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The effect of heat treatment on colostral and newborn calf redox status and oxylipid biomarkers

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ABSTRACT

Newborn calves experience altered redox balance upon transition to extrauterine life. In addition to its nutritional value, colostrum is rich in bioactive factors, including pro- and antioxidants. The objective was to investigate differences in pro- and antioxidants as well as oxidative markers in raw and heat-treated (HT) colostrum and in the blood of calves fed either raw or HT colostrum. Eleven colostrum samples (≥8 L) of Holstein cows were each divided into a raw or HT (60°C, 60 min) portion. Both treatments were stored for <24 h at 4°C and tube-fed in a randomized-paired design at 8.5% of body weight to 22 newborn female Holstein calves within 1 h after birth. Colostrum samples were obtained before feeding, and calf blood samples were taken immediately before feeding (0 h) and at 4, 8, and 24 h after feeding. All samples were analyzed for reactive oxygen and nitrogen species (RONS) and antioxidant potential (AOP), from which the oxidant status index (OSi) was calculated. In 0-, 4-, and 8-h plasma samples, targeted fatty acids (FA) were analyzed using liquid chromatography-mass spectrometry, and oxylipids and isoprostanes (IsoP) using liquid chromatography-tandem mass spectrometry. Results for RONS, AOP, and OSi were analyzed by mixed-effects ANOVA or mixed-effects repeated-measures ANOVA, for colostrum and calf blood samples, respectively, whereas FA, oxylipid, and IsoP were analyzed using false discovery rate–adjusted analysis of paired data. Compared with control, HT colostrum showed lower RONS [least squares means (LSM) 189, 95% confidence interval (95% CI): 159–219 vs. 262, 95% CI: 232–292) relative fluorescence units] and OSi (7.2, 95% CI: 6.0–8.3 vs. 10.0, 95% CI: 8.9–11.1), but AOP remained unchanged (26.7, 95% CI: 24.4–29.0 vs. 26.4, 95% CI: 24.1–28.7 Trolox equivalents/μL). Changes in colostrum oxidative markers due to heat treatment were minor. No changes in RONS, AOP, OSi, or oxidative markers were detected in calf plasma. In both groups of calves, plasma RONS activity declined considerably at all postfeeding time points compared with precolostral values, and AOP reached its maximum 8 to 24 h after feeding. Generally, oxylipid and IsoP plasma abundance reached nadirs at 8 h postcolostrum in both groups. Overall, effects due to heat treatment on redox balance of colostrum and newborn calves and on oxidative biomarkers were minimal. In this study, heat treatment of colostrum reduced RONS activity but did not lead to detectable changes in calf oxidative status overall. This indicates that there were only minor changes in colostral bioactive components that could alter newborn redox balance and markers of oxidative damage.

Key words: colostrum, heat treatment, redox balance, oxylipid

INTRODUCTION

Newborn mammals have to rapidly adapt to extrauterine respiration to secure an independent oxygen supply (Hillman et al., 2012). The beginning of autonomous breathing is associated with a period of alteration of the acid-base balance and increase in reactive oxygen and nitrogen species (**RONS**; Massip, 1980), and the timing and magnitude of this increase depends on the duration and ease of the birthing process (Vannucchi et al., 2019). In addition to the pro-oxidant load, the newborn calf's inherent antioxidant capacity and resulting oxidative balance will determine the presence and the degree of oxidative stress (Abuelo et al., 2014). Colostrum provides both substrates and enzymes for RONS production but, at the same time, supplies several antioxidant factors to the newborn calf (Przybylska et al., 2007). Therefore, the balance of pro- and antioxidants absorbed from colostrum likely affects the oxidative status of the newborn calf (Abuelo et al., 2014). Oxidative stress as a result of an imbalance of pro- and antioxidants has been shown to have a negative effect on calf health and

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immune competence in early life (Cuervo et al., 2021). This has motivated researchers to seek enrichment of colostrum with antioxidants such as n-3 fatty acids and α-tocopherol to boost the antioxidant capacity of colostrum (Opgenorth et al., 2020b).

Heat treatment of colostrum before feeding is a management method used on dairy farms to reduce the potential for transmission of milk-borne pathogens to the newborn calf and to reduce bacterial contamination, which is known to interfere with immunoglobulin absorption (Godden et al., 2006). Whereas supplementation of colostrum with antioxidants has received some attention recently, the effect of this postharvest thermal treatment on colostral pro- and antioxidant capacity has not been a focus of research to date. Recent studies of colostral proteins have begun to show altered abundance of several colostral components with possible biological activity as pro- or antioxidants, such as vitamin A binding protein, amine oxidase, and lactoperoxidase, following heat treatment (Tacoma et al., 2017; Mann et al., 2020a). Calligaris et al. (2004) showed that heat treatment of milk at 80°C affected redox potential with an increase in pro-oxidant activity and a reduction in antioxidant activity. Although milk likely behaves differently from colostrum, and 80°C is above the standard colostrum heat treatment temperature (60°C), these results warrant further investigation of the effects of heat treatment on colostral redox status. In contrast, concentrations of selected important antioxidants such as serum vitamin A and E in calves fed either raw or heat-treated (60 min at 60°C) colostrum did not change (Johnson et al., 2007), which may indicate that at least these colostrum components are unaffected by the thermal processing.

Based on the available data, we hypothesized that heating of colostrum to 60°C for 60 min changes the concentrations of colostral pro-oxidants, antioxidants, or both, and thereby the oxidative balance of newborn calves fed with either raw or heat-treated colostrum. Oxylipids and isoprostanes (**IsoP**; prostaglandin-like compounds) are produced from fatty acid precursors and are generated when PUFA become modified, for example, through exposure of free radicals, making oxylipids an accessible utility as biomarkers of oxidative stress (Mavangira and Sordillo, 2018). Therefore, the primary objective of this explorative study was to determine whether heat treatment of colostrum alters the concentration of pro- and antioxidants and the oxylipid profile of colostrum. The secondary objective was to investigate whether differences resulting from heat treatment, if present, extend to the overall oxidative status of the calves fed heat-treated or raw colostrum.

MATERIALS AND METHODS

Colostrum and Calf Samples

Samples analyzed in this study were part of a larger study described previously and used the sample size of animals determined as described (Mann et al., 2020a,b). All animal procedures were reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2018-0021; Ithaca, NY). In brief, colostrum samples were harvested from fresh cows housed on a commercial dairy farm in New York State. Cows eligible for inclusion had to have a minimum dry period of 28 d, appear clinically healthy at the time of first milking, and could not have experienced dystocia. Colostrum harvest occurred within 8 h of calving in a 4-stall herringbone parlor (DeLaval International AB). First-milking colostrum yield of each cow had to be at least 8 L and ≥22% Brix on a digital refractometer (Palm Abbe, Misco) to qualify for this study. Each colostrum sample was gently and thoroughly mixed and split in half using disposable bags (Perfect Udder, Dairy Tech Inc.). One bag of each colostrum was immediately placed on ice (raw; n = 11) and the other was heat treated (heat; n = 11) for 60 min at 60°C in a commercial pasteurizer (Dairy Tech Inc.). Temperature of the water bath during the heat treatment was monitored with a Bluetooth device (TR42, T&D Corp.). After the cool-down phase of the pasteurizer, heat-treated colostrum bags were also placed on ice and then moved to a 4°C refrigerator. Within 24 h of harvest, colostrum was fed to calves as described below, and samples were taken immediately before feeding to reflect composition of the colostrum at feeding time. Colostrum samples were snap frozen in liquid nitrogen using sterile containers, placed briefly at −20°C until transport to the laboratory, and stored at −80°C until further analysis. For further details on sample handling and storage, see Mann et al. (2020a).

Calves were enrolled and fed with colostrum as described in detail elsewhere (Mann et al., 2020b). In brief, pairs of female Holstein calves born within ≤4 h of each other were enrolled in the study if they appeared clinically healthy, were born without assistance, and had a birth weight between 34 and 47 kg. Within each pair, treatment of raw or heat-treated colostrum was randomly assigned by study personnel using randomizing software available online and fed, on average, 2 h apart with a maximum difference of 4 h of each other (Mann et al., 2020b). Researchers were not blinded to treatment allocation. Regardless of treatment, colostrum was first warmed in a 43°C water bath and then fed at 8.5% of birth BW to the calf within 1 h

of birth using an esophageal feeder. Calves were moved to a group-housed barn after the 8-h sampling time point and offered free-choice, heat-treated whole milk. Blood samples (serum, EDTA plasma) from each calf enrolled in the study were obtained from the jugular vein immediately before feeding and at 4, 8, and 24 h relative to feeding and processed on-farm, snap frozen in liquid nitrogen, and transported on dry ice to the laboratory where they remained stored at −80°C until further analysis. Samples were sent on dry ice to Michigan State University (East Lansing) for sample analysis as described below.

Ratio of Total RONS and Total Antioxidant Activity

The ratio of total RONS and the total antioxidant activity (antioxidant potential, **AOP**) were assayed in colostrum, as well as in serum samples from calves at 0, 4, 8, and 24 h. The concentration of RONS was determined using a commercial assay (OxiSelect, Cell Biolabs Inc.), and AOP was determined using the method described by Re et al. (1999). Redox balance was assessed as the ratio of pro-oxidant to total antioxidant defenses (RONS:AOP), also called the oxidant status index (**OSi**; Abuelo et al., 2013), as it accurately detects changes in oxidant status. An increase in the ratio suggests a higher risk for oxidative stress due to increased pro-oxidant production or antioxidant depletion (Abuelo et al., 2013).

Preparation of Samples for Liquid Chromatography Analysis

Targeted MUFA, PUFA, and SFA were analyzed with liquid chromatography-MS (**LC-MS**), and oxylipids and IsoP were quantified using LC-tandem MS (LC-MS/MS). Plasma obtained at 0, 4, and 8 h (1 mL) and colostrum (2 mL) samples were thawed on crushed ice and mixed with an antioxidant-reducing agent and cyclooxygenase inhibitor mixture at 4 μL/mL to prevent degradation of preformed oxylipids and prevent ex vivo lipid peroxidation. The antioxidant-reducing agent mixture consisted of 50% methanol, 25% ethanol, and 25% water with 0.9 m*M* butylated hydroxytoluene, 0.54 m*M* EDTA, 3.2 m*M* tetraphenylporphyrin, and 5.6 m*M* indomethacin (all from Sigma Aldrich). Samples were combined with a 15-μL mixture of deuterated internal standards containing 0.25 μ*M* 5(S)-hydroxyeicosatetraenoic acid-d_8, 0.25 μ*M* 15(S)-hydroxyeicosatetraenoic acid-d_8, 0.5 μ*M* 8(9)-epoxyeicosatrienoic acid-d_{11}, 0.5 μ*M* prostaglandin E_2-d_9, and 0.25 μ*M* 8,9-dihydroxyeicosatrienoic acid-d_{11}. Afterward, acetonitrile was added to yield a 60% acetonitrile solution. Samples were vortexed for 2 min, incubated at room temperature for 15 min, and centrifuged at 4,816 × *g* for 20 min at 4°C. Supernatant was diluted with HPLC water to yield a 20% acetonitrile solution. Solid-phase extraction was carried out using Phenomenex Strata-X 33-μm polymeric reversed-phase 200 mg/3 mL columns preconditioned with 6 mL of methanol followed by 6 mL of HPLC water. Supernatants were loaded into the columns and then washed with 20% methanol and eluted with a 10:90 mixture of methanol and acetonitrile with 2% formic acid. Volatile solvents were dissolved using a Savant SpeedVac (Thermo Fisher Scientific), and residues were reconstituted in methanol, mixed at a 2:1 ratio with HPLC water, and stored in chromatography vials at −20°C until analysis within 1 wk.

Chromatography Determination of IsoP, Oxylipids, and Fatty Acids

Chromatography analyses were conducted following previously published protocols (Putman et al., 2018, 2019). Quantification of IsoP and oxylipids in plasma and colostrum were carried out on a Waters Xevo TQ-S tandem quadrupole mass spectrometer using multiple reaction monitoring. Chromatography separation of oxylipids was performed with a Waters Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 150 mm) held at 50°C and autosampler held at 10°C. Mobile phase A was water containing 0.1% acetic acid, mobile phase B was acetonitrile, and mobile phase C was methanol; the flow rate was 0.3 mL/min. The gradient initial phase was A:B 80:20 to 1 min, changing to A:B:C 50:30:20 to 7 min, changing to A:B:C 1:80:19 to 7.01 min, changing back to initial phase and holding until 10 min. For IsoP, chromatography separation was performed with an Ascentis Express C18 HPLC column, held at 50°C, with autosampler held at 10°C. Mobile phase A was water containing 0.1% acetic acid, mobile phase B was acetonitrile, and mobile phase C was methanol; the flow rate was 0.3 mL/min. Liquid chromatography separation took 15 min with linear gradient steps programmed as follows (A:B ratio): time 0 to 0.5 min (99:1), to (60:40) at 2.0 min, to (20:80) at 8.0 min, to (1:99) at 9.0 min, 0.5 min held at (1:99) until min 13.0; then returned to (99:1) at 13.01 min and held at this condition until 15.0 min. Data analysis was performed by generating 7-point linear curves with commercial standards (Cayman Chemical). The curves for IsoP and oxylipids were 5-fold dilutions ranging from 100 to 0.01 n*M*. The linear curves generated produced R^2 values of 0.99 with percent deviations of less than 100%. Concentrations of IsoP and oxylipids were detected using electrospray ionization in negative-ion mode. Cone voltages and col-

lision voltages were optimized for each analyte using Waters QuanOptimize software, and data analysis was carried out with Waters TargetLynx software.

All fatty acids (**FA**) were quantified using reverse-phase LC on a Waters Acquity UPLC using a Supelco Ascentis Express C18 10 cm × 2.1 mm, 2.7 μm column, with a flow rate of 0.35 mL/min at 50°C. The single quadrupole MS was in electrospray negative ionization mode, and voltage was −3 kV with the turbo ion spray source temperature at 450°C. The gradient mobile phase was programmed in the following manner (A:B:D ratio): 45:22:33, time 0 to 0.2 min, to 80:19:1 at 4.0 min and held until 5.0 min, to 45:22:33 at 6.0 min and held until 8.0 min. In this gradient, mobile phase A was acetonitrile, B was methanol, and D was 0.1% formic acid. Fatty acids were quantified by matching mass-1 and retention time with corresponding deuterated internal standard abundance and calibrated to a linear 7-point standard curve ranging from 5-fold dilution ranging from 500 to 0.001 μM ($R^2 > 0.99$) using Waters Empower 3 software.

Statistical Analysis

RONS, AOP, and OSi. All statistical analyses were performed in JMP Pro (v. 16.0.0, SAS Institute Inc.) unless otherwise specified. The differences between concentrations of RONS and AOP and the resulting OSi of paired colostrum samples were analyzed using mixed-effects ANOVA with the fixed effect of treatment and random effect of pair.

The differences between concentrations of RONS, AOP, and the resulting OSi of paired longitudinal calf serum samples were analyzed in repeated-measures ANOVA with fixed effect of time (4, 8, 24 h), treatment, and their interaction; random effect of calf nested in pair; and baseline values at 0 h as covariates. Differences at baseline were evaluated using mixed-effects ANOVA with the fixed effect of treatment and random effect of animal nested within pair and presented separately.

For ANOVA, model assumptions of normality and homoscedasticity of residuals were assessed visually. Pairwise comparisons were adjusted for multiple comparisons using the Tukey procedure.

Oxylipids. Concentrations of the 37 detected IsoP, FA, and oxylipids in colostrum were analyzed for differences between treatments using false discovery rate (**FDR**)–adjusted analysis of paired data in MetaboAnalyst 5.0 (Pang et al., 2022). Data were first normalized using the normalization by sum feature, square root-transformed, and scaled using the Pareto scaling feature. Success of data normalization was inspected visually. Statistical differences between treatments were explored by fold-change in pairs of heat-treated compared with raw colostrum and defined as differences >2.0-fold change, or by FDR-adjusted paired *t*-tests with $P < 0.05$.

Concentrations of the 39 detected IsoP, FA, and oxylipids in calf plasma were analyzed accordingly with the following difference: data were normalized using the normalization by sum feature, log-transformed, and scaled using the Pareto scaling feature. Success of data normalization was inspected visually. Statistical differences were defined as FDR-adjusted *P*-values from 2-way ANOVA with $P < 0.05$.

RESULTS

Colostrum

Figure 1 shows treatment differences between the 11 colostrum pairs for RONS, AOP, and OSi. Heat treatment was associated with a significantly lower RONS activity ($P = 0.001$) but no association was found with AOP ($P = 0.68$). As a consequence of the difference in

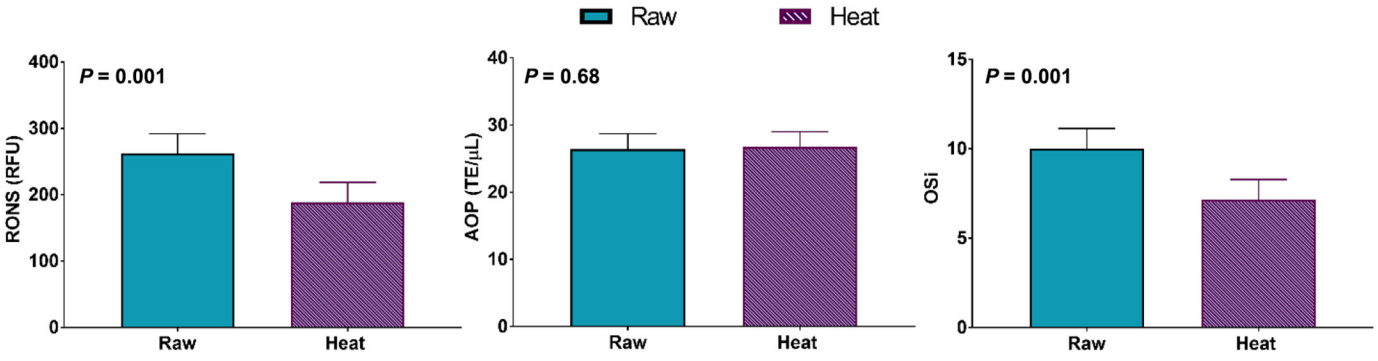

Figure 1. Least squares means and 95% CI of reactive oxygen and nitrogen species (RONS, relative fluorescence units, RFU) activity, total antioxidant potential [AOP; Trolox equivalents (TE)/μL], and the resulting oxidant stress index (OSi) in 11 paired colostrum samples that were either cooled immediately after harvest (Raw) or heat treated at 60°C for 60 min before cooling (Heat). *P*-values are from mixed-effects ANOVA.

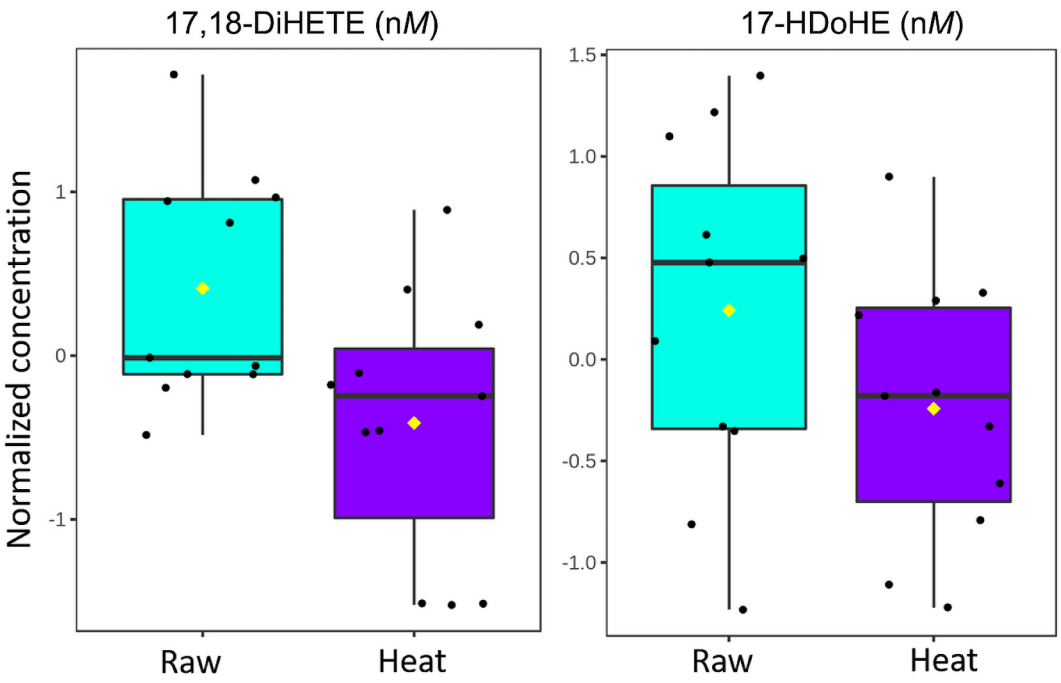

Figure 2. Results of liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of oxylipids showing analytes with a fold change ≥2 of normalized concentrations in 11 paired colostrum samples that were either cooled immediately after harvest (Raw) or heat treated at 60°C for 60 min before cooling (Heat). Box and whisker plots were produced in MetaboAnalyst 5.0 (Pang et al., 2022). The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, the median is indicated by the middle line of the box, the mean of the group's values is shown by the yellow diamond, and whiskers extend to 1.5 times the interquartile range. 17,18-DiHETE = 17,18-dihydroxyeicosatetraenoic acid; 17-HDoHE = 17-hydroxydocosahexaenoic acid.

RONS, OSi was also significantly lower in heat-treated colostrum ($P = 0.001$).

Supplemental Table S1 (https://hdl.handle.net/1813/112244) shows the raw distribution of all analytes detected by LC/MS or LC-MS/MS in colostrum samples. Heat treatment was associated with a decrease of >2-fold change between paired samples in 2 analytes: the arachidonic acid derivative 17,18-dihydroxyeicosatetraenoic acid (17–18-DiHETE; 0.1-fold) and the docosahexaenoic acid (DHA) derivative 17-hydroxydocosahexaenoic acid (17-HDoHE, 0.45-fold; Figure 2). Paired *t*-tests did not yield any significant features.

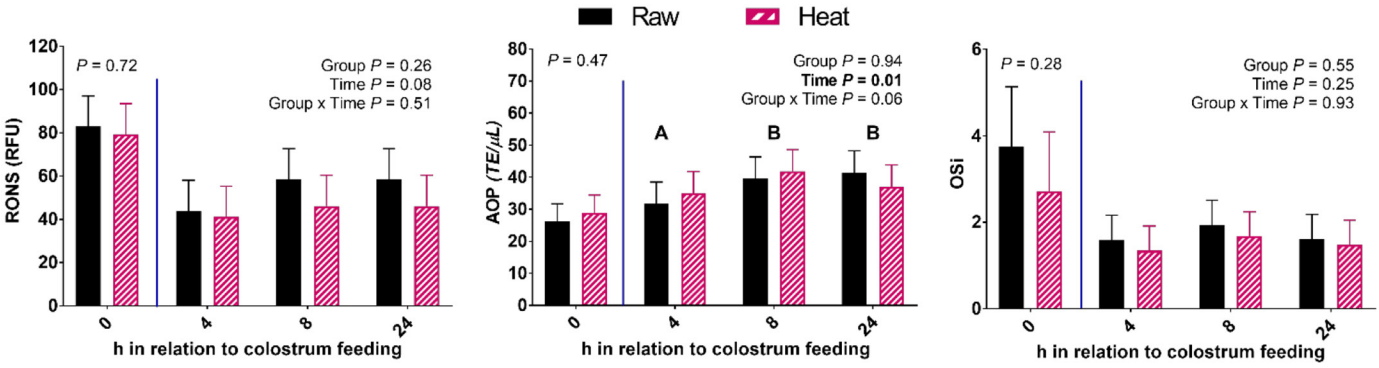

Figure 3. Least squares means and 95% CI of reactive oxygen and nitrogen species (RONS, relative fluorescence units, RFU), total antioxidant potential [AOP; Trolox equivalents (TE)/μL], and the resulting oxidant stress index (OSi) in plasma samples from 11 pairs of calves that were either fed colostrum that was not heat-treated (Raw) or heat treated at 60°C for 60 min before feeding. Samples were taken immediately before colostrum feeding (0 h) and 4, 8, and 24 h later. *P*-values from mixed-effects repeated-measures ANOVA for differences between 4, 8, and 24 h, adjusting for baseline (0 h) values; pairwise comparisons of time points with different letters differ at Tukey's corrected $P < 0.05$. Differences at baseline were evaluated using mixed-effects ANOVA without the repeated effect and presented separately to the left of the vertical purple lines.

Plasma

Figure 3 shows the analysis for treatment and time points in the serum of 11 pairs of calves that received either heat-treated or raw colostrum. No treatment differences were found for RONS, AOP, and OSi ($P > 0.26$), but AOP increased in both groups to reach the maximum value at 8 and 24 h relative to feeding of colostrum, whereas RONS declined in the postcolostral measurements in both groups, resulting in an overall decrease in OSi after 0 h.

Supplemental Table S2 (https://hdl.handle.net/1813/112244) shows the raw distribution of all analytes detected by LC-MS or LC-MS/MS in calf plasma samples. None of the analytes differed statistically between treatments but several differences were observed over time. Figure 4 shows normalized abundance of the 3 FA detected by LC-MS that were different over time, with 2 FA increasing (dihomo-γ-linolenic acid, DGLA; linoleic acid, LA), and one declining (docosapentaeonic acid, **DPA**) by 8 h. Figures 5 to 7 show normalized abundance of analytes detected by LC-MS/MS that were different over time, including 4 IsoP (Figure 5), 12 arachidonic acid metabolites (Figure 6), and 6 linoleic acid and 3 DHA metabolites (Figure 7). Generally, when a time difference was found, IsoP and oxylipids were lowest at the 8-h time point.

DISCUSSION

The primary objective was to determine whether heat treatment of colostrum altered the concentrations of pro- and antioxidants and the oxylipid profile of colostrum. Overall, only small differences were found when analyzing all analytes of interest. The most prominent difference between heat-treated and raw colostrum was the lower RONS activity after heat treatment, resulting in a lower OSi. Relatively little is known about RONS sources and oxylipid composition of bovine colostrum to date. Reactive oxygen and nitrogen species are typically produced by cells, including immune cells, that produce reactive oxygen species and reactive nitrogen species during oxidative burst (Gostner et al., 2013; Yang et al., 2013). Given that cells do not survive the heat treatment process, it is conceivable that the difference in RONS activity is due to the difference in live cell numbers. Colostrum also has cell-independent sources of reactive oxygen species; for example, through xanthine oxidase (Przybylska et al., 2007). Heat treatment of colostrum has been shown to alter enzyme abundance (Mann et al., 2020a). Although we lack understanding of the enzymes that may be important in regulating redox balance in colostrum, it is plausible that the difference in RONS might stem from lower activity of the enzymes responsible for their production. Alternatively, heat treatment might directly degrade certain RONS. The results of our study are in contrast to those by Dias et al. (2020), who found that total antioxidant capacity decreased due to heat treatment compared with raw bovine milk. However, the antioxidation composition of mature milk is different from that of colostrum, with overall higher antioxidant capacity (Przybylska et al., 2007), which may explain

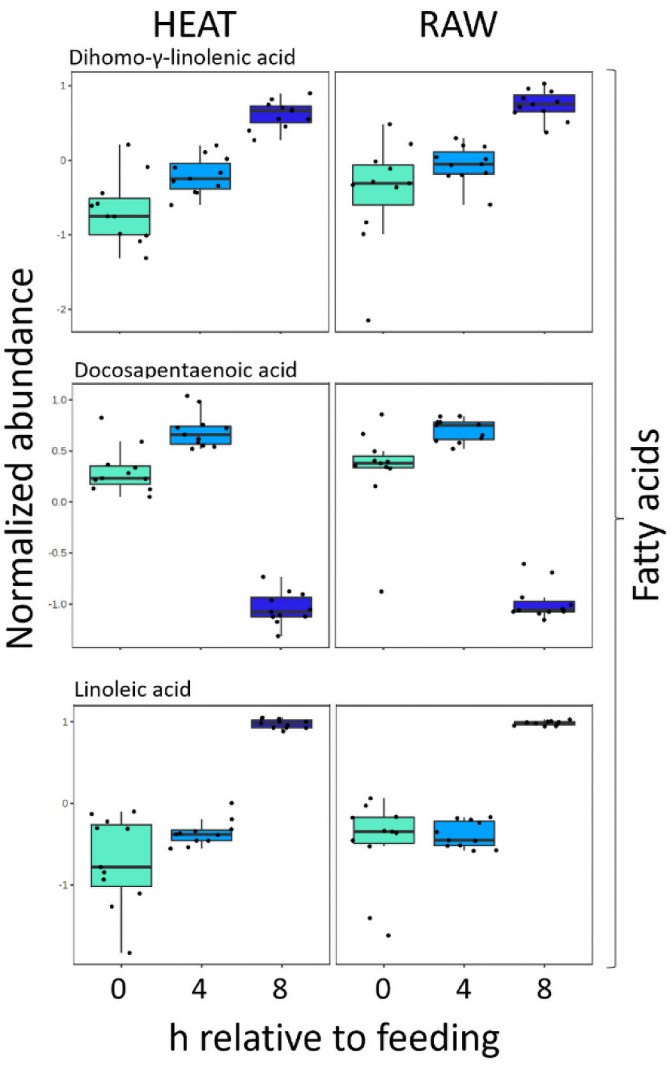

Figure 4. Results of normalized abundances of fatty acids in calf plasma analyzed by liquid chromatography-mass spectrometry, showing analytes with a false discovery rate–adjusted P-value ≤ 0.05 for the effect of time in 2 groups of calves that were fed either untreated colostrum (Raw, n = 11) or colostrum that was heat treated at 60°C for 60 min (Heat, n = 11) at 8.5% of birth BW within 1 h after birth using an esophageal feeder. Samples were taken immediately before colostrum feeding (0 h) and 4 and 8 h later. Box and whisker plots were produced in MetaboAnalyst 5.0 (Pang et al., 2022). The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, the median is indicated by the middle line of the box, and whiskers extend to 1.5 times the interquartile range.

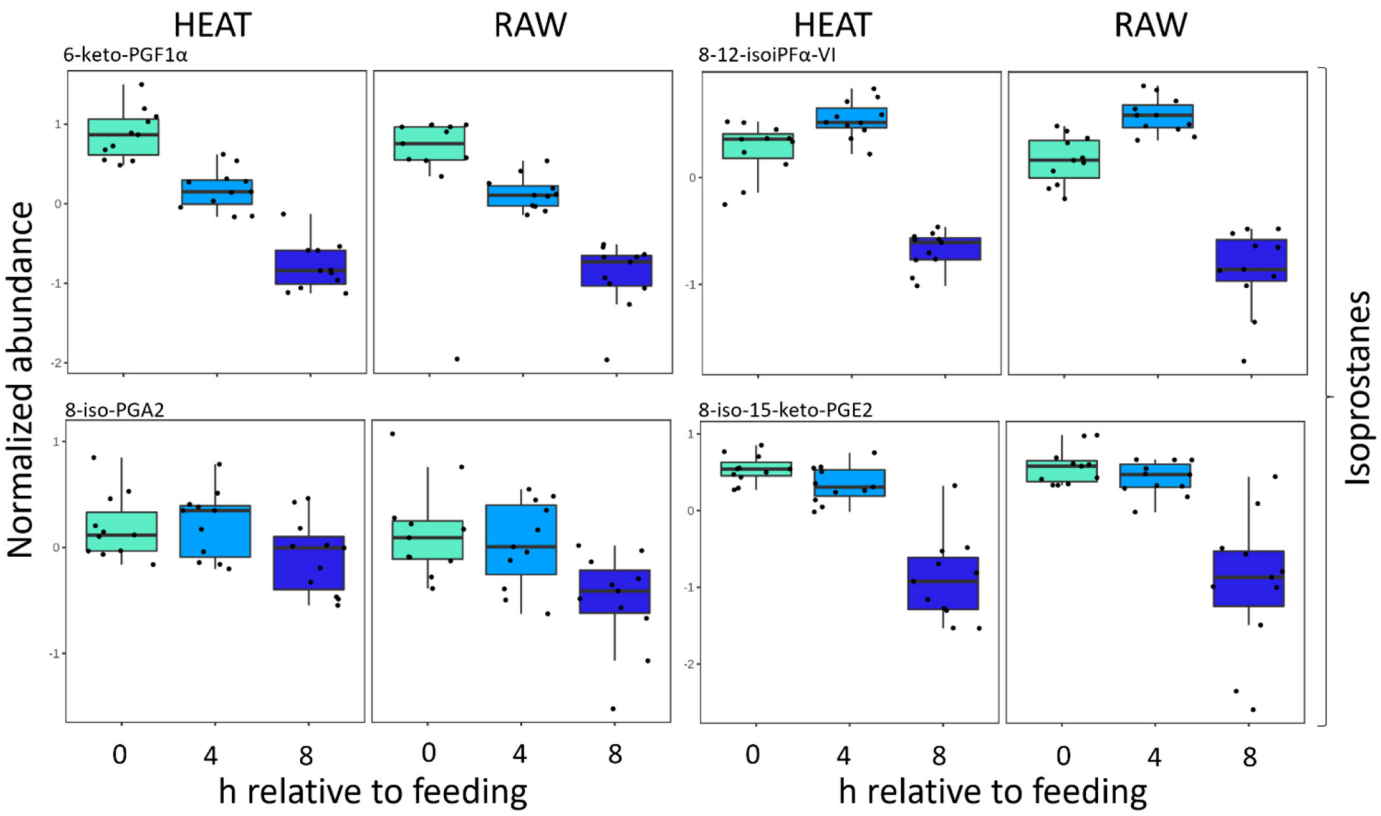

Figure 5. Results of normalized abundances of isoprostanes (IsoP) in calf plasma analyzed by liquid chromatography-tandem mass spectrometry, showing analytes with a false discovery rate–adjusted *P*-value ≤0.05 for the effect of time in 2 groups of calves that were fed either untreated colostrum (Raw, n = 11) or colostrum that was heat treated at 60°C for 60 min (Heat, n = 11) at 8.5% of birth BW within 1 h after birth using an esophageal feeder. Samples were taken immediately before colostrum feeding (0 h) and 4 and 8 h later. Box and whisker plots were produced in MetaboAnalyst 5.0 (Pang et al., 2022). The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, the median is indicated by the middle line of the box, and whiskers extend to 1.5 times the interquartile range. 6-keto-PGF1α = 6-keto-prostaglandin $F_{1\alpha}$; 8,12-isoiPFα-VI = 8,12-iso-iP$F_{2\alpha}$-VI isoprostane; 8-iso-PGA2 = 8-iso-prostaglandin A2; 8-iso-15-keto-PGE2 = 8-iso-15-keto prostaglandin E2.

the observed differences between our study and results of Dias et al. (2020) in whole milk.

This may be the first study describing oxylipid composition of bovine colostrum; the oxylipid profile of colostrum is equally poorly understood in other species. Overall, 5 different prostaglandins and 25 oxylipids were detected in colostrum, 1 less in each category compared with those detected in calf plasma. This demonstrates that colostrum has an inherent oxylipid profile, which is likely predominantly derived from the dam but can be altered by postharvest procedures, as we demonstrate in this study. Heat treatment decreased the abundance of arachidonic acid derivative 17,18-dihydroxy-eicosatetraenoic acid (17–18-DiHETE) and the DHA derivative 17-hydroxy-docosahexaenoic acid (17-HDoHE). Industrial heat treatment of bovine milk for human consumption reduced certain oxylipids at high (72°C, 15 s) and ultra-high (135–150°C, 2–6 s) temperatures, but not at pasteurization temperature (63°C, 30 min; Dias et al., 2020). Given the paucity of data on oxylipids in milk, the reasons for this difference are currently unclear. However, the stability of lipids depends on several intrinsic and extrinsic factors (Shahidi and Zhong, 2010). We speculate, therefore, that matrix composition differences between colostrum and milk could make these oxylipids more susceptible to thermal degradation at pasteurization temperatures.

The secondary objective was to investigate whether differences resulting from heat treatment, if present, extend to the overall redox status of the calves fed heat-treated or raw colostrum. The bovine neonate is exposed to an oxygen-rich environment after birth, which may overwhelm inherent antioxidative capacity, leading to oxidative stress immediately after calving (Gaál et al., 2006; Przybylska et al., 2007). Abuelo et al. (2014) investigated colostrum redox balance and concluded that it affected the calf's oxidative status after feeding. Differences in RONS and subsequently in OSi between

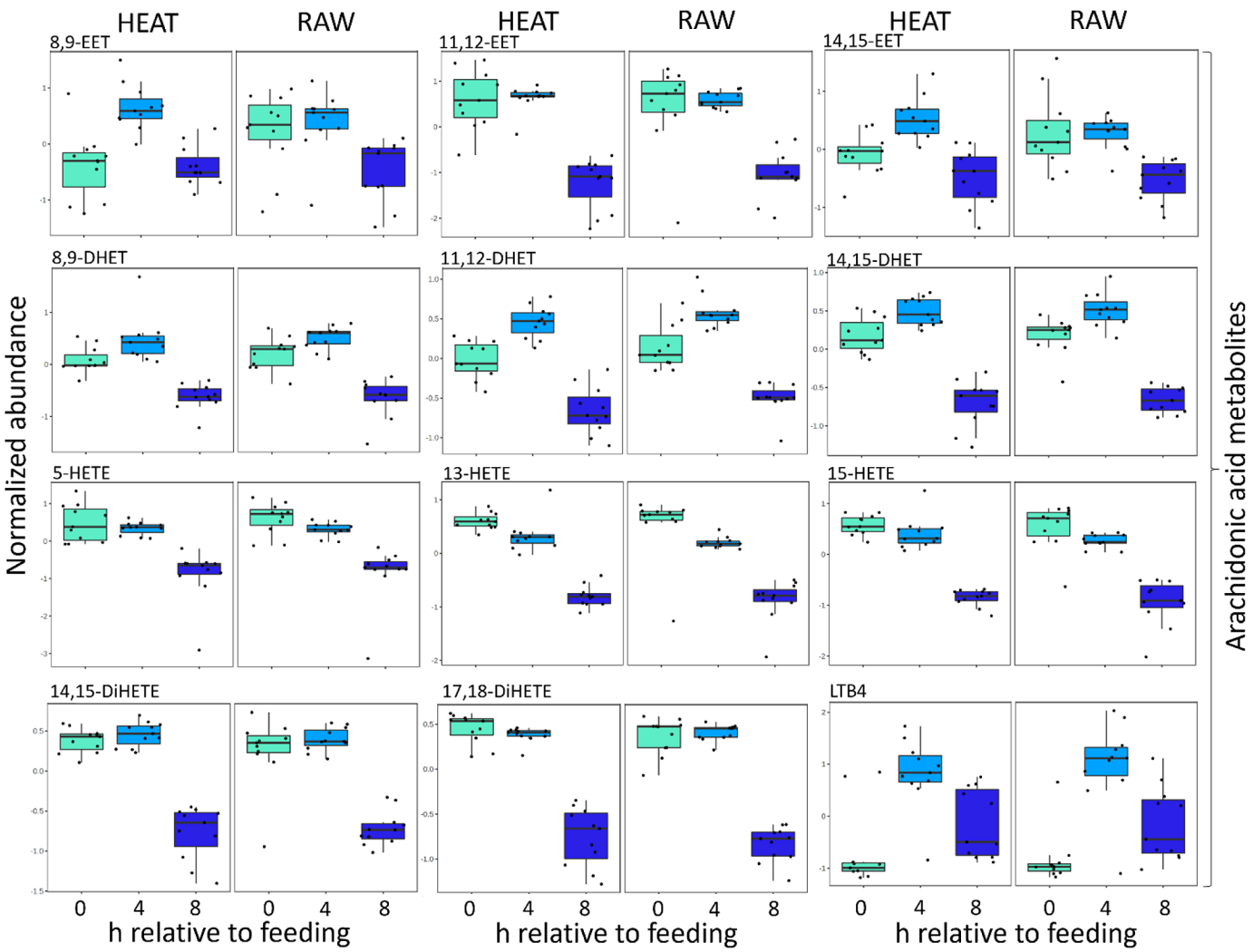

Figure 6. Results of normalized abundances of arachidonic acid-derived oxylipids in calf plasma analyzed by liquid chromatography-tandem mass spectrometry, showing analytes with a false discovery rate–adjusted *P*-value of ≤0.05 for the effect of time in 2 groups of calves that were fed either untreated colostrum (Raw, n = 11) or colostrum that was heat treated at 60°C for 60 min (Heat, n = 11) at 8.5% of birth BW within 1 h after birth using an esophageal feeder. Samples were taken immediately before colostrum feeding (0 h) and 4 and 8 h later. Box and whisker plots were produced in MetaboAnalyst 5.0 (Pang et al., 2022). The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, the median is indicated by the middle line of the box, and whiskers extend to 1.5 times the interquartile range. EET = epoxyeicosatrienoic acid; DHET = dihydroxyeicosatrienoic acid; HETE = hydroxyeicosatetraenoic acid; DiHETE = dihydroxyeicosatetraenoic acid; LTB4 = leukotriene B4.

the 2 colostrum treatments did not transfer to measurable differences in calves fed with this colostrum. This could be due to the differences in pro- and antioxidants in colostrum being too small to result in a biological effect in the calf or to the calf's inherent oxidative balance being influenced only mildly by the difference in redox status of colostrum. As expected, AOP increased and RONS decreased after colostrum feeding. Previous work in neonates showed that RONS decreased and AOP increased on the day after birth compared with values obtained before colostrum feeding in calves, regardless of n-3 FA supplementation of colostrum (Opgenorth et al., 2020b).

Oxylipid concentrations at 3 time points during the first day of life were in the same nanomolar range of concentrations as previously reported for neonates (Opgenorth et al., 2020b). Parallel to the lack of differences in overall OSi between treatments, we found no difference in biomarkers of oxidative status, including direct biomarkers of oxidative damage (prostaglandins) between treatments. However, oxylipid and IsoP abundance of many of the studied analytes was generally

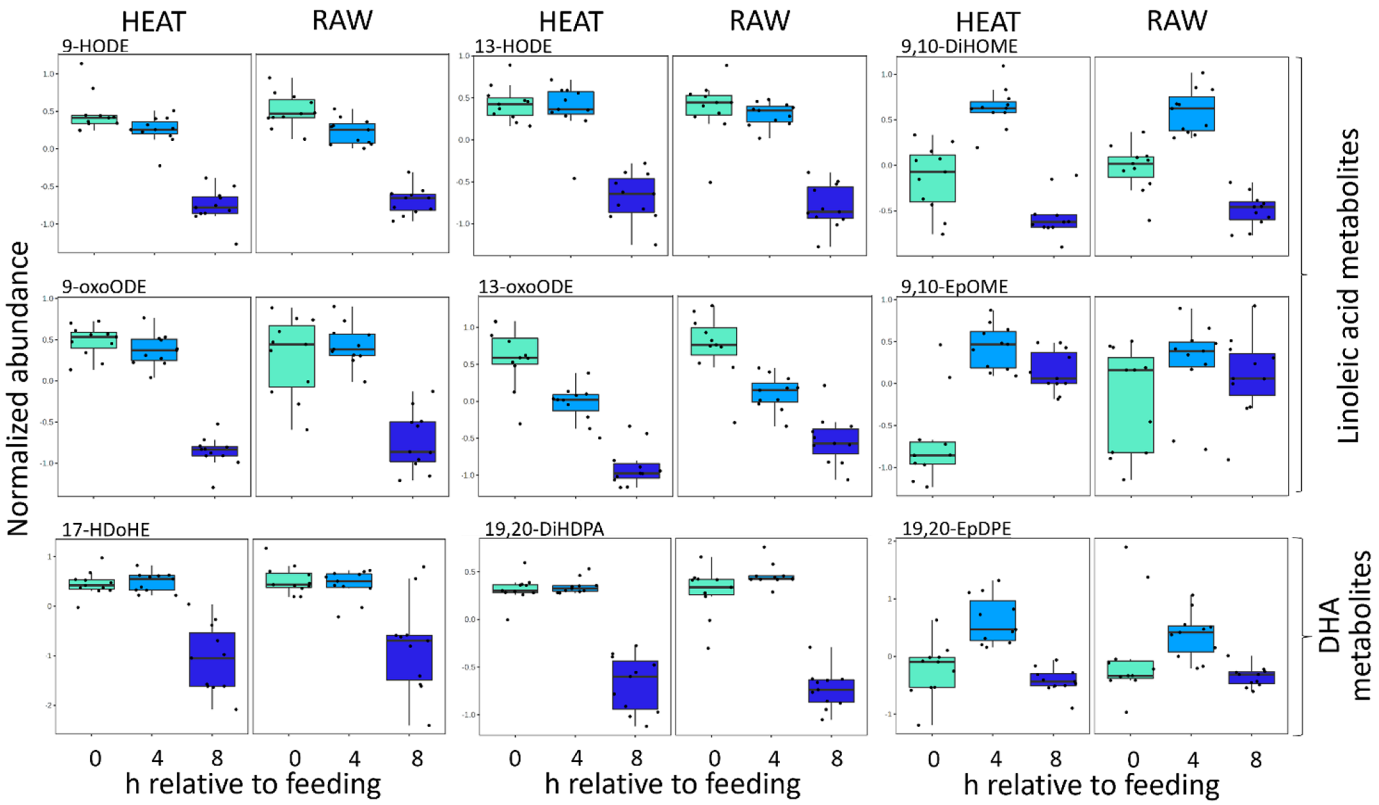

Figure 7. Results of normalized abundances of linoleic acid- or docosahexaenoic acid (DHA)-derived oxylipids in calf plasma analyzed by liquid chromatography-tandem mass spectrometry, showing analytes with a false discovery rate–adjusted *P*-value of ≤0.05 for the effect of time in 2 groups of calves that were fed either untreated colostrum (Raw, n = 11) or colostrum that was heat treated at 60°C for 60 min (Heat, n = 11) at 8.5% of birth BW within 1 h after birth using an esophageal feeder. Samples were taken immediately before colostrum feeding (0 h) and 4 and 8 h later. Box and whisker plots were produced in MetaboAnalyst 5.0 (Pang et al., 2022). The lower and upper limits of the boxes represent the 25th and 75th percentiles, respectively, the median is indicated by the middle line of the box, and whiskers extend to 1.5 times the interquartile range. HODE = hydroxy-octadecadienoic acid; DiHOME = dihydroxyoctadecenoic acid; oxoODE = oxooctadecadienoic acid; EpOME = epoxyoctadecenoic acid; HDoHE = hydroxydocosahexaenoic acid; DiHDPA = dihydroxydocosapentaenoic aci; EpDPE = epoxydocosapentaenoic acid.

reduced at 8 h after colostrum feeding, indicating that production of these markers was lower at this time compared with the time directly after birth or 4 h after colostrum feeding, or that they were removed from circulation at a higher rate. Whether this change is due to factors ingested with colostrum or simply a decline over time cannot be answered with this study. A follow-up study comparing colostrum-fed and colostrum-deprived calves is warranted to answer this question. Additionally, previous research suggests that the redox changes associated with colostrum ingestion, if present, are transient, and calves experience subsequent redox imbalances throughout the preweaning stage (Abuelo et al., 2014).

In both groups, 2 FA increased very robustly after colostrum feeding: dihomo-γ-linolenic acid and linoleic acid. A large increase in plasma linolenic acid and incorporation of that FA into phospholipids was previously observed (Opgenorth et al., 2020a,b) and was most pronounced when colostrum was supplemented with fish and flaxseed oil, indicating that the rapid rise in these FA was due to intestinal uptake. Colostrum fed in our study contained DPA in the low micromolar range, approximately 100-fold higher than the precolostral plasma concentrations (a finding consistent with the human literature; Ulu et al., 2019), possibly leading to the initial increase at 4 h. The reasons for the decline in DPA after an initial increase are not clear. However, DPA is a substrate for inflammatory pro-resolving mediators (Vik et al., 2017; Drouin et al., 2019); therefore, the initially absorbed DPA could have been consumed in ameliorating the inflammation associated with the birthing process.

This study reports findings from a single farm regarding biomarkers of oxidative status and response to heat treatment. Colostrum composition varies by farm, geographic location, and feeding and management practices; therefore, future work should aim to further

extend both the findings in raw colostrum composition and the response to heat treatment.

CONCLUSIONS

Colostrum contained a similar set of oxylipids as those detected in blood, albeit at different concentrations. Heat treatment of colostrum altered the redox balance and oxylipid composition minimally, but reduced the overall activity of reactive oxygen and nitrogen species, leading to an overall reduced OSi of heat-treated versus raw colostrum. In this small-scale study, differences in colostrum did not translate into detectable differences in any measured parameters describing redox status or oxylipid profile in colostrum-fed calves. Colostrum may have contributed to the rapid increase in antioxidant potential within hours after feeding; however, this hypothesis needs to be tested in future studies using a control group deprived of colostrum. Overall, effects due to heat treatment on redox balance of colostrum and newborn calves as well as biomarkers of oxidative stress were minimal.

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