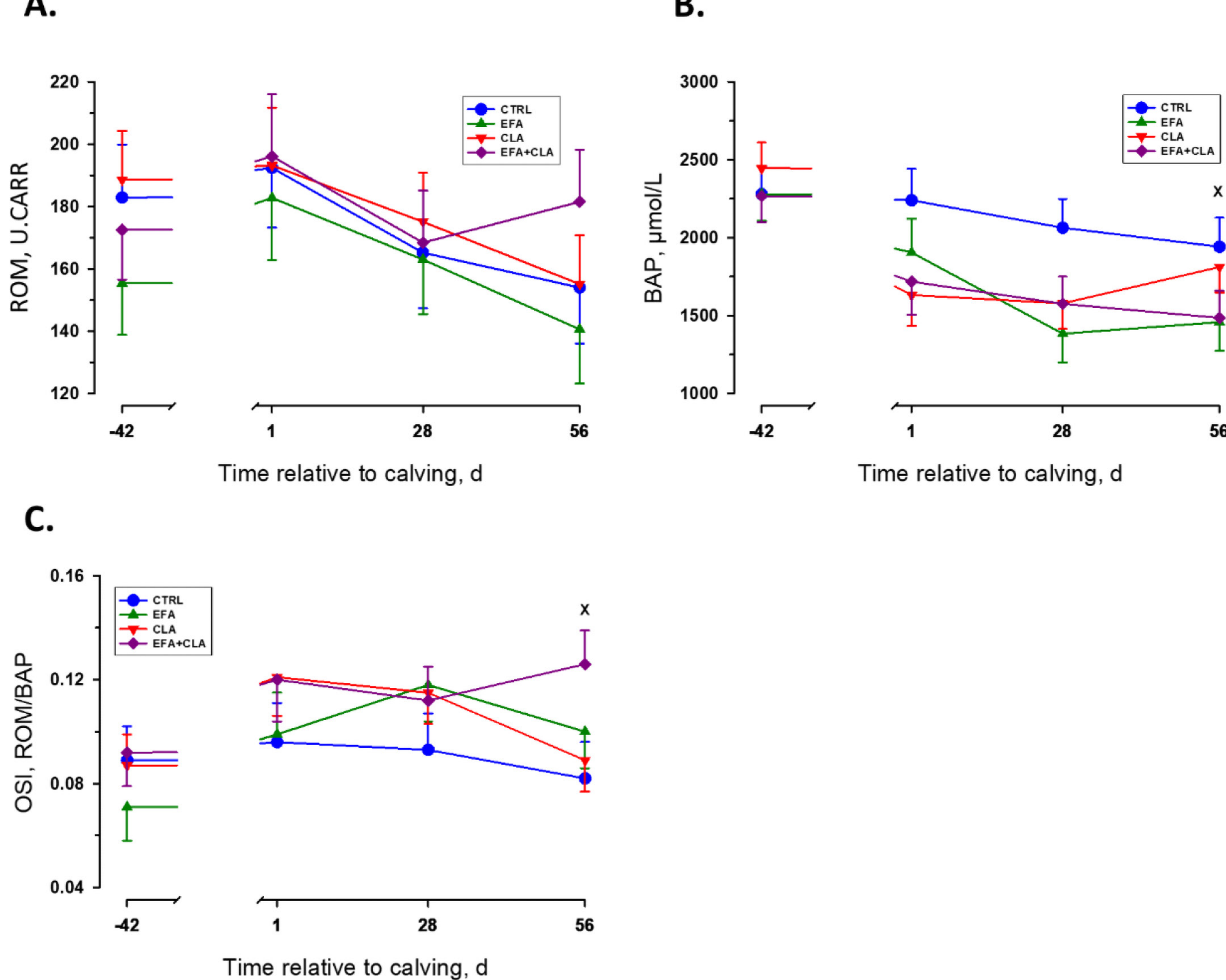

Figure 5. Plasma markers of oxidative status including (A) reactive oxygen metabolites (ROM, Carratelli units: U.CARR), (B) biological antioxidant potential (BAP, μmol/L), and (C) oxidative stress index (OSI: ROM/BAP) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; X = EFA effect at the respective time point. Statistically significant ($P < 0.05$) effects for the concentration of ROM (time), BAP (time, EFA), and OSI (time).

Pearson Correlation of Oxidative Status and Inflammation Markers with Plasma Metabolites

Correlations between oxidative and inflammatory parameters and metabolites are presented in Supplemental Figure S1 https://doi.org/10.5281/zenodo.7539752; Veshkini et al., 2023). In this study, plasma GPX-1 levels (r = −0.38, $P < 0.001$) and erythrocyte SOD-1 (r = −0.6, $P < 0.001$) were negatively correlated with erythrocyte GPX-1, whereas SH was positively correlated (r = 0.47, $P < 0.001$) with erythrocyte GPX-1. There was a positive correlation between tocopherol and high-density lipoprotein (**HDL**; r = 0.58, $P < 0.001$), low-density lipoprotein (**LDL**; r = 0.59, $P < 0.001$), and cholesterol (r = 0.57, $P < 0.001$), as well as between retinol and HDL (r = 0.64, $P < 0.001$), LDL (r = 0.68, $P < 0.001$) and cholesterol (r = 0.6, $P < 0.001$). Additionally, tocopherol (r = 0.7, $P < 0.001$) and retinol (r = 0.48, $P < 0.001$) were positively correlated with β-carotene. The plasma HP level was negatively correlated with retinol (r = −0.62, $P < 0.001$) and tocopherol (r = −0.58, $P < 0.001$) and positively correlated with plasma GPX-1 (r = 0.55, $P < 0.05$). Moreover, there was a positive correlation between retinol and insulin-like growth factor (IGF)-I (r = 0.44) and a negative

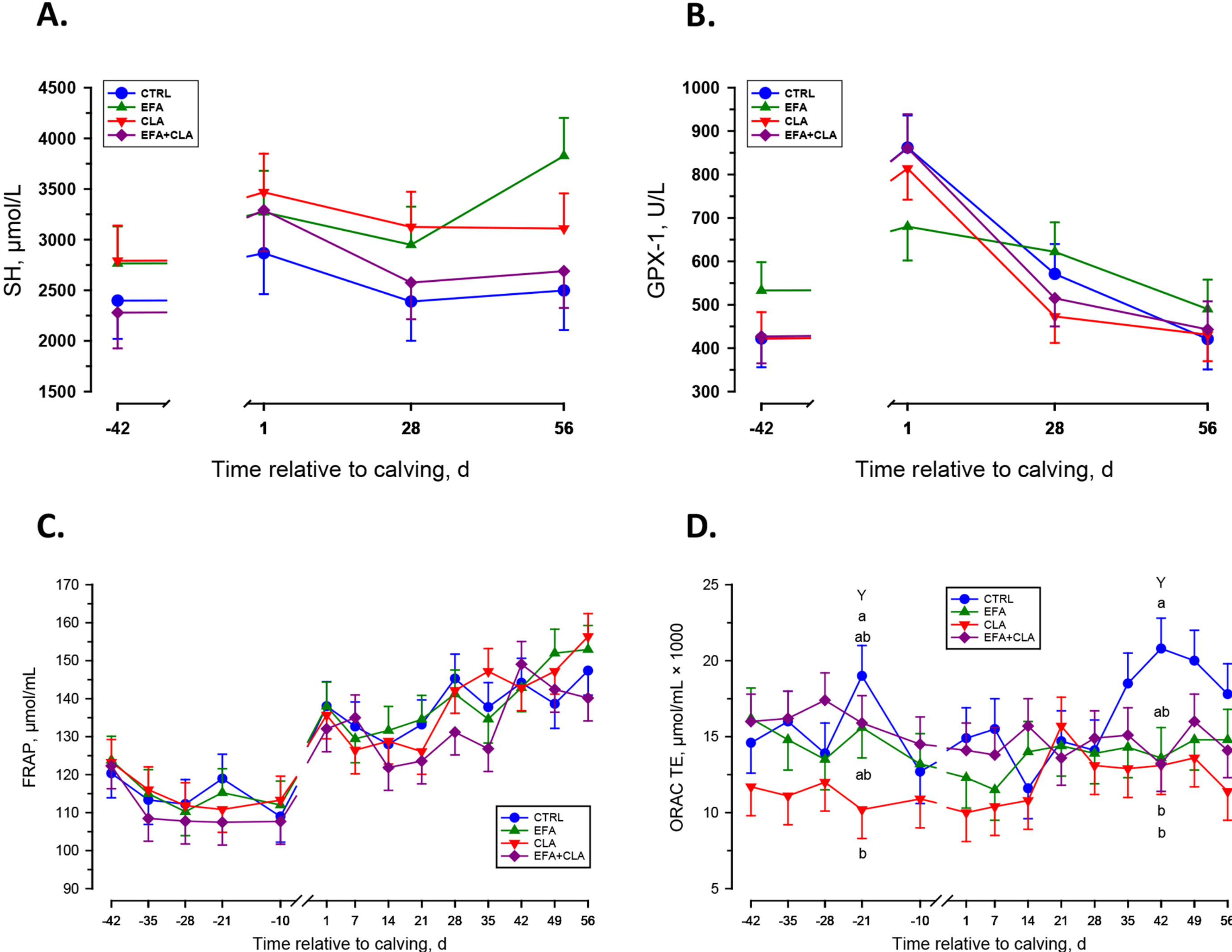

Figure 6. Plasma markers of oxidative status including (A) thiol groups (SH, µmol/L), (B) glutathione peroxidase 1 (GPX-1, U/L), (C) ferric reducing antioxidant power (FRAP, µmol/L), and (D) oxygen radical absorbance capacity (ORAC; TE = trolox equivalent; µmol/L × 1,000) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; Y = CLA effect at the respective time point. a,b: LSM with different letters differ ($P < 0.05$) at the respective time point. Statistically significant ($P < 0.05$) effects for the concentration of SH, GPX-1, FRAP, and ORAC (time).

correlation between β-carotene and SH (r = −0.42, $P < 0.001$).

DISCUSSION

Oxidative stress and inflammation are 2 integral and intimately linked parts of the transition period, which are often compromised in high-yielding dairy cows fed maize silage-based TMR (Trevisi and Minuti, 2018). This study demonstrated the positive correlation between oxidative stress and inflammation during the transition to lactation in dairy cows, although further studies are needed to verify their chronic relationship (or OxInflammation). Supplementing diets with natural anti-inflammatory and antioxidative substances could enhance metabolic and immunological responses and decrease the incidence of disorders and diseases in transition dairy cows. Herein, we investigated the effects of supplementation with EFA (ALA) or CLA, or both, on the markers affecting oxidative stress and hematological markers in transition dairy cows.

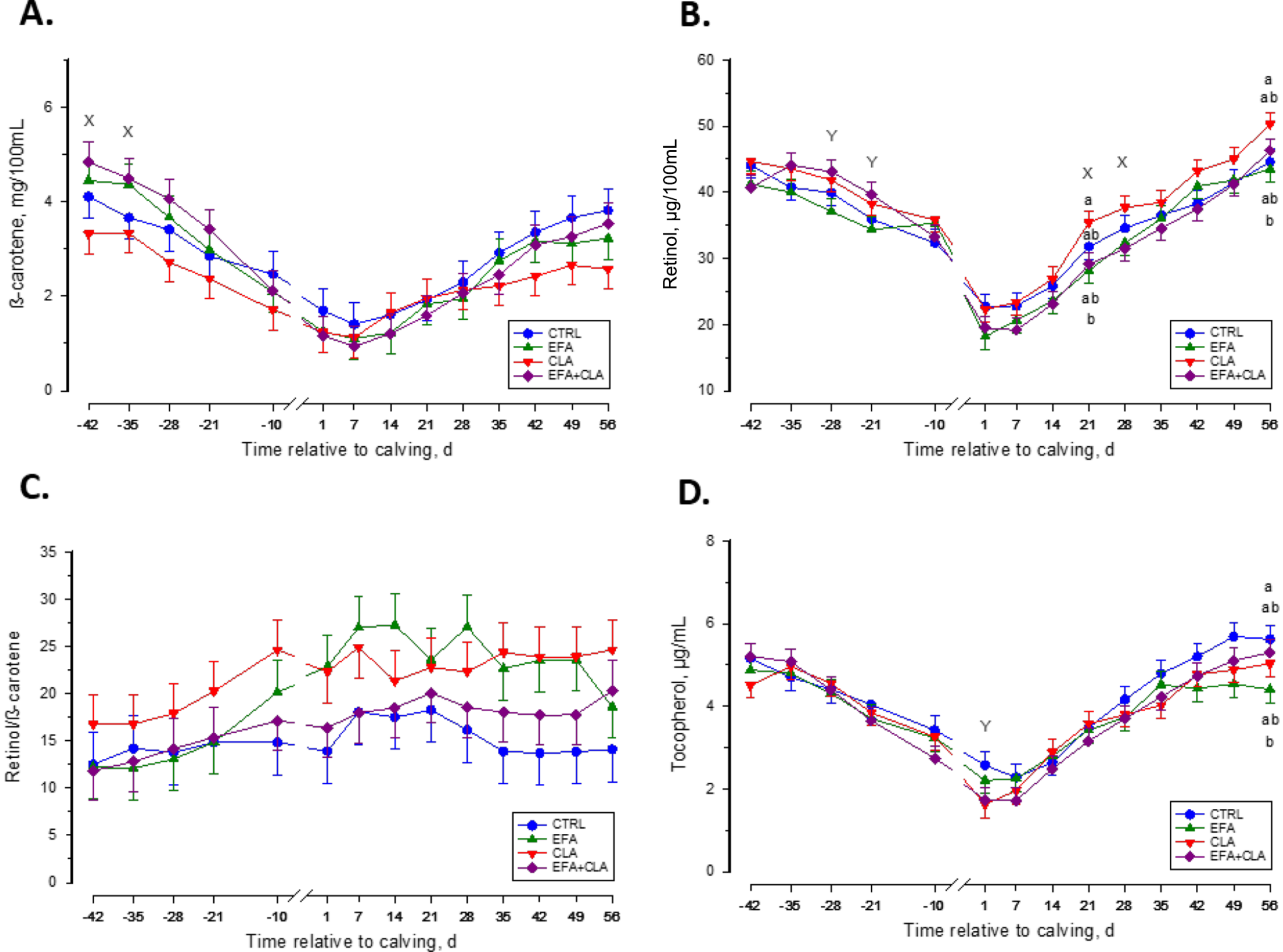

Figure 7. Plasma concentration of lipid-soluble antioxidants consisting of (A) β-carotene (μg/mL), (B) retinol (μg/100 mL), (C) retinol/β-carotene ratio, and (D) tocopherol (μg/mL) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE; LSM with different letters (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 β-carotene (time), retinol (time, EFA), retinol/β-carotene ratio (time, EFA × CLA), and tocopherol (time).

Immunohematological and Oxidative Status of Dairy Cows During the Transition from Pregnancy to Lactation

It should be noted that the obtained hematological results were in the range suggested as reference values for hematological parameters of dairy cows during the transition period (Moretti et al., 2017; Vallejo-Timarán et al., 2020). Plasma HCT and HGB levels, as well as leukocyte and erythrocyte counts, peaked the day after calving but suddenly dropped at d 28 PP. It is not unexpected to observe an erythrocyte peak at d 1 PP because HCT and HGB levels are directly proportional to erythrocyte counts. In accordance, there was a gradual increase in MCV and MCH observed from AP to calving and then a gradual decline from calving to d 56 PP. Higher MCV and MCH reflect higher HGB. A rise in the level of HGB in response to increased oxygen demand in the body stimulates the production of erythrocytes and erythropoietin in kidney tissue (Pittman, 2011). Our results agree with Lamp et al. (2015), who reported decreased HCT at d 22 PP compared with d −21 AP.

The profound immunohematological change around parturition was indicative of systemic inflammation, resulting in erythrocyte release from the spleen the day after calving (Engan and Schagatay, 2015). In healthy individuals, heme is largely intracellular and complexed

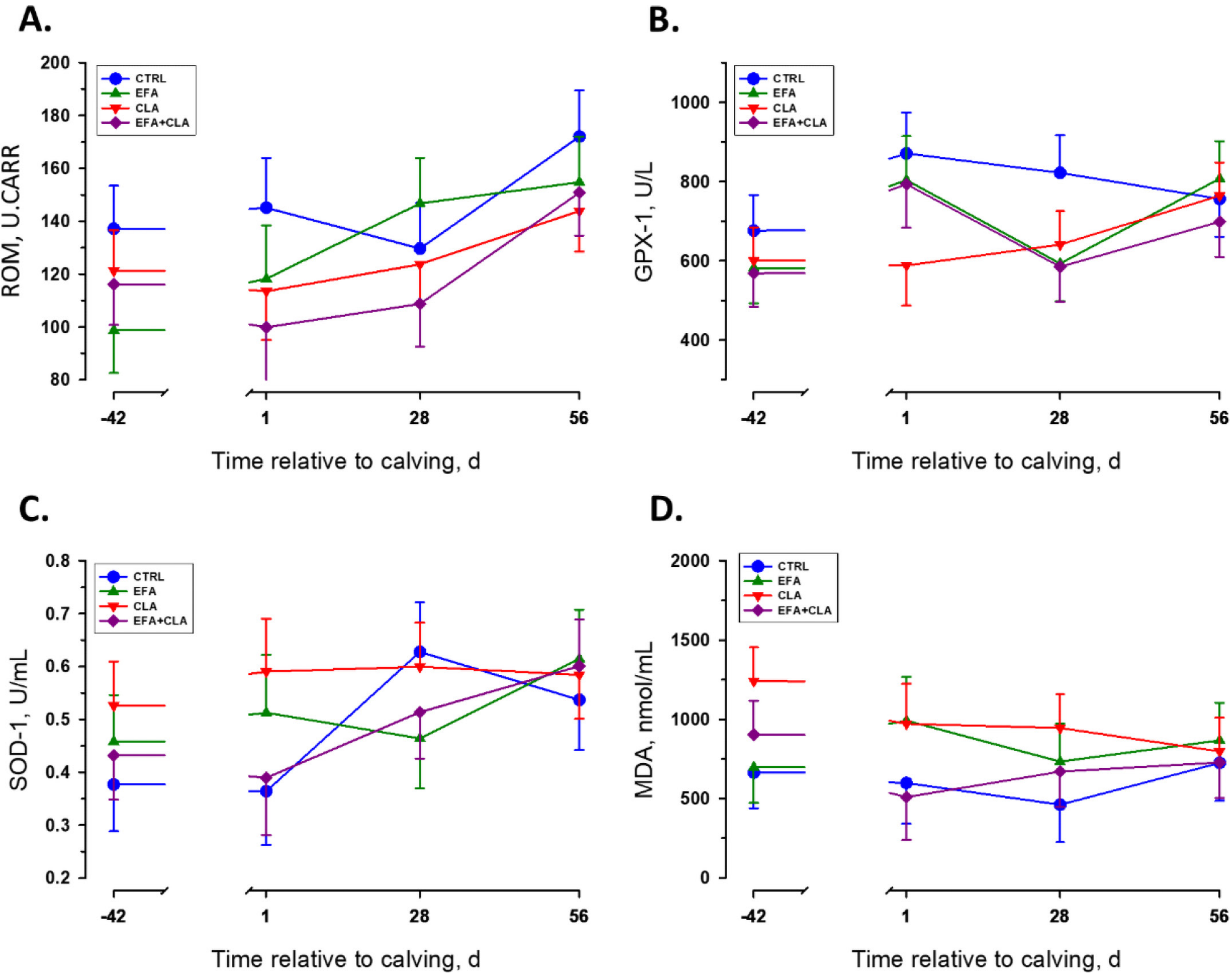

Figure 8. Erythrocyte markers of oxidative status including (A) reactive oxygen metabolites (ROM, Carratelli units: U.CARR), (B) glutathione peroxidase 1 (GPX-1, U/L), (C) superoxide dismutase (SOD-1, U/mL), and (D) malonaldehyde (MDA, nmol/mL) in dairy cows that received fatty acid supplementation (● CTRL, control, n = 9; ▲ EFA, essential fatty acids, n = 9; ▼ CLA, n = 10; ◆ EFA + CLA, n = 10) during the transition from late gestation to early lactation. Data are presented as the LSM ± SE. A statistically significant ($P < 0.05$) effect was observed for the plasma ROM concentration (time) and MDA (EFA × CLA).

to hemoglobin within erythrocytes (Kell and Pretorius, 2018). After erythrocytes are lysed, HGB and heme are released into the circulation, where HP and hemopexin capture them, respectively (Kell and Pretorius, 2018). As previously reported, HP is elevated immediately after parturition in dairy cows, which may contribute to HGB elimination (Gnott et al., 2020). Extracellular HGB has been previously suggested to be an initiator of inflammation in association with injuries (Fang et al., 2013) and diseases (Gram et al., 2013). Spontaneous autooxidation of ferrous (Fe^{2+}) HGB to ferric (Fe^{3+}) HGB, superoxide, and ferryl (Fe^{4+}) HGB releases free heme and ROS, which in turn induce bilirubin generation as a result of heme degradation by heme oxygenase and biliverdin reductase (Gram et al., 2013). HGB and free heme are involved in the humoral compartment of the innate immune system, immunoglobulins, and complement system regulation (Repessé et al., 2012; Anderson et al., 2018), and their accumulation in plasma enhances the production of ROS by activating the Toll-like receptor 4 signaling pathway (Nader et al., 2020). In this regard, we have previously reported that the plasma concentration of bilirubin was at the highest level in early lactation dairy cows (Gnott et al., 2020).

An oxidative stress state is evident at calving by induced ROM and plasma OSI and decreased BAP com-

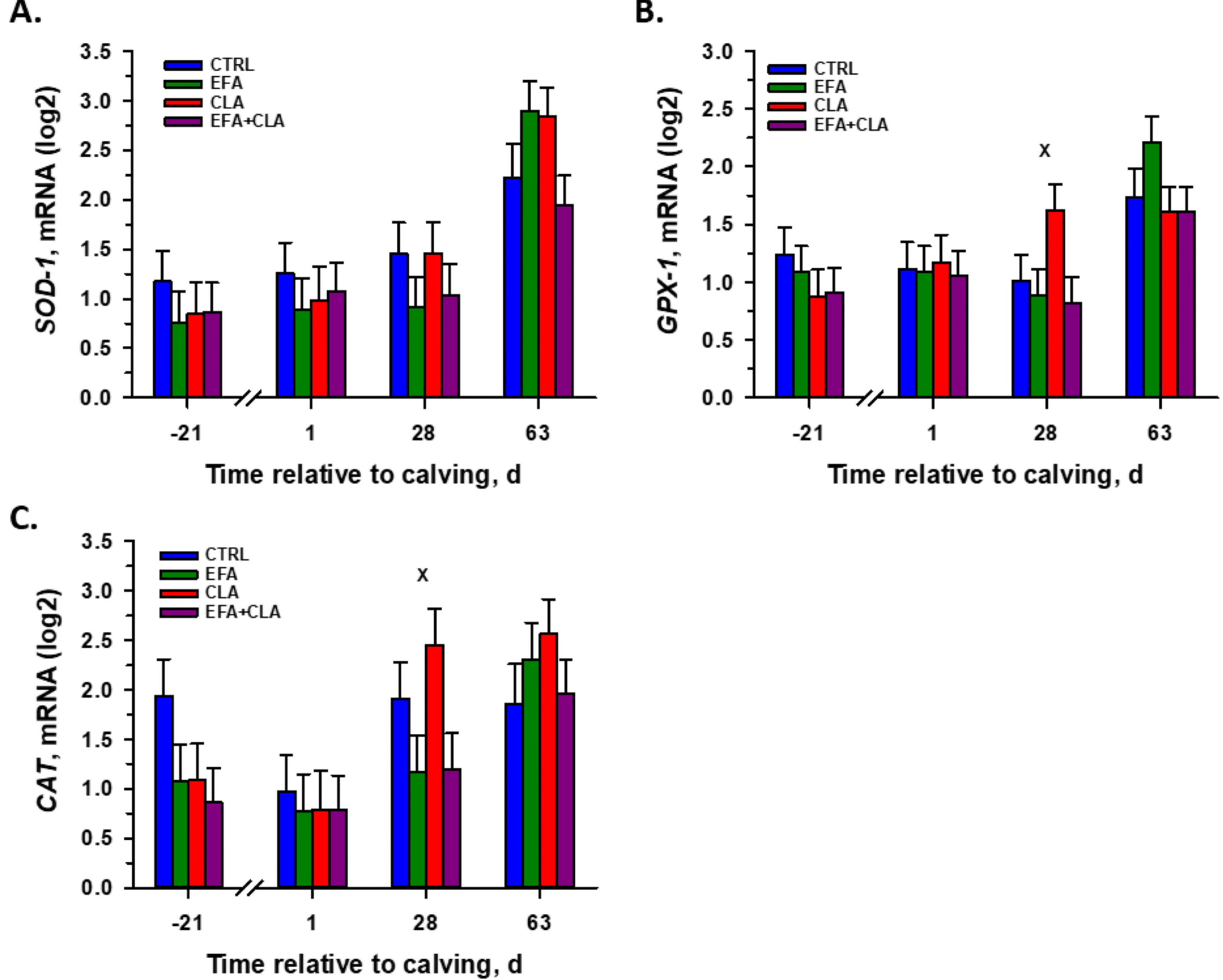

Figure 9. Relative transcript abundance of (A) superoxide dismutase (*SOD-1*), (B) glutathione peroxidase 1 (*GPX-1*), and (C) catalase (*CAT*) in the liver of dairy cows that received fatty acid supplementation (blue bars, CTRL, control, n = 9; green bars, EFA, essential fatty acids, n = 9; red bars, CLA, n = 10; purple bars, EFA + CLA, n = 10) during transition from late gestation to early lactation. Data are presented as the LSM ± SE of fold-change normalized to the geometric mean of the reference gene. X = EFA effect at the respective time point. Statistically significant ($P < 0.05$) effects for the relative hepatic mRNA expression of *SOD-1*, *GPX-1*, and *CAT* (time).

pared with AP. The results are consistent with some of the previous studies reporting induced oxidative stress during early lactation in dairy cows (Bernabucci et al., 2002, 2005; Castillo et al., 2006; Abuelo et al., 2013; Rizzo et al., 2013; Gong and Xiao, 2016; Tsuchiya et al., 2020). There was an increase in the antioxidant indicators FRAP, SH, and GPX-1 in PP compared with AP, partly because of an increase in oxygen status postcalving and partly as a consequence of an altered PP diet. Previously, it was suggested that there is a relationship between bilirubin and FRAP (Liermann et al., 2021). However, bilirubin was not correlated with FRAP in this study but was correlated with plasma GPX-1 (Supplemental Figure S1). Unlike OSI, there was no significant effect of treatment, time, or their interaction on the ROM/FRAP ratio (results not shown). There was no significant correlation between BAP and FRAP assays, which is consistent with previous report that found no statistically significant correlation between the 2 assays in human plasma (Jansen and Ruskovska, 2013).

Erythrocyte GPX-1 increased and stayed constant in PP, whereas ROM gradually increased in PP until d 56. In contrast, MDA and SOD-1 markers were not

affected by time. SOD-1 and GPX-1 are 2 interconnected enzymatic antioxidants (Irato and Santovito, 2021) that protect periparturient cows against excessive oxidative stress. In accordance, Bernabucci et al. (2005, 2009) reported increased plasma GPX-1 as well as erythrocyte SH and SOD-1 around calving compared with the dry-off period, which is associated with a higher degree of oxidative stress at parturition. However, the selected time points are not exactly matched to our experimental design; therefore, a direct comparison cannot be made.

During the early PP period, there was a lower concentration of lipid-soluble antioxidants in the plasma compared with AP. The serum concentrations of retinol (>20 μg/100 mL), tocopherol (>2 mg/100 mL), and β-carotene (>0.3 μg/mL) were within healthy limits, according to McMurray and Rice (1982) and Herdt and Stowe (1991). Decreased concentrations of vitamins might be reflective of reduced feed intake and disrupted lipid metabolism but might also be a result of systemic inflammation. The association of inflammation with antioxidant vitamins has also been established in human studies; for instance, inflammation causes impaired retinol absorption and retinol deficiency (Rubin et al., 2017). The reduction in plasma retinol during inflammation is mainly caused by the impaired synthesis of its carrier (retinol binding protein), as previously observed (Bertoni and Trevisi, 2013), while the reduction in vitamin E and β-carotene during inflammation is partly explained by the reduced synthesis of lipoproteins as the main carriers for their distribution in peripheral tissues (Bertoni et al., 2008).

Time-affected markers support our hypothesis that transition dairy cows were in a state of systemic inflammation and oxidative stress peaking at calving time. This is in line with Horst et al. (2021), who suggested that early lactation dairy cows experience a state of inflammation and immune activation due to tissue damage and remodeling in critical tissues such as the liver, uterus, mammary glands, and adipose tissues at parturition. During parturition, enzymatic antioxidant activity increased, while nonenzymatic antioxidants activity decreased.

Immunohematological and Oxidative Status of Dairy Cows Affected by Abomasal Infusion of EFA or CLA, or Both

Supplementing EFA or CLA, or both, had minor effects on the immunohematological parameters and markers of oxidative status compared with CTRL, but the main differences were observed between the EFA and CLA groups themselves. In agreement, previous studies reported that in vivo proliferation of rats RBC (Nnamonu et al., 2020) and in vitro proliferation of bovine peripheral blood mononuclear cells (Lacetera et al., 2007) were not affected by n-3 FA administration. Moreover, other studies reported that various dosages of CLA did not affect mitogen-stimulated cell proliferation in bovines (Renner et al., 2012) or RBC and white blood cells proliferation in pigs (Bassaganya-Riera et al., 2001). Nevertheless, an in vitro study demonstrated a dose-dependent (20 to 148 μmol/L FA mixture containing palmitic acid, palmitoleic acid, stearic acid, and oleic acid) antiproliferative effect of FA (in general) on bovine PMBC (Renner et al., 2013). In particular, a recent in vitro study showed that CLA (10–500 μM 50:50 mixtures of *trans*-10,*cis*-12, and *cis*-9,*trans*-11 CLA) had immunomodulatory effects and interacted synergistically to reduce apoptosis and increase inflammatory respiratory bursts in bovine monocytes under inflammatory conditions but did not affect chemotaxis, phagocytosis, or killing activity of immune cells (Ávila et al., 2020). Inconsistent results could be explained by the differences in experimental design and in the particular FA dosages.

A lower level of hematological markers was observed in the CLA-treated groups, suggesting that CLA was more effective than EFA at reducing early lactation systemic inflammation and erythrocyte release from the spleen. This was clearly shown by the induction of lower lymphocyte and atypical lymphocyte concentrations in the CLA group than in the EFA group. Moreover, recent research on human patients with coronary artery disease has proposed that mean platelet volume, combined with RDW and platelet count, are predictors of joint inflammation and oxidative stress status (Vukicevic et al., 2021), which also supports lower oxidative stress status in the CLA group compared with the EFA group.

There was no strong evidence that FA treatments altered plasma or RBC oxidative markers, although EFA supplementation compared with non-EFA decreased the relative expression of oxidative markers at the hepatic level in a time-dependent manner. We have previously reported in mid-lactation dairy cows that the plasma markers of oxidative and immune status, including BAP, ROM, ROM/BAP ratio, MDA, FRAP, ORAC, SH, and GPX-1, were not affected by EFA or CLA treatment (Haubold et al., 2020). There is controversy over the effect of EFA on oxidative stress markers. For instance, a previous study showed that a high dosage of flaxseed (5–15%, as a rich source of ALA) supplementation did not alter the plasma concentrations of GPX-1 and CAT in dairy cows (Schogor et al., 2013). However, the authors suggested that flaxseed supplementation can improve the oxidative status of dairy cows through decreased thiobarbituric acid-reactive

substance (**TBARS**) production and the induction of nuclear factor (erythroid-derived 2)-like 2 (**NFE2L2**) transcript abundance in mammary tissue (Schogor et al., 2013). These markers, TBARS and NFE2L2, were not measured in our study. In another study, plasma GPX-1 activity tended to decrease, and the mammary mRNA abundance of *CAT*, *GPX-1*, *GPX-3,* and *SOD-3* was lower in dairy cows infused daily into the abomasum with 500 g of flax oil (Côrtes et al., 2012). In contrast, Ponnampalam et al. (2019) reported that dietary flax meal or flaxseed supplementation in sheep was positively correlated with GPX-1 and SOD-2 gene expression and enzyme activity in muscles. Enhanced antioxidative capacity and cell protection were observed in bovine mammary epithelial cells treated with a 50 μmol/L *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and CLA mixture (50:50 CLA isomers; Basiricò et al., 2015). In a recent study by Ma et al. (2021), the pretreatment of bovine mammary epithelial cells with 50 μmol/L *cis*-9,*trans*-11 CLA reduced the formation of ROS and TBARS and enhanced the expression of antioxidative enzymes such as heme oxygenase 1 and the mRNA expression of NAD(P)H quinone dehydrogenase 1, thioredoxin reductase, and glutathione-disulfide reductase when exposed to LPS-induced oxidative challenge. Furthermore, a very recent study showed that intrajugular infusion of fish oil (rich in EPA and DHA) lowered plasma HP concentrations at d 2 and 3 PP, suggesting an anti-inflammatory effect (Mezzetti et al., 2022). Other plasma inflammation-related biomarkers, including plasma concentrations of bilirubin, aspartate aminotransferase – glutamate oxaloacetate transaminase and γ-glutamyl transferase, alkaline phosphatase, globulin, ceruloplasmin, albumin, paraoxonase, and thiol groups, were not affected by treatment (Mezzetti et al., 2022). These inconsistencies may have been attributed to the FA composition of the basal diet, the FA concentration of the supplements, the duration of the treatment, and the physiological status of the cow and may vary in different tissues. In this regard, a recent study reported that a mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA at the chosen dosage, formulation, and application period had marginal antioxidative activity in lactating dairy cows (Hanschke et al., 2016).

Immediately after calving, the production of oxidative molecules increases dramatically in dairy cows as a consequence of the calving process and the huge increase in milk synthesis (Castillo et al., 2003). In this context, it is important to buffer the oxidative molecules with endogenous and exogenous molecules. From our results, we have not found any improvement in the availability of antioxidant molecules in plasma with EFA and CLA supplements. A trend toward a higher retinol to β-carotene ratio was observed with CLA supplementation in the whole experiment. Nevertheless, CLA treatment seems to have increased circulating retinol levels more quickly, suggesting a better resilience of the liver during the physiological distress of PP. These data support that inflammatory processes in CLA treatment seem less severe and have not impaired the synthesis of common liver proteins such as retinol binding protein, resulting in a higher level of retinol in peripheral tissues (Bertoni et al., 2008; Trevisi and Minuti, 2018). However, CLA treatment did not affect the plasma concentration of albumin that is synthesized in large amounts in the liver (L. Vogel, M. Gnott, and H. M. Hammon, unpublished data). β-Carotene, a precursor of vitamin A, is a lipid-soluble antioxidant capable of quenching ROS and reducing oxidative stress (Nishino et al., 2017). Accordingly, we have previously suggested that EFA treatment may have stabilized the β-carotene plasma concentration to protect against oxidative damage (Haubold et al., 2020). Nevertheless, the current results revealed that it is not effective during early lactation oxidative stress, as EFA-treated groups did not differ in their β-carotene concentrations from the other groups.

One possible explanation for the lower effectiveness of EFA and CLA in the prevention of the oxidative situation around parturition relates to the pathophysiology of lactation and the transition period. According to Mezzetti et al. (2020) and Horst et al. (2021), systemic inflammation around parturition is an integral part of the metabolic and immune adaptations that induce and then further amplify oxidative damage through positive feedback. In the transition period, constant oxidative reagent production is a natural outcome of metabolic adaptation and could explain the ineffectiveness of FA supplementation in reducing oxidative markers. The validity of this hypothesis will, however, require further studies under specific experimental designs.

CONCLUSIONS

Collectively, the results revealed that early lactation dairy cows were in an oxidative stress state as an integral part of metabolic and immune adaptation that disappeared after a few weeks of lactation. Oxidative stress and inflammatory markers showed a strong positive correlation. In terms of immunohematological and oxidative status markers, EFA and CLA had a minor time-dependent effect. Compared with CLA, supplementation with EFA increased immunohematological peak levels at the onset of lactation and induced lower expression of antioxidant markers in the liver during the PP period. Despite time-dependent differences between groups, we found no strong evidence that EFA or CLA supplementation improved antioxidant and im-

munohematology parameters in dairy cows during the transition period.

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REFERENCES

Abou-Rjeileh, U., and G. A. Contreras. 2021. Redox regulation of lipid mobilization in adipose tissues. Antioxidants 10:1090. https://doi.org/10.3390/antiox10071090.

Abuelo, A., J. Hernández, J. L. Benedito, and C. Castillo. 2013. Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 7:1374–1378. https://doi.org/10.1017/S1751731113000396.

Abuelo, A., J. Hernández, J. L. Benedito, and C. Castillo. 2019. Redox biology in transition periods of dairy cattle: Role in the health of periparturient and neonatal animals. Antioxidants 8:20. https://doi.org/10.3390/antiox8010020.

Alam, S.-I., M.-W. Kim, F. A. Shah, K. Saeed, R. Ullah, and M.-O. Kim. 2021. Alpha-linolenic acid impedes cadmium-induced oxidative stress, neuroinflammation, and neurodegeneration in mouse brain. Cells 10:2274. https://doi.org/10.3390/cells10092274.

Anderson, H. L., I. E. Brodsky, and N. S. Mangalmurti. 2018. The evolving erythrocyte: Red blood cells as modulators of innate immunity. J. Immunol. 201:1343–1351. https://doi.org/10.4049/jimmunol.1800565.

Ávila, G., C. Catozzi, D. Pravettoni, G. Sala, P. Martino, G. Meroni, C. Lecchi, and F. Ceciliani. 2020. In vitro effects of conjugated linoleic acid (CLA) on inflammatory functions of bovine monocytes. J. Dairy Sci. 103:8554–8563. https://doi.org/10.3168/jds.2020-18659.

Basiricò, L., P. Morera, D. Dipasquale, A. Tröscher, A. Serra, M. Mele, and U. Bernabucci. 2015. Conjugated linoleic acid isomers strongly improve the redox status of bovine mammary epithelial cells (BME-UV1). J. Dairy Sci. 98:7071–7082. https://doi.org/10.3168/jds.2015-9787.

Bassaganya-Riera, J., R. Hontecillas-Magarzo, K. Bregendahl, M. Wannemuehler, and D. Zimmerman. 2001. Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. J. Anim. Sci. 79:714–721. https://doi.org/10.2527/2001.793714x.

Benzie, I. F., and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239:70–76. https://doi.org/10.1006/abio.1996.0292.

Bernabucci, U., L. Basiricò, D. Pirazzi, F. Rueca, N. Lacetera, E. Lepri, and A. Nardone. 2009. Liver apolipoprotein B 100 expression and secretion are down-regulated early postpartum in dairy cows. Livest. Sci. 125:169–176. https://doi.org/10.1016/j.livsci.2009.04.006.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci. 85:2173–2179. https://doi.org/10.3168/jds.S0022-0302(02)74296-3.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017–2026. https://doi.org/10.3168/jds.S0022-0302(05)72878-2.

Bertoni, G., and E. Trevisi. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Vet. Clin. North Am. Food Anim. Pract. 29:413–431. https://doi.org/10.1016/j.cvfa.2013.04.004.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300–3310. https://doi.org/10.3168/jds.2008-0995.

Bionaz, M., E. Vargas-Bello-Pérez, and S. Busato. 2020. Advances in fatty acids nutrition in dairy cows: From gut to cells and effects on performance. J. Anim. Sci. Biotechnol. 11:110. https://doi.org/10.1186/s40104-020-00512-8.

Birben, E., U. M. Sahiner, C. Sackesen, S. Erzurum, and O. Kalayci. 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9–19. https://doi.org/10.1097/WOX.0b013e3182439613.

Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele, and C. T. Wittwer. 2009. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55:611–622. https://doi.org/10.1373/clinchem.2008.112797.

Cao, G., and R. L. Prior. 1999. Measurement of oxygen radical absorbance capacity in biological samples. Methods Enzymol. 299:50–62. https://doi.org/10.1016/S0076-6879(99)99008-0.

Castillo, C., J. Hernández, M. López-Alonso, M. Miranda, and J. Luís. 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: Preliminary observations. Arch. Tierzucht 46:227–233. https://doi.org/10.5194/aab-46-227-2003.

Castillo, C., J. Hernández, I. Valverde, V. Pereira, J. Sotillo, M. L. Alonso, and J. L. Benedito. 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res. Vet. Sci. 80:133–139. https://doi.org/10.1016/j.rvsc.2005.06.003.

Cesarone, M. R., G. Belcaro, M. Carratelli, U. Cornelli, M. T. De Sanctis, L. Incandela, A. Barsotti, R. Terranova, and A. Nicolaides. 1999. A simple test to monitor oxidative stress. Int. Angiol. 18:127–30. https://doi.org/10.1016/j.rvsc.2005.06.003.

Côrtes, C., M. F. Palin, N. Gagnon, C. Benchaar, P. Lacasse, and H. V. Petit. 2012. Mammary gene expression and activity of antioxidant enzymes and concentration of the mammalian lignan enterolactone in milk and plasma of dairy cows fed flax lignans and infused with flax oil in the abomasum. Br. J. Nutr. 108:1390–1398. https://doi.org/10.1017/S0007114511006829.

Dipasquale, D., L. Basiricò, P. Morera, R. Primi, A. Tröscher, and U. Bernabucci. 2018. Anti-inflammatory effects of conjugated linoleic acid isomers and essential fatty acids in bovine mammary epithelial cells. Animal 12:2108–2114. https://doi.org/10.1017/S1751731117003676.

DLG (Deutsche Landwirtschafts-Gesellschaft, German Agricultural Society). 2013. Leitfaden zur Berechnung des Energiegehaltes bei Einzel-und Mischfuttermitteln für die Schweine-und Rinderfutter-

ung (Guidelines for calculation of energy content of single and mixed feedstuff for pigs and cattle). Stellungnahme des DLG-Arbeitskreises Futter und Futterung.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259–2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3.

Elgersma, A. 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges, and future perspectives. Eur. J. Lipid Sci. Technol. 117:1345–1369. https://doi.org/10.1002/ejlt.201400469.

Engan, H., and E. Schagatay. 2015. "Spleen contraction and hemoconcentration" regarding the review "Hemoconcentration and hemostasis during acute stress: Interacting and independent effects" by Austin et al. 2011. Ann. Behav. Med. 49:634–635. https://doi.org/10.1007/s12160-015-9707-2.

Essid, E., Y. Dernawi, and E. Petzinger. 2012. Apoptosis induction by OTA and TNF-α in cultured primary rat hepatocytes and prevention by silibinin. Toxins (Basel) 4:1139–1156. https://doi.org/10.3390/toxins4111139.

Fang, H., P.-F. Wang, Y. Zhou, Y.-C. Wang, and Q.-W. Yang. 2013. Toll-like receptor 4 signaling in intracerebral hemorrhage-induced inflammation and injury. J. Neuroinflammation 10:794. https://doi.org/10.1186/1742-2094-10-27.

Fontes, J. D., F. Rahman, S. Lacey, M. G. Larson, R. S. Vasan, E. J. Benjamin, W. S. Harris, and S. J. Robins. 2015. Red blood cell fatty acids and biomarkers of inflammation: A cross-sectional study in a community-based cohort. Atherosclerosis 240:431–436. https://doi.org/10.1016/j.atherosclerosis.2015.03.043.

GFE Gesellschaft für Ernährungsphysiologie (German Society of Nutrition Physiology). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder (Recommended energy and nutrient supply of dairy cows and growing cattle). Vol. 8. DLGVerlag.

GFE Gesellschaft für Ernährungsphysiologie (German Society of Nutrition Physiology). 2008. New equations for predicting metabolisable energy of grass and maize products for ruminants. Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology. Proc. Soc. Nutr. Physiol. 17:191–198.

GFE Gesellschaft für Ernährungsphysiologie (German Society of Nutrition Physiology). 2009. New equations for predicting metabolisable energy of compound feeds for cattle. Communications of the Committee for Requirement Standards of the Society of Nutrition Physiology. Proc. Soc. Nutr. Physiol. 18:143–146.

Gnott, M., L. Vogel, C. Kröger-Koch, D. Dannenberger, A. Tuchscherer, A. Tröscher, E. Trevisi, T. Stefaniak, J. Bajzert, A. Starke, M. Mielenz, L. Bachmann, and H. M. Hammon. 2020. Changes in fatty acids in plasma and association with the inflammatory response in dairy cows abomasally infused with essential fatty acids and conjugated linoleic acid during late and early lactation. J. Dairy Sci. 103:11889–11910. https://doi.org/10.3168/jds.2020-18735.

Gong, J., and M. Xiao. 2016. Selenium and antioxidant status in dairy cows at different stages of lactation. Biol. Trace Elem. Res. 171:89–93. https://doi.org/10.1007/s12011-015-0513-2.

Gram, M., S. Sveinsdottir, K. Ruscher, S. R. Hansson, M. Cinthio, B. Åkerström, and D. Ley. 2013. Hemoglobin induces inflammation after preterm intraventricular hemorrhage by methemoglobin formation. J. Neuroinflammation 10:867. https://doi.org/10.1186/1742-2094-10-100.

Hanschke, N., M. Kankofer, L. Ruda, M. Höltershinken, U. Meyer, J. Frank, S. Dänicke, and J. Rehage. 2016. The effect of conjugated linoleic acid supplements on oxidative and antioxidative status of dairy cows. J. Dairy Sci. 99:8090–8102. https://doi.org/10.3168/jds.2015-10685.

Haubold, S., C. Kröger-Koch, A. Starke, A. Tuchscherer, A. Tröscher, H. Kienberger, M. Rychlik, U. Bernabucci, E. Trevisi, and H. M. Hammon. 2020. Effects of abomasal infusion of essential fatty acids and conjugated linoleic acid on performance and fatty acid, antioxidative, and inflammatory status in dairy cows. J. Dairy Sci. 103:972–991. https://doi.org/10.3168/jds.2019-17135.

Herdt, T. H., and H. D. Stowe. 1991. Fat-soluble vitamin nutrition for dairy cattle. Vet. Clin. North Am. Food Anim. Pract. 7:391–415. https://doi.org/10.1016/S0749-0720(15)30796-9.

Horst, E. A., S. K. Kvidera, and L. H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance—A critical evaluation of traditional dogmas. J. Dairy Sci. 104:8380–8410. https://doi.org/10.3168/jds.2021-20330.

Irato, P., and G. Santovito. 2021. Enzymatic and non-enzymatic molecules with antioxidant function. Antioxidants 10:579. https://doi.org/10.3390/antiox10040579.

Jansen, E. H., and T. Ruskovska. 2013. Comparative analysis of serum (anti)oxidative status parameters in healthy persons. Int. J. Mol. Sci. 14:6106–6115. https://doi.org/10.3390/ijms14036106.

Kaska, L., A. Mika, P. Stepnowski, M. Proczko, K. Ratnicki-Sklucki, T. Sledzinski, E. Goyke, and J. Swierczynski. 2014. The relationship between specific fatty acids of serum lipids and serum high sensitivity C-reactive protein levels in morbidly obese women. Cell. Physiol. Biochem. 34:1101–1108. https://doi.org/10.1159/000366324.

Kell, D. B., and E. Pretorius. 2018. No effects without causes: The iron dysregulation and dormant microbes hypothesis for chronic, inflammatory diseases. Biol. Rev. Camb. Philos. Soc. 93:1518–1557. https://doi.org/10.1111/brv.12407.

La Terra, S., V. M. Marino, M. Manenti, G. Licitra, and S. Carpino. 2010. Increasing pasture intakes enhances polyunsaturated fatty acids and lipophilic antioxidants in plasma and milk of dairy cows fed total mix ration. Dairy Sci. Technol. 90:687–698. https://doi.org/10.1051/dst/2010100.

Lacetera, N., G. Kuzminsky, P. Morera, and L. Basiricò. 2007. Fatty acids affect proliferation of peripheral blood mononuclear cells in dairy cows. Ital. J. Anim. Sci. 6 (Suppl.):434–436. https://doi.org/10.4081/ijas.2007.1s.434.

Lamp, O., M. Derno, W. Otten, M. Mielenz, G. Nürnberg, and B. Kuhla. 2015. Metabolic heat stress adaption in transition cows: differences in macronutrient oxidation between late-gestating and early-lactating German Holstein dairy cows. PLoS One 10:e0125264. https://doi.org/10.1371/journal.pone.0125264.

Liermann, W., K. L. Uken, C. Schäff, L. Vogel, M. Gnott, A. Tuchscherer, E. Trevisi, T. Stefaniak, H. Sauerwein, A. Tröscher, and H. M. Hammon. 2021. Effects of a maternal essential fatty acid and conjugated linoleic acid supplementation during late pregnancy and early lactation on hematologic and immunological traits and the oxidative and anti-oxidative status in blood plasma of neonatal calves. Animals (Basel) 11:2168. https://doi.org/10.3390/ani11082168.

Lopreiato, V., M. Mezzetti, L. Cattaneo, G. Ferronato, A. Minuti, and E. Trevisi. 2020. Role of nutraceuticals during the transition period of dairy cows: A review. J. Anim. Sci. Biotechnol. 11:96. https://doi.org/10.1186/s40104-020-00501-x.

Ma, N., G. Wei, H. Zhang, H. Dai, A. C. Roy, X. Shi, G. Chang, and X. Shen. 2021. *Cis*-9, *trans*-11 CLA alleviates lipopolysaccharide-induced depression of fatty acid synthesis by inhibiting oxidative stress and autophagy in bovine mammary epithelial cells. Antioxidants 11:55. https://doi.org/10.3390/antiox11010055.

McMurray, C. H., and D. A. Rice. 1982. Vitamin E and selenium deficiency diseases. Ir. Vet. J. 36:57–67.

Mezzetti, M., M. Bionaz, and E. Trevisi. 2020. Interaction between inflammation and metabolism in periparturient dairy cows. J. Anim. Sci. 98:155–174. https://doi.org/10.1093/jas/skaa134.

Mezzetti, M., F. Piccioli-Cappelli, A. Minuti, and E. Trevisi. 2022. Effects of an intravenous infusion of emulsified fish oil rich in long-chained omega-3 fatty acids on plasma total fatty acids profile, metabolic conditions, and performances of postpartum dairy cows during the early lactation. Front. Vet. Sci. 9. https://doi.org/10.3389/fvets.2022.870901.

Moretti, P., S. Paltrinieri, E. Trevisi, M. Probo, A. Ferrari, A. Minuti, and A. Giordano. 2017. Reference intervals for hematological and biochemical parameters, acute phase proteins, and markers of oxidation in Holstein dairy cows around 3 and 30 days after calving. Res. Vet. Sci. 114:322–331. https://doi.org/10.1016/j.rvsc.2017.06.012.

Moscovici Joubran, A., K. M. Pierce, N. Garvey, L. Shalloo, and T. F. O'Callaghan. 2021. Invited review: A 2020 perspective on pasture-based dairy systems and products. J. Dairy Sci. 104:7364–7382. https://doi.org/10.3168/jds.2020-19776.

Nader, E., M. Romana, and P. Connes. 2020. The red blood cell—Inflammation vicious circle in sickle cell disease. Front. Immunol. 11:454. https://doi.org/10.3389/fimmu.2020.00454.

Nishino, A., H. Yasui, and T. Maoka. 2017. Reaction and scavenging mechanism of β-carotene and zeaxanthin with reactive oxygen species. J. Oleo Sci. 66:77–84. https://doi.org/10.5650/jos.ess16107.

Nnamonu, E. I., B. O. Mgbenka, B. I. Ezewudo, E. C. Mbegbu, C. S. Ezechukwu, and G. C. Ugwu. 2020. Omega-3 fatty acids as feed supplement modulates blood formation and body weight in *Rattus norvegicus* model. J. Basic Appl. Zool. 81:14. https://doi.org/10.1186/s41936-020-00155-1.

Opgenorth, J., L. M. Sordillo, and M. J. VandeHaar. 2020. Colostrum supplementation with n-3 fatty acids and α-tocopherol alters plasma polyunsaturated fatty acid profile and decreases an indicator of oxidative stress in newborn calves. J. Dairy Sci. 103:3545–3553. https://doi.org/10.3168/jds.2019-17380.

Pittman, R. N. 2011. Integrated systems physiology: From molecule to function to disease. Pages 59–78 in Regulation of Tissue Oxygenation. Morgan and Claypool. https://doi.org/10.4199/C00029ED1V01Y201103ISP017.

Ponnampalam, E. N., V. Vahedi, K. Giri, P. Lewandowski, J. L. Jacobs, and F. R. Dunshea. 2019. Muscle antioxidant enzymes activity and gene expression are altered by diet-induced increase in muscle essential fatty acid (α-linolenic acid) concentration in sheep used as a model. Nutrients 11:723. https://doi.org/10.3390/nu11040723.

Renner, L., S. Kersten, A. Duevel, H.-J. Schuberth, and S. Dänicke. 2013. Effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid, linoleic acid, phytanic acid, and the combination of various fatty acids on proliferation and cytokine expression of bovine peripheral blood mononuclear cells. Nutrients 5:2667–2683. https://doi.org/10.3390/nu5072667.

Renner, L., J. Pappritz, R. Kramer, S. Kersten, G. Jahreis, and S. Dänicke. 2012. Fatty acid profile and proliferation of bovine blood mononuclear cells after conjugated linoleic acid supplementation. Lipids Health Dis. 11:63. https://doi.org/10.1186/1476-511X-11-63.

Repessé, Y., J. D. Dimitrov, I. Peyron, F. Moshai, L. Kiger, S. Dasgupta, S. Delignat, M. C. Marden, S. V. Kaveri, and S. Lacroix-Desmazes. 2012. Heme binds to factor VIII and inhibits its interaction with activated factor IX. J. Thromb. Haemost. 10:1062–1071. https://doi.org/10.1111/j.1538-7836.2012.04724.x.

Revskij, D., S. Haubold, T. Viergutz, C. Kröger-Koch, A. Tuchscherer, H. Kienberger, M. Rychlik, A. Tröscher, H. M. Hammon, H.-J. Schuberth, and M. Mielenz. 2019. Dietary fatty acids affect red blood cell membrane composition and red blood cell ATP release in dairy cows. Int. J. Mol. Sci. 20:2769. https://doi.org/10.3390/ijms20112769.

Rizzo, A., E. Ceci, M. Pantaleo, M. Mutinati, M. Spedicato, G. Minoia, and R. L. Sciorsci. 2013. Evaluation of blood and milk oxidative status during early postpartum of dairy cows. Animal 7:118–123. https://doi.org/10.1017/S1751731112001048.

Rubin, L. P., A. C. Ross, C. B. Stephensen, T. Bohn, and S. A. Tanumihardjo. 2017. Metabolic effects of inflammation on vitamin A and carotenoids in humans and animal models. Adv. Nutr. 8:197–212. https://doi.org/10.3945/an.116.014167.

Schogor, A. L. B., M.-F. Palin, G. T. dos Santos, C. Benchaar, P. Lacasse, and H. V. Petit. 2013. Mammary gene expression and activity of antioxidant enzymes and oxidative indicators in the blood, milk, mammary tissue, and ruminal fluid of dairy cows fed flax meal. Br. J. Nutr. 110:1743–1750. https://doi.org/10.1017/S0007114513001220.

Trevisi, E., and A. Minuti. 2018. Assessment of the innate immune response in the periparturient cow. Res. Vet. Sci. 116:47–54. https://doi.org/10.1016/j.rvsc.2017.12.001.

Tsuchiya, Y., N. Kawahara, Y.-H. Kim, T. Ichijo, and S. Sato. 2020. Changes in oxidative stress parameters in healthy and diseased Holstein cows during the transition period in Yamagata Prefecture, Japan. J. Vet. Med. Sci. 82:955–961. https://doi.org/10.1292/jvms.20-0024.

Valacchi, G., F. Virgili, C. Cervellati, and A. Pecorelli. 2018. Oxinflammation: From subclinical condition to pathological biomarker. Front. Physiol. 9:858. https://doi.org/10.3389/fphys.2018.00858.

Vallejo-Timarán, D., J. Montoya-Zuluaga, V. Castillo-Vanegas, and J. Maldonado-Estrada. 2020. Parity and season affect hematological, biochemical, and milk parameters during the early postpartum period in grazing dairy cows from high-tropics herds. Heliyon 6:e04049. https://doi.org/10.1016/j.heliyon.2020.e04049.

Veshkini, A., M. Gnott, L. Vogel, C. Kröger-Koch, A. Tuchscherer, A. Tröscher, U. Bernabucci, E. Trevisi, A. Starke, M. Mielenz, L. Bachmann, and H. M. Hammon. 2023. Supplementary data to publication, Abomasal infusion of essential fatty acids and conjugated linoleic acid during late pregnancy and early lactation affects immunohematological and oxidative stress markers in dairy cows. Data set. Zenodo. https://doi.org/10.5281/zenodo.7539752.

Vogel, L., M. Gnott, C. Kroger-Koch, D. Dannenberger, A. Tuchscherer, A. Troscher, H. Kienberger, M. Rychlik, A. Starke, L. Bachmann, and H. M. Hammon. 2020. 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. J. Dairy Sci. 103:7431–7450. https://doi.org/10.3168/jds.2019-18065.

Vukicevic, P., A. Klisic, V. Neskovic, L. Babic, A. Mikic, N. Bogavac-Stanojevic, M. Matkovic, S. Putnik, N. Aleksic, and J. Kotur-Stevuljevic. 2021. New markers of platelet activation and reactivity and oxidative stress parameters in patients undergoing coronary artery bypass grafting. Oxid. Med. Cell. Longev. 2021:8915253. https://doi.org/10.1155/2021/8915253.

Wang, X., T. Wu, S. Yan, B. Shi, Y. Zhang, and X. Guo. 2019. Influence of pasture or total mixed ration on fatty acid composition and expression of lipogenic genes of longissimus thoracis and subcutaneous adipose tissues in albas white cashmere goats. Ital. J. Anim. Sci. 18:111–123. https://doi.org/10.1080/1828051X.2018.1490632.

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