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Correlation of oxidative stress-related indicators with milk composition and metabolites in early lactating dairy cows

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Abstract

Background: In highly intensive dairy farms, cows often suffer from metabolic disorders that cause severe oxidative stress.

Objectives: This study aimed to observe correlations and associations of oxidative stress-related indicators with milk compositions and metabolites.

Methods: Twenty-two multiparous Holstein dairy cows in early lactation were randomly selected from a commercial dairy farm. The morning milk was collected for composition and metabolites analysis. Blood was sampled via the tail vein to analyze oxidative stress-related indicators (reactive oxygen species, ROS; catalase, CAT; superoxide dismutase, SOD; glutathione peroxidase, GPX; malondialdehyde, MDA) and metabolites.

Results: Results showed that ROS were positively correlated with CAT, GPX, SOD, and MDA. However, the levels of CAT, GPX, and SOD were negatively related to milk fat ($P < 0.05$). Nineteen serum and 7 milk metabolites were selected from detectable metabolites according to their correlations with ROS, CAT, GPX, and SOD ($P < 0.05$). Metabolic pathway analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database revealed that these metabolites are primarily involved in the metabolic pathways of carbohydrates and amino acids.

Conclusions: This study gave us a better understanding on oxidative stress that ROS not only increased oxidative damage (MDA) in dairy cows, but also altered some metabolites involved in amino acid and carbohydrate metabolism.

KEYWORDS
antioxidants, dairy cows, metabolites, reactive oxygen species

1 | INTRODUCTION

Oxidative stress refers to the imbalance between oxidative and antioxidant effects in the body. Reactive oxygen species (ROS) are metabolites formed during mitochondrial energy production (Radi, 2018). Under normal physiological conditions, ROS production and clearance are in dynamic equilibrium, and ROS can be maintained at extremely low levels that are beneficial and harmless (Ludin et al., 2014). However, excessive ROS can lead to oxidative damage of cells, leading to pathological processes and even cell death (Mavangira & Sordillo, 2018). From the perspective of physiological changes, cows experienced a lot of oxidative stress in early lactation. A large amount of physiological pressure for milk synthesis is accompanied by an increase in high energy demand and oxygen demand and increases the production of ROS (Cadenas & Davies, 2000). During the transition of lactating animals from early lactation to middle lactation, a large number of metabolic and physiological adaptations may occur, which may lead to dysfunction and inflammatory of the host (Turk et al., 2012; Zahrazadeh et al., 2018; Zhao et al., 2019). A variety of defence systems are involved in cells to prevent the increase of ROS, including enzymatic and non-enzymatic mechanisms. The non-enzymatic antioxidants obtained from dietary sources include carotenoids, tocopherols, vitamin D, phenolic acids, and flavonoids can help reduce oxidative damage in mammals (Ratnam et al., 2006). Enzymatic antioxidants exist in forage crops such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) are easily inactivated during processing and cannot play their role in animals. Enzymatic antioxidants synthesized in the animal body seemed to play a vital role in the defence of ROS. The emergence of metabolomics has provided us with a new opportunity to identify animal health and the quality of animal products (Nie et al., 2016). Gas chromatography-mass spectrometry (GC-MS) is a powerful technique in metabolomics. Zhang et al. (2013) used GC-MS to identify several metabolites as potential biomarkers for distinguishing cows that had clinical ketosis, sub-clinical ketosis from clinically normal. Another study found that serum ophthalmic acid is an indicator of glutathione decline in the liver and maybe use as new biomarkers of oxidative stress (Soga et al., 2006). This study aimed to observe correlations and associations of oxidative stress-related indicators (ROS, SOD, GPX, and CAT) with milk compositions and metabolites (serum and milk). Therefore, 22 multiparous Holstein dairy cows in early lactation were randomly selected from a commercial dairy farm. Malondialdehyde (MDA) is one of the lower compounds formed by ROS-induced polyunsaturated fatty acids and was used as an oxide to evaluate oxidative stress in this study (Kapuy et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Experimental design and sample collection

Twenty-two disease-free multiparous (parity, 2.55 ± 0.11, 10 for two parity, 12 for three parity) Holstein cows (days in milk, 91.64 ± 1.71; dry matter intake, 22.17 ± 1.09 kg) were randomly selected from a commercial dairy farm (herd population, n = 2134) in Heilongjiang Province, P. R. China. Water was allowed ad libitum and cows were fed with total mixed ration three times a day after milking (Table 1) in cross ventilation barns. Milking was performed three times a day at 9:00 AM, 3:00 PM, and 9:00 PM, and the milk yield (39.46 ± 1.93 kg) was recorded. Milk samples (50 ml) were collected at 9:00 AM and stored at −40°C. Tail vein blood (10 ml) was sampled after morning milking when the cows took the total mixed ration (TMR) and centrifuged at 2500 r/min for 10 min. The serum was collected and stored at −80°C until analysis.

TABLE 1 Ingredients and the chemical composition of total mixed ration (TMR)

Item	
Ingredients (% dry matter)	
Corn silage	35.37
Corn (finely ground)	20.09
Soybean meal	16.84
Molasses	2.64
Premix[1]	2.24
Oat hay	14.58
DDGS	8.24
Chemical composition (% dry matter)[2]	
Crude protein	16.16
Starch	26.72
Crude fat	3.47
Neutral Detergent fibre (NDF)	29.16
Acid detergent fibre (ADF)	16.74
Net energy for lactation (NEL), Mcal/kg	1.78

[1]Premix composition (per kilogram): Ca 16.5 mg, P 3 mg, Mg 5.4 mg, Na 10.4 mg, Cl 7.7 mg, Zn 1600 mg, Cu 540 mg, Mn 600 mg, Se 15 mg, Co 10 mg, I 32 mg, vitamin A 360 IU, vitamin D 90 IU, vitamin E 2160 IU.
[2]Calculated according to the Feed Composition and Nutritive Values in China (2017).

2.2 | Milk composition and serum oxidation status analysis

The fat, protein, and lactose contents of the milk samples were determined using a milk composition analyzer (LA, Lactosan). Briefly, the concentrations of serum ROS, CAT, GPX, SOD, and MDA were measured using ELISA kits according to the instructions (Shanghai Enzyme-linked Biotechnology Co., Ltd.). The calibration standards are assayed at the same time as the samples, and the intensity of the colour is measured using a spectrophotometer (UVmini-1280; Shimadzu Instrument Co., Ltd.). The concentration of individual parameter in the samples is then determined by comparing the optical density of the samples to the standard curve.

2.3 | Serum metabolites analyzed by GC-MS

Serum (100 μl) was mixed with 300 μl of methanol and chloroform solution (3:1) and 20 μl of L-2-chlorophenyl alanine (1 mg/ml stock in dH_2O). After shaking, the samples were centrifuged at 12,000 r/min for 10 min. The supernatant was collected and vacuum freeze-dried at −45°C (ALPHA 1-2LD PLUS; Marin Christ). Then, 60 μl of pyridine solution of methoxyamine hydrochloride (15 mg/ml) was added and incubated for 120 min in a 37°C water bath (TW20; Julaba, Seelbach). Then, 60 μl of BSTFA (containing 1% TMCS) was added and incubated for 60 min in a 70°C water bath. The supernatant (1 μl) was collected for GC-MS (TSQ 8000; Thermo) analysis with anelastic quartz capillary column (30 m × 0.25 mm × 0.25 μm; HP-5MS; Agilent Technologies, Inc.). All injections were done in the split less mode with 1 μl injected volume, and an oven ramp beginning at 70°C (hold for 4 min), then increasing at a programme rate to 270°C with a hold time of 5 min. Helium (carrier gas) was used at a rate of 1.0 ml/min. The electron impact (EI) ionization mode used was at 70 eV. The split ratio was 10:1, the solvent delay time was 3 min, and mass scanning range was 50–550 m/z.

2.4 | Milk metabolites analyzed by GC-MS

Milk (10 ml) was ultrasonically homogenized (SB 25-12 DTN; Scientz). Then, 100 μl sample was mixed with 250 μl methanol, 125 μl chloroform, and 20 μl of L-2-chlorophenyl alanine (1 mg/ml stock in dH2O). After shaking, 380 μl of chloroform and 90 μl of potassium chloride aqueous solution (14.8 mg/ml) were added. The samples were centrifuged at 12,000 r/min. Note that 250 μl each of the upper (chloroform layer) and lower (water layer) solutions was taken to vacuum freeze-dry at −45°C. Then, 60 μl of pyridine solution of methoxyamine hydrochloride (10 mg/ml) was added in each sample and incubated for 120 min in a 37°C water bath. Then, 80 μl of BSTFA (containing 1% TMCS) was added and incubated for 60 min in a 70°C water bath. The supernatant (1 μl) was collected for GC-MS. The samples were performed using GC-MS with anelastic quartz capillary column as described above. An oven ramp beginning at 80°C (hold for 1 min), then increasing at a programme rate to 275°C with a hold time of 5 min.

2.5 | Statistical analysis

Pearson analysis was conducted by using the R version 3.5.3. The correlation significance level of all statistical analysis was set to $p < 0.05$, and the trend was set to $0.05 < p < 0.10$. The selected metabolites were introduced into Metaboanalyst 4.0 (http://www.metaboanalyst.ca) for path enrichment analysis and path topology analysis. Significant impact pathways were defined as $p < 0.05$. In addition, databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) help reveal metabolic disorders.

3 | RESULTS

3.1 | Correlation of oxidative stress-related indicators with milk composition and metabolites (serum and milk)

Figure 1 shows the Pearson correlation among 37 detectable serum metabolites, five oxidative stress-related indicators, and four milk-related traits. A large number of metabolites were significantly correlated with oxidative stress-related indicators ($p < 0.05$) and milk production traits ($p < 0.05$). Their correlations are explained in detail below. ROS was significantly positively correlated with GPX and SOD (Table 2; $0.46 < r < 0.53$, $p < 0.05$), and positively correlated with CAT and MDA (Table 2; $0.39 < r < 0.42$, $p < 0.10$). Seven serum metabolites including L-leucine, L-isoleucine, butanoic acid, L-valine, L-methionine, D-lactic acid, and acetic acid were significantly negatively correlated with ROS (Table 3; $-0.61 \leq r \leq -0.45$, $p < 0.05$). Four milk metabolites including galactinol, undecane, cis-aconitate, ethanol were significantly positively correlated with ROS (Table 3; $0.44 \leq r \leq 0.52$, $p < 0.05$). Milk fat yield was significantly negatively correlated with CAT, GPX, and SOD (Table 4; $-0.64 \leq r \leq -0.49$, $p < 0.05$). The CAT, GPX, and SOD had significant correlations with most of the serum and milk metabolites (Table 5; $p < 0.05$). Among them, serum L-leucine, L-isoleucine, L-valine, and L-methionine are highly negative correlated with CAT, GPX, and SOD ($-0.72 \leq r \leq -0.57$, $p < 0.05$). Milk metabolites including galactinol, cis-aconitate, and succinic acid have significant positively correlated with CAT, GPX, and SOD ($-0.79 \leq r \leq -0.52$, $p < 0.05$). Milk glycine was related to SOD ($r = -0.37$, $p = 0.09$) and GPX ($r = -0.38$, $p = 0.08$). In addition, Table 5 showed serum L-isoleucine ($r = 0.39$, $p = 0.07$), L-methionine ($r = 0.33$, $p = 0.13$), and butanoic acid ($r = 0.35$, $p = 0.12$) had positive correlations with milk fat yield. And milk galactinol ($r = -0.68$, $p = 0.00$), succinic acid ($r = -0.41$, $p = 0.06$), pentanedioic acid ($r = -0.63$, $p = 0.00$), and s-2-methyl-1-butanol ($r = -0.39$, $p = 0.08$) were negatively correlated with milk fat yield.

3.2 | Metabolites for pathway analysis

According to the above correlation analysis (Tables 3 and 5; $p < 0.05$), it was found that these 19 serum metabolites and 7 milk metabolites were significantly correlated with ROS and/or three antioxidase (Figures 2a and 3a). Affected metabolic pathways of these metabolites were analyzed by path enrichment analysis and path topology analysis (Figures 2b–d and 3b). In Figure 2b, metabolic pathways involved in ROS-related metabolites were mainly related to pyruvate metabolism ($p = 0.00$, impact value = 0.07). In Figure 2c, metabolic pathways involved in both ROS and antioxidase-related metabolites mainly included valine, leucine, and isoleucine biosynthesis ($p = 0.00$, impact value = 0.25); aminoacyl-tRNA biosynthesis ($p = 0.00$, impact value = 0.14); valine, leucine, and isoleucine degradation ($p = 0.00$, impact value = 0.06); and butanoate metabolism ($p = 0.05$, impact value = 0.07). In Figure 2d, metabolic

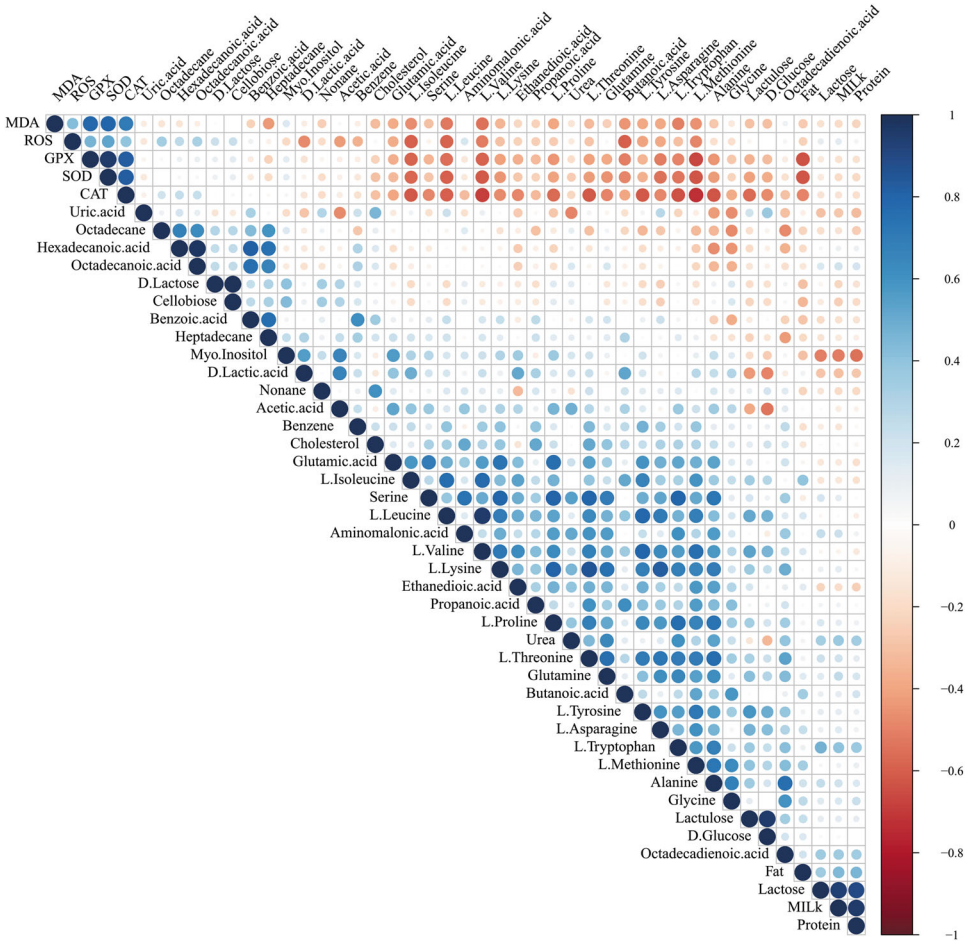

FIGURE 1 Correlation analysis among oxidative stress-related indicators, milk composition, and serum metabolites in early lactating dairy cows. The size of the circle represents the absolute value of the correlation, blue and red represent positive and negative correlations, respectively, and a blank indicates that the two variables had no correlation

TABLE 2 Correlation of reactive oxygen species (ROS)[1] with other oxidative stress-related indicators and milk composition

	Range	Mean	SEM	r	p-Value
Oxidative stress-related indicators					
MDA (nmol/ml)	6.82–22.34	10.34	0.81	0.42	0.05
CAT (U/ml)	110.91–298.29	171.44	9.54	0.39	0.07
GPX (IU/ml)	734.72–1908.06	1054.76	57.81	0.46	0.03
SOD (U/ml)	141.88–354.22	210.27	11.11	0.53	0.01
Milk composition					
Milk yield (kg/d)	21.38–52.70	39.46	1.93	0.13	0.57
Fat (kg/d)	0.34–2.56	1.27	0.12	−0.23	0.30
Protein (kg/d)	0.59–1.56	1.18	0.06	0.05	0.82
Lactose (kg/d)	0.75–2.58	1.61	0.09	0.11	0.61

Abbreviations: CAT, catalase; GPX, glutathione peroxidase; MDA, malondialdehyde; *r*, correlation coefficient; SOD, superoxide dismutase, SEM, standard error of mean.

[1]ROS: reactive oxygen species, the range is 196.18–787.26 IU/ml.

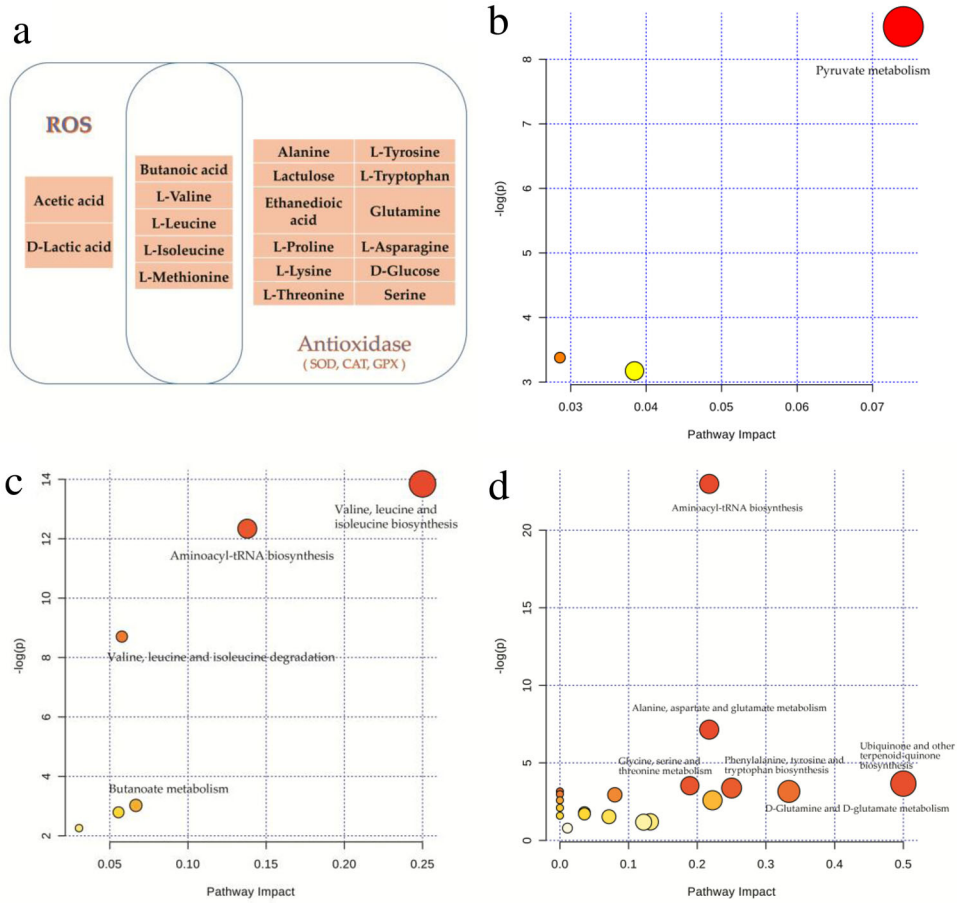

FIGURE 2 Screened serum metabolites and their metabolome view. (a) Classification of 19 metabolites. Two metabolites related to reactive oxygen species (ROS) only, five metabolites related to ROS and antioxidase in common, and 12 metabolites related to antioxidase only. (b) Serum metabolites related to ROS and their metabolic pathway. (c) Serum metabolites related to both ROS and antioxidase and their metabolic pathways. (d) Serum metabolites related to antioxidase and their metabolic pathways. The *Y*-axis is based on the *p*-value (from path enrichment analysis), and the *X*-axis is based on the path impact value (from path topology analysis). When colour in the figure is darker and the bubble area is larger, this indicates that the metabolites in the corresponding pathway are more varied

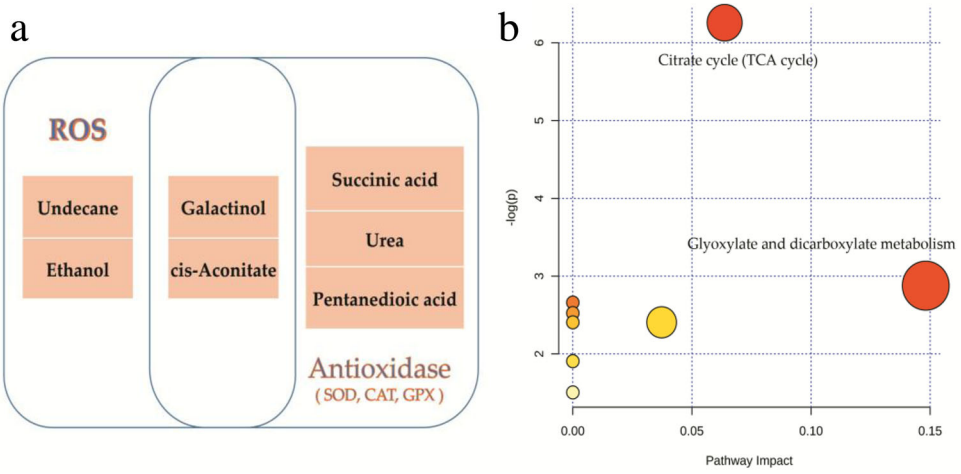

FIGURE 3 Screened milk metabolites and their metabolome view. (a) Classification of seven metabolites. Two metabolites related to reactive oxygen species (ROS) only, two metabolites related to ROS and/or antioxidase in common, and three metabolites related to antioxidase only. (b) Metabolic pathways affected by oxidative stress-related indicators (ROS, CAT, GPX, and SOD)

TABLE 3 Correlation of reactive oxygen species (ROS) with metabolites

Metabolites	Range	Mean	SEM	r	p-Value
Serum metabolites					
L-Leucine	3.34–8.51	6.07	0.33	−0.61	0.00
L-Isoleucine	1.77–8.13	4.20	0.32	−0.60	0.00
Butanoic acid	0.18–0.42	0.29	0.01	−0.60	0.00
L-Valine	4.31–11.89	8.22	0.45	−0.51	0.02
L-Methionine	0.43–1.06	0.81	0.04	−0.48	0.02
D-Lactic acid	90.44–149.33	113.27	2.68	−0.48	0.02
Acetic acid	0.15–0.37	0.26	0.01	−0.45	0.03
Milk metabolites					
Galactinol	5.05–24.9	13.96	1.32	0.52	0.01
Undecane	1.64–2.13	1.89	0.02	0.46	0.03
cis-Aconitate	3.95–10.3	6.47	0.35	0.45	0.04
Ethanol	3.63–4.28	3.89	0.04	0.44	0.04

Abbreviation: SEM, standard error of mean

TABLE 4 Correlation of other oxidative stress-related indicators with milk yield and composition

	CAT		GPX		SOD	
	r	*p*-Value	*r*	*p*-Value	*r*	*p*-Value
Milk yield (kg/d)	−0.15	0.51	−0.16	0.47	−0.19	0.40
Fat (kg/d)	−0.49	0.02	−0.64	0.00	−0.63	0.00
Protein (kg/d)	−0.17	0.44	−0.17	0.46	−0.21	0.35
Lactose (kg/d)	−0.15	0.49	−0.12	0.58	−0.13	0.57

Abbreviations: CAT, catalase; GPX, glutathione peroxidase; *r*, correlation coefficient; SOD, superoxide dismutase.

pathways involved in antioxidase-related metabolites mainly included aminoacyl-tRNA biosynthesis ($p = 0.00$, impact value = 0.22); alanine, aspartate, and glutamate metabolism ($p = 0.00$, impact value = 0.22); ubiquinone and other terpenoid-quinone biosynthesis ($p = 0.03$; impact value = 0.05); glycine, serine, and threonine metabolism ($p = 0.03$, impact value = 0.19); phenylalanine, tyrosine, and tryptophan biosynthesis ($p = 0.03$, impact value = 0.25); and D-glutamine and D-glutamate metabolism ($p = 0.04$, impact value = 0.33). In Figure 3b, the main metabolic pathways related to serum ROS, CAT, GPX, and SOD include citrate cycle ($p = 0.00$; impact value = 0.06), and glyoxylate and dicarboxylate metabolism ($p = 0.07$; impact value = 0.15). KEGG database revealed that these metabolic pathways are mainly related to the carbohydrate pathway and the amino acid metabolism pathway (Table 6). Besides, metabolism of other amino acids, metabolism of cofactors and vitamins, and translation (genetic information processing) are also affected.

4 | DISCUSSION

Oxidative stress is thought to be an imbalance between the generation and elimination of free radicals due to increased oxidants and/or insufficient antioxidant. The positive correlation between ROS and MDA indicated that cows with high serum ROS content may suffer more oxidative damage than cows with low ROS content. The levels of several antioxidase found in these animals including CAT, GPX, and SOD increased simultaneously with ROS. Moolchandani (2018) found that increased SOD activity in early and middle lactation is a hallmark of oxidative stress. In this experiment, the relatively higher serum CAT, GPX, and SOD activity may be due to the physiological upgrade of this enzyme, trying to neutralize/mitigate the challenge of ROS. In fact, oxidative stress increases the susceptibility of cows to metabolic disorders (Zahrazadeh et al., 2018, Zhao et al., 2019). This study found that some serum and milk metabolites were significantly correlated with these oxidative stress-related indicators. These metabolites are mainly related to the metabolic pathways of amino acids and carbohydrates.

4.1 | Correlation of oxidative stress-related indicators with serum metabolites

ROS is a by-product of the energy generated by the oxidative phosphorylation process, and its increase reflects an increase in animal demand for energy. This study found that the metabolic pathway related to ROS

TABLE 5 Correlation of other oxidative stress-related indicators and milk fat with metabolites

	CAT		GPX		SOD		Milk fat	
	r	*p*-Value	*r*	*p*-Value	*r*	*p*-Value	*r*	*p*-Value
Serum metabolites								
Alanine	−0.62	0.00	−0.45	0.04	−0.42	0.05	0.21	0.36
L-Valine	−0.69	0.00	−0.59	0.00	−0.61	0.00	0.16	0.48
L-Leucine	−0.58	0.01	−0.57	0.01	−0.61	0.00	0.19	0.40
L-Isoleucine	−0.61	0.00	−0.59	0.00	−0.57	0.01	0.39	0.07
L-Proline	−0.56	0.01	−0.44	0.04	−0.41	0.06	0.06	0.81
Serine	−0.48	0.02	−0.34	0.12	−0.29	0.19	−0.11	0.64
L-Threonine	−0.62	0.00	−0.42	0.05	−0.42	0.05	0.07	0.77
L-Methionine	−0.72	0.00	−0.66	0.00	−0.62	0.00	0.33	0.13
L-Asparagine	−0.49	0.02	−0.51	0.02	−0.55	0.01	0.24	0.28
Glutamine	−0.50	0.02	−0.38	0.08	−0.45	0.04	0.14	0.53
L-Lysine	−0.48	0.03	−0.41	0.06	−0.45	0.04	0.01	0.96
L-Tyrosine	−0.62	0.00	−0.35	0.11	−0.35	0.11	0.07	0.77
L-Tryptophan	−0.63	0.00	−0.49	0.02	−0.53	0.01	0.08	0.72
Butanoic acid	−0.39	0.07	−0.51	0.02	−0.48	0.02	0.35	0.12
Ethanedioic acid	−0.49	0.02	−0.36	0.10	−0.27	0.22	0.14	0.54
D-Glucose	−0.48	0.03	−0.32	0.15	−0.37	0.09	0.16	0.48
Lactulose	−0.56	0.01	−0.35	0.11	−0.42	0.05	0.24	0.29
Milk metabolites								
Galactinol	0.68	0.00	0.79	0.00	0.73	0.00	−0.68	0.00
cis-Aconitate	0.52	0.01	0.67	0.00	0.74	0.00	−0.3	0.18
Succinic acid	0.59	0.00	0.59	0.00	0.58	0.01	−0.41	0.06
Glycine	−0.23	0.31	−0.38	0.08	−0.37	0.09	0.01	0.96
Urea	0.19	0.40	0.48	0.03	0.49	0.02	−0.33	0.14
Pentanedioic acid	0.38	0.08	0.45	0.04	0.35	0.11	−0.63	0.00
s-2-Methyl-1-butanol	0.28	0.21	0.37	0.09	0.32	0.15	−0.39	0.08

Abbreviations: CAT, catalase; GPX, glutathione peroxidase; *r*, correlation coefficient; SOD, superoxide dismutase.

TABLE 6 Affected metabolic pathways based on Kyoto Encyclopedia of Genes and Genomes (KEGG) classification

Pathway name	Class
Valine, leucine, and isoleucine biosynthesis (serum)[1]	Amino acid metabolism
Valine, leucine, and isoleucine degradation (serum)	
Alanine, aspartate, and glutamate metabolism (serum)	
Phenylalanine, tyrosine, and tryptophan biosynthesis (serum)	
Glycine, serine, and threonine metabolism (serum)	
Pyruvate metabolism (serum)	Carbohydrate metabolism
Butanoate metabolism (serum)	
Galactose metabolism (milk)	
Citrate cycle (TCA cycle) (milk)	
Glyoxylate and dicarboxylate metabolism (milk)	
D-Glutamine and D-glutamate metabolism (serum)	Metabolism of other amino acids
Ubiquinone and other terpenoid-quinone biosynthesis (serum)	Metabolism of cofactors and vitamins
Aminoacyl-tRNA biosynthesis (serum)	Translation

[1]Serum: changes in metabolic pathways using serum metabolites; milk: changes in metabolic pathways using milk metabolites.

alone is primarily pyruvate metabolism (D-lactic acid). Compared with L-lactic acid, D-lactic acid is less studied. From the KEGG database, it was found that pyruvate is a bridge substance between D-lactic acid and tricarboxylic acid cycle. The conventional view is that D-lactic acid is not well metabolized by mammals. But there are also opinions that D-lactic acid can be metabolized in humans, rats, and cattle (Julia et al., 2005; Harmon et al., 1984). The metabolism and excretion of D-lactic acid in mammals are still not clear. Whether the significant negative correlation between D-lactic acid and ROS is related to energy generated in cow deserves further analysis. In butanoate metabolism, butanoic acid is first converted to butyryl-CoA and then enters the TCA cycle through crotonoyl-CoA. L-leucine, L-isoleucine, and L-valine can provide energy to animals by the TCA cycle as well. In addition, L-leucine, L-isoleucine, and L-valine were negatively related to antioxidase. From the KEGG database, it was found that L-leucine, L-isoleucine, and L-valine were involved in the synthesis of aminoacyl-tRNA and may provide precursors for protein synthesis. The low levels of L-leucine, L-isoleucine, and L-valine may be attributed to the synthesis of antioxidase in dairy cows. van Dijk et al. (2016) found that a leucine-rich whey diet intervention can improve muscle function and quality in aging mice lacking antioxidases. Jiang et al. (2017) found that dietary supplementation with leucine reduces MDA and ROS levels and improves the synthesis of SOD, GPX, and CAT. As mentioned above, aminoacyl-tRNA biosynthesis seemed to be an important metabolic pathway for cow antioxidases synthesis. Therefore, antioxidase synthesis is partially depended on these amino acids. L-Methionine was also found negatively related to ROS and antioxidases in this study which may attributed to its side chain that has a sulphur atom (with two lone electron pairs) (Xu et al., 2017). It was reported that methionine metabolism can alter oxidative stress resistance via the pentose phosphate pathway (Campbell et al., 2016). Moreover, increasing the supply of methionine and arginine was effective in attenuating the proinflammatory response in bovine mammary epithelial cells in vitro (Dai et al., 2020). L-Tyrosine is a precursor of the synthetic ubiquinone-n substance in the ubiquinone and other terpenoid-quinone biosynthesis pathways. Ubiquinone (UQ), also known as coenzyme Q, is an electron carrier in oxidative phosphorylation and is related to antioxidases (Grivennikova et al., 2017). Changes in the metabolism of amino acids and carbohydrates may be due to adjustments made by animals in response to antioxidase synthesis.

4.2 | Correlation of oxidative stress-related indicators with milk metabolites

Succinic acid and cis-aconitate are important metabolic cycling compounds in the TCA cycle. Their changes indicated that oxidative stress could enhance the TCA cycle of breast cells. This study found that glycine has a negative correlation with serum GPX and SOD, indicating that oxidative stress has changed the metabolism of glycine in mammary gland cells of dairy cows. KEGG revealed that glycine is involved in the glyoxylate and dicarboxylate metabolism in which cis-aconitate and glycine can be converted into each other. Glycine can provide not only energy for the metabolism of mammary gland cells, but also a precursor for protein synthesis and some enzyme synthesis. Senthilkumar et al. (2004) found that antioxidase activities (GPX, SOD, and CAT) in the liver and brain of glycine supplemented rats were significantly increased. Wang et al. (2014) found that dietary glycine under conditions of oxidative stress and glycine deficiency provides a biochemical mechanism for improving gut health in pigs by increasing glutathione (GSH) concentration. It was reported that glycine could protect diabetic β-cells against damage caused by oxidative stress by increasing glycine transporter-1-mediated synthesis of GSH and by reducing glycine receptor-mediated ROS production (Chen et al., 2018). According to the above studies, glycine seemed to be an important amino acid regulating oxidative stress in animal cells.

4.3 | Correlation of oxidative stress-related indicators and metabolites with milk composition

The correlation between milk fat and ROS was weak, while antioxidases were significantly negatively correlated with milk fat yield. Several serum metabolites (butanoic acid, L-isoleucine, and L-methionine) were found negatively correlated with antioxidase (CAT, SOD, and GPX), but positively correlated with milk fat yield. Butanoic acid is a well-known precursor of milk fat synthesis. For healthy ruminants, supplementing isoleucine and methionine could increase milk (Robinson et al., 1999) or lactose (Xu et al., 2019) production. There were few reports on the effect of L-isoleucine and L-methionine supplementation on lactation performance of dairy cows under oxidative stress. However, it was reported that unhealthy cows (such as with inflammation or oxidative stress) need multiple nutrients to repair damage and indirectly lose some of their lactation properties (Li et al., 2021, Memon et al., 2019, Sordillo & Raphael, 2013, Zahrazadeh et al., 2018).

5 | CONCLUSIONS

This study gave us a better understanding on oxidative stress that ROS not only increased oxidative damage (MDA) in dairy cows, but also altered some metabolites involved in amino acid and carbohydrate metabolism. Further studies are needed to provide more details on the mechanisms of the oxidative stress damage in digestive function and mammary gland health. And the roles of L-isoleucine and L-leucine in oxidative stress alleviation and their potential as biomarkers for the evaluation of oxidative stress status are undergoing now in our lab.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

ETHICS STATEMENT

Animals were managed according to the guidelines for the care and use of experimental animals of Jilin Agricultural University (JLAUACUC-2018-018)

AUTHOR CONTRIBUTIONS

Sen Zheng was involved in data curation, methodology, and writing-original draft. Guixin Qin was involved in supervision and writing-review & editing. Yuguo Zhen was involved in conceptualization and project administration. Xuefeng Zhang was involved in resources and software. Xue Chen, Jianan Dong, and Chunlai Li were involved in formal analysis. Natnael Demelash Aschalew was involved in writing-review & editing. Tao Wang and Zhe Sun were involved in conceptualization, supervision, writing-original draft, and writing-review & editing. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

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REFERENCES

Cadenas, E., & Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radical Biology and Medicine*, *29*, 222–230. https://doi.org/10.1016/s0891-5849(00)00317-8

Campbell, K., Vowinckel, J., Keller, M. A., & Ralser, M. (2016). Methionine metabolism alters oxidative stress resistance via the pentose phosphate pathway. *Antioxidants & Redox Signaling*, *24*, 543–547. https://doi.org/10.1089/ars.2015.6516

Chen, L., Zhang, J. Q., Li, C. H., Wang, Z. W., Li, J. J., Zhao, D., Wang, S., Zhang, H., Huang, Y., & Guo, X. (2018). Glycine Transporter-1 and glycine receptor mediate the antioxidant effect of glycine in diabetic rat islets and INS-1 cells. *Free Radical Biology Medicine*, *123*, 53–61 https://doi.org/10.1016/j.freeradbiomed.2018.05.007

Dai, H., Coleman, D. N., Hu, L., Martinez-Cortés, I., Wang, M., Parys, C., Shen, X., & Loor, J. J. (2020). Methionine and arginine supplementation alter inflammatory and oxidative stress responses during lipopolysaccharide challenge in bovine mammary epithelial cells in vitro. *Journal of Dairy Science*, *103*(1), 676–689. https://doi.org/10.3168/jds.2019-16631

Grivennikova, V. G., Kozlovsky, V. S., & Vinogradov, A. D. (2017). Respiratory complex II: ROS production and the kinetics of ubiquinone reduction. *Biochimica et Biophysica Acta - Bioenergetics*, *1858*, 109–117. https://doi.org/10.1016/j.bbabio.2016.10.008

Harmon, D. L., Britton, R. A., & Prior, R. L. (1984). In vitro rates of oxidation and gluconeogenesis from l(+)- and d(−)lactate in bovine tissues. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, *77*, 365–368. https://doi.org/10.1016/0305-0491(84)90344-4

Jiang, W. D., Deng, Y. P., Zhou, X. Q., Liu, Y., Jiang, J., Kuang, S. Y., Tang, L., Wu, P., Zhang, Y.-N., & Feng, L. (2017). Towards the modulation of oxidative damage, apoptosis and tight junction protein in response to dietary leucine deficiency: A likely cause of ROS-induced gill structural integrity impairment. *Fish & Shellfish Immunology*, *70*, 609–620. https://doi.org/10.1016/j.fsi.2017.09.049

Julia, B. E., Jonathan, M. N., & Gordon, A. Z. (2005). D-Lactate in human and ruminant metabolism. *Journal of Nutrition*, *135*, 1619–1625. https://doi.org/10.1093/jn/135.7.1619

Kapuy, O., Papp, D., Vellai, T., Banhegyi, G., & Korcsmaros, T. (2018). Systems-level feedbacks of NRF2 controlling autophagy upon oxidative stress response. *Antioxidants*, *7*, 39. https://doi.org/10.3390/antiox7030039

Li, H., Zhang, Y., Li, R., Wu, Y., Zhang, D., Xu, H., Zhang, Y., & Qi, Z. (2021). Effect of seasonal thermal stress on oxidative status, immune response and stress hormones of lactating dairy cows. *Animal Nutrition*, *7*(1), 216–223. https://doi.org/10.1016/j.aninu.2020.07.006

Ludin, A., Gur-Cohen, S., Golan, K., Kaufmann, K. B., Itkin, T., Medaglia, C., Lu, X.-J., Ledergor, G., Kollet, O., & Lapidot, T. (2014). Reactive oxygen species regulate hematopoietic stem cell self-renewal, migration and development, as well as their bone marrow microenvironment. *Antioxidants & Redox Signaling*, *21*, 1605–1619. https://doi.org/10.1089/ars.2014.5941

Mavangira, V., & Sordillo, L. M. (2018). Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. *Research in Veterinary Science*, *116*, 4–14. https://doi.org/10.1016/j.rvsc.2017.08.002

Memon, M. A., Wang, Y., Xu, T. L., Ma, N. N., Zhang, H. M., Roy, A. C., Ul Aabdin, Z., & Shen, X. Z. (2019). Lipopolysaccharide induces oxidative stress by triggering MAPK and Nrf2 signalling pathways in mammary glands of dairy cows fed a high-concentrate diet. *Microbial Pathogenesis*, *128*, 268–275. https://doi.org/10.1016/j.micpath.2019.01.005

van Dijk, M., Dijk, F. J., Bunschoten, A., van Dartel, D. A. M., van Norren, K., Walrand, S., Jourdan, M., Verlaan, S., & Luiking, Y. (2016). Improved muscle function and quality after diet intervention with leucine-enriched whey and antioxidants in antioxidant deficient aged mice. *Oncotarget*, *7*, 17338–55. https://doi.org/10.18632/oncotarget.7800

Moolchandani, A. (2018). A Review: Oxidative stress during lactation in dairy cattle. *Journal of Dairy & Veterinary Sciences*, *5*, 555669. https://doi.org/10.19080/jdvs.2018.05.555669

Nie, W., Yan, L., Lee, Y. H., Guha, C., Kurland, I. J., & Lu, H. (2016). Advanced mass spectrometry-based multi-omics technologies for exploring the pathogenesis of hepatocellular carcinoma. *Mass Spectrometry Reviews*, *35*, 331–349. https://doi.org/10.1002/mas.21439

Radi, R. (2018). Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proceedings of the National Academy of Sciences*, *115*, 5839–5848. https://doi.org/10.1073/pnas.1804932115

Ratnam, D. V., Ankola, D. D., Bhardwaj, V., Sahana, D. K., & Kumar, M. (2006), Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. *Journal of Controlled Release*, *113*, 189–207. https://doi.org/10.1016/j.jconrel.2006.04.015

Robinson, P. H., Chalupa, W., Sniffen, C. J., Julien, W. E., Sato, H., Fujieda, T., Watanabe, K., & Suzuki, H. (1999). Influence of postruminal supplementation of methionine and lysine, isoleucine, or all three amino acids on intake and chewing behavior, ruminal fermentation, and milk and milk component production. *Journal of Animal Science*, *77*, 2781–2792. https://doi.org/10.2527/1999.77102781x

Senthilkumar, R., Viswanathan, P., & Nalini, N. (2004). Effect of glycine on oxidative stress in rats with alcohol induced liver injury. *Pharmazie*, *59*, 55–60.

Soga, T., Baran, R., Suematsu, M., Ueno, Y., Ikeda, S., Sakurakawa, T., Kakazu, Y., Ishikawa, T., Robert, M., Nishioka, T., & Tomita, M. (2006). Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *Journal of Biological Chemistry*, *281*, 16768–16776. https://doi.org/10.1074/jbc.M601876200

Sordillo, L. M., & Raphael, W. (2013). Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. *Veterinary Clinics of North America. Food Animal Practice*, *29*, 267–278. https://doi.org/10.1016/j.cvfa.2013.03.002

Turk, R., Piras, C., Kovacic, M., Samardzija, M., Ahmed, H., De Canio, M., Urbani, A., Meštrić, Z. F., Soggiu, A., Bonizzi, L., & Roncada, P. (2012). Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis. *Journal of Proteomics*, *75*, 4412–4428. https://doi.org/10.1016/j.jprot.2012.05.021

Wang, W., Wu, Z., Lin, G., Hu, S., Wang, B., Dai, Z., & Wu, G. (2014). Glycine stimulates protein synthesis and inhibits oxidative stress in pig small intestinal epithelial cells. *Journal of Nutrition*, *144*, 1540–1548. https://doi.org/10.3945/jn.114.194001

Xu, L. B., Hanigan, M. D., Lin, X. Y., Li, M. M., Yan, Z. G., Hu, Z. Y., & Wang, Z. H. (2019). Effects of jugular infusions of isoleucine, leucine, methionine, threonine, and other amino acids on insulin and glucagon concentrations, mammalian target of rapamycin (mTOR) signaling, and lactational performance in goats. *Journal of Dairy Science*, *102*, 9017–9027. https://doi.org/10.3168/jds.2018-16102

Xu, N., Chen, G., & Liu, H. (2017). Antioxidative categorization of twenty amino acids based on experimental evaluation. *Molecules*, *22*, 2066. https://doi.org/10.3390/molecules22122066

Zahrazadeh, M., Riasi, A., Farhangfar, H., & Mahyari, S. A. (2018). Effects of close-up body condition score and selenium-vitamin E injection on lactation performance, blood metabolites, and oxidative status in high-producing dairy cows. *Journal of Dairy Science*, *101*, 10495–10504. https://doi.org/10.3168/jds.2017-14196

Zhang, H., Wu, L., Xu, C., Xia, C., Sun, L., & Shu, S. (2013). Plasma metabolomic profiling of dairy cows affected with ketosis using gas chromatography/mass spectrometry. *BMC Veterinary Research*, *9*, 186. https://doi.org/10.1186/1746-6148-9-186

Zhao, W., Chen, X., Xiao, J., Chen, X. H., Zhang, X. F., Wang, T., Zhen, Y. G., & Qin, G. X. (2019). Prepartum body condition score affects milk yield, lipid metabolism, and oxidation status of Holstein cows. *Asian-Australasian Journal of Animal Sciences*, *12*, 1889–1896. https://doi.org/10.5713/ajas.18.0817

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