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Heat stress affects the milk yield, milk composition, serum oxidative status, and metabolites of Holstein cows during mid-lactation

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Abstract: Seasonal heat stress and metabolic disorders during mid-lactation are the main factors limiting production in Holstein cows, and several proteins and molecules involved in metabolic pathways are altered in response to stress. We investigated the effects of heat stress on the milk yield, milk composition, serum oxidative status, and metabolites in Holstein cows during mid-lactation to identify biomarkers associated with heat stress in serum and milk. Holstein cows with similar body condition scores (3.0 ± 0.25), parity (2.5 ± 0.5), and lactation days (115 ± 5 days) were selected in August (heat stress, HS, n = 20) 2017 and March 2018 (non-heat stress, NHS, n = 20). Milk yield was recorded daily and serum was collected on days 1, 31, and 61. Serum and milk metabolites were analysed by gas chromatography-mass spectrometry on day 1. The results showed a significantly lower average daily milk yield in the HS group than in the NHS group ($P < 0.05$). The milk compositions of fat (%), lactoprotein, lactose yield, and milk solid-not-fat in the HS group were significantly lower than in the NHS group on days 1, 31, and 61 ($P < 0.05$). The levels of malondialdehyde were higher, whereas those of superoxide dismutase and glutathione peroxidase were lower in the serum of the HS group ($P < 0.05$) than that of the NHS group. The serum concentrations of D-glucose,

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9,12-octadecadienoic acid, and D-lactose were significantly higher in the NHS group than in the HS group ($P < 0.05$). The concentrations of lactic acid and milk urea nitrogen in the NHS group were lower than those in the HS group ($P < 0.05$). The present data suggest that metabolic biomarkers are closely associated with heat stress in the serum and milk, which provides a basis for evaluating indicators of heat stress occurrence in mid-lactation cows.

Keywords: lactation performance; serum and milk metabolites; oxidative stress; dairy cow

During hot weather (summer), temperature and humidity are key factors in heat stress (HS). A temperature-humidity index of 72 is considered the threshold for HS in lactating cows (Armstrong 1994). The occurrence of HS increases sweating and drinking water, and then causes the rumen to be full of water, which leaves insufficient space for feed, leading to a reduction in feed intake, rumination, and nutrient absorption, increasing the energy required to maintain homeostasis (Atrian and Shahryar 2012). Gantner et al. (2019) reported that HS decreased the daily milk yield and milk composition of Holstein cows, which was specifically reflected by a decrease in milk fat and milk protein. In addition to milk production, the influence of HS on oxidative status and metabolites has also been investigated. Bernabucci et al. (2002) reported that HS increased oxidative stress. Calamari et al. (1999) indicated that HS impairs blood antioxidant activity in dairy cows during mid-lactation. In addition, Liu et al. (2017) analysed milk lipid metabolites using liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry (GC-MS) and found that heat stress alters the triacylglycerol profile and synthesis of fatty acids (FAs) in milk. According to Tian et al. (2016), HS induces metabolic disorders and affects potential biomarkers as reflected by altered mammary gland function.

Abeni et al. (2007) indicated that the milk yield of cows in mid-lactation was more susceptible to heat stress than that during early lactation. Yan et al. (2021) reported that multiparous cows, especially those above the second parity, exhibited respiration rates that were more sensitive to heat stress than those of primiparous cows. However, there are few published analyses of the effects of HS on metabolites, particularly serum and milk metabolites. Therefore, in the present study, we aimed to explore the effects of HS on lactational performance, oxidative status, and metabolites levels in multiparous cows during mid-lactation.

MATERIAL AND METHODS

The experiment was approved by the Jilin Agricultural University Animal Care and Use Committee (JLAU-ACUC2018-008, Changchun, China).

Animals and experimental design

This experiment was carried out on a commercial dairy farm in Shandong Province, China (118°5'E, 38°15'N, 8.8 m altitude). A total of 40 Holstein dairy cows with similar body condition scores (3.0 ± 0.25), parity (2.5 ± 0.5), lactation days (115 ± 5 days), and milk yield were selected in August 2017 (heat stress group, HS; $n = 20$), and March 2018 (non-heat stress group, NHS; $n = 20$). Animals in the same group were raised in the same barn, 300 m in length and 31.0 m in width. Fans were installed 2.5 m above the ground and 6.0 m apart in the long axis direction and the fan operated when the air temperature was above 20 °C. The cows were fed a total mixed ration (TMR) and a lactation diet (Table 1) with clean water *ad libitum* during the experimental period. The cows were milked at approximately 6:00, 12:00, 18:00, and 0:00, and individual milk yields were recorded daily. Air temperature and humidity were recorded three times per day at 1.5 m above the ground at both ends and in the middle of the cowshed. The average daily temperature humidity index (THI) was calculated using the equation:

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.005\,5 \times \times RH) \times (1.8 \times T_{db} - 26.8)] \quad (1)$$

where:

T_{db} – dry-bulb temperature (°C);

RH – relative humidity (%) (Chen et al. 2022).

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Table 1. Ingredients and chemical composition of the experimental diets

Ingredients	DM (%)
Oat grass	3.37
Alfalfa hay	6.73
Cottonseed	5.66
Brewer's grains	2.19
Beet	2.25
Corn silage	25.09
Molasses	2.39
Corn meal	25.57
Fat powder	2.10
Distillers dried grains with soluble	0.99
Soya bean meal	8.05
Cotton seed meal	4.65
Expanded soybean	1.65
Premix[1]	2.91
$NaHCO_3$	0.73
Yeast culture	0.07
Methionine	0.04
Mycotoxin adsorbent	0.04
Concentrate supplement	5.59
Chemical composition (%DM)	
Crude protein	16.20
Starch	25.10
Ether extract	5.20
Metabolizable energy (Mcal/kg)	2.76
Net energy for lactation (Mcal/kg)	1.78

[1]Premix composition (per kilogram): vitamin A, 360 000 IU; vitamin D_3, 135 000 IU; vitamin E, 2 160 IU; Cu, 510 mg; Zn, 2 300 mg; Mn, 630 mg; Co, 10 mg; I, 35 mg; Se, 15 mg; Ca, 150 g; P, 30 g; NaCl, 120 g

Sample collection and analysis

Milk and serum samples were collected on days 1, 31, and 61. Milk yield was monitored daily and milk composition was assessed using a milk analyzer (MCC; Lactoscan, Nova Zagora, Bulgaria). Blood was individually sampled from the coccygeal veins 3–4 h after the morning feed. Serum was collected after centrifuging at 3 000 rpm at 4 °C for 10 minutes. Superoxide dismutase (SOD), glutathione peroxidase (GSX-Px), and malondialdehyde (MDA) levels were analysed using commercial bovine ELISA kits (Shanghai Enzyme-Linked Biotechnology Co., Ltd, Shanghai, China). Serum and milk metabolites were analysed by GC-MS.

GC/MS analysis

Serum samples were prepared as described previously (Chen et al. 2019). Milk (15 ml) was ultrasonically homogenised for 15 min, and a 100 µl sample was mixed with 250 µl methyl alcohol and 125 µl chloroform. After shaking for 1 min, 380 ul of chloroform and 90 µl of potassium chloride solution (14.8 g/l) were added. After standing for approximately 10 min at –20 °C, the mixed solution was centrifuged at 12 000 × *g* at 4 °C for 10 min. Liquid chloroform and water layers (250 ml each) were dried by vacuum freeze-drying at –55 °C. An 80 µl solution of methoxyamine hydrochloride and pyridine was added to the serum samples and the solution was heated in a water bath at 37 °C for 120 minutes. Then, 80 µl of BSTFA was added and the entire solution was incubated in a water bath at 60 °C for 60 minutes. The samples were centrifuged at 13 000 × *g* at 4 °C for 5 min, and then 50 µl of supernatant from each sample was transferred to a GC vial.

Each sample (1 µl) was injected into an Agilent 7890/5975C system (Agilent Technologies, Santa Clara, CA, USA) for GC-MS analysis. Detailed information regarding the fused silica capillary column, carrier gas, ion source, and temperature program was the same as that reported by Chen et al. (2019).

The temperature program for milk analysis was as follows: water layer – the temperature of the column was maintained at 80 °C for 0.5 min, then increased to 180 °C at a rate of 11 °C/min for 2 min, increased to 240 °C at a rate of 6 °C/min for 4 min, increased to 270 °C at a rate of 5 °C/min, and ultimately increased to 270 °C at a rate of 1 °C/min for 5 minutes.

Chloroform layer – the temperature of the column was maintained at 80 °C for 6 min, then increased to 270 °C at a rate of 5 °C/min, and increased to 270 °C at a rate of 1 °C/min for 5 minutes.

Statistical analysis

Data on milk yield, milk composition, and the oxidative stress index were analysed using independent *t*-tests and SPSS software (v17.0; SPSS Statistics for Windows, Chicago, IL, USA). Statistical significance was set at $P < 0.05$. Metabolite data were obtained by comparison with the NIST11 library,

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deduced in EXCEL, and organised into a matrix format. Principal component analysis (PCA) was performed using SIMCA software v13.0 (Sartorius AG, Göttingen, Germany). Discriminating metabolites were selected according to their S-plots and variable importance in the projection values (VIP > 1), and Student's *t*-test ($P < 0.05$) was used to determine the significance of metabolites between the HS and NHS groups. Candidate metabolites were analysed using Metaboanalyst v4.0 (http://www.metaboanalyst.ca) for path enrichment and topology analysis. Metabolic pathway analysis was performed by inputting the identified substances into the Kyoto Encyclopedia of Genes and Genomes website (KEGG, http://www.kegg.com). Spearman's correlation analysis was used to evaluate the correlation between blood metabolism parameters and the oxidative stress index, milk metabolism parameters, and milk composition.

RESULTS

Milk yield and composition

The THI in the HS group gradually decreased with increasing lactation duration (Figure 1). During the experimental period, the THI of the HS group was > 72 on days 1 to 30 and reached 81.87 on day 8, suggesting that the cows were undergoing heat stress, while the THI of the NHS group was < 72 throughout the experiment. The average daily milk yield in the NHS group was higher than that in the HS group throughout the experimental period. According to Table 2, the average milk yield in the NHS group was significantly higher than that in the HS group on days 1, 31, and 61. Compared with the NHS group, milk yield, milk fat composition (%), lactoprotein, lactose yield, and milk solids-not-fat (MSNF) in the HS group were significantly lower on days 1, 31, and 61 ($P < 0.05$); however, no significant difference was found in lactose between the two groups ($P > 0.05$).

Compared with the NHS group, milk yield and composition in the HS group decreased on days 1, 31, and 61, with an average decrease of 9.46%, 9.39%, and 14.79%, respectively (Figure 2). The reduction in milk lactose yield in the HS group was more pronounced than that of milk fat composition, lactoprotein, and MSNF when compared with the NHS group, and decreased by 9.88%, 9.56%, and 14.47% on days 1, 31, and 61, respectively. The percent decline in milk yield and composition on day 61 was the highest relative to that at other times during the experimental period. Figure 3 shows the changes in milk yield and composition in the NHS and HS groups on day 61 compared with day 1. Milk yield in both the NHS and HS groups was lower on day 61 than on day 1 during the experimental period, however, the decrease in milk yield was more pronounced in the HS group. The milk lactose yield and MSNF in both the NHS and HS groups decreased, with a decrease of 7.11% and 11.84% on day 61, respectively, compared with day 1. The milk fat composition and lactoprotein content in the NHS group increased by 1.08% and 0.93%, respectively; however,

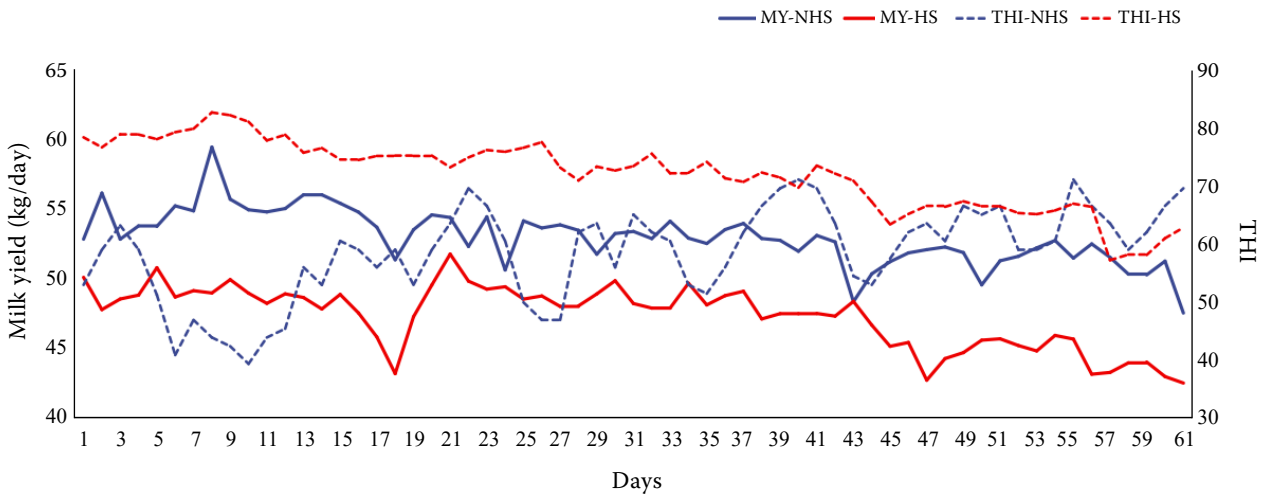

Figure 1. Average daily milk yield from days 1 to 61 during the experimental period
HS = heat-stressed; MY = milk yield; NHS = non-heat-stressed; THI = temperature-humidity index

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Table 2. Effect of heat stress on milk yield and composition of dairy cows during mid-lactation

	Group	Day 1	Day 31	Day 61
Average milk yield (kg/day)	NHS	53.80 ± 1.34*	53.23 ± 0.50*	49.74 ± 1.39*
	HS	49.15 ± 1.21	48.66 ± 0.97	43.33 ± 0.64
Fat (%)	NHS	3.69 ± 0.27*	3.67 ± 0.34*	3.73 ± 0.29*
	HS	3.48 ± 0.33	3.47 ± 0.30	3.45 ± 0.23
Lactoprotein (%)	NHS	3.21 ± 0.11*	3.19 ± 0.12*	3.24 ± 0.14*
	HS	3.08 ± 0.10	3.07 ± 0.13	3.05 ± 0.10
Lactose (%)	NHS	4.70 ± 0.20	4.71 ± 0.15	4.73 ± 0.16
	HS	4.63 ± 0.16	4.66 ± 0.19	4.65 ± 0.15
Lactose yield (kg/day)	NHS	2.53 ± 0.06*	2.51 ± 0.02*	2.35 ± 0.07*
	HS	2.28 ± 0.06	2.27 ± 0.05	2.01 ± 0.03
Solid-not-fat (%)	NHS	8.84 ± 0.22*	8.79 ± 0.19*	8.83 ± 0.22*
	HS	8.57 ± 0.16	8.60 ± 0.20	8.56 ± 0.24

HS = heat-stressed; NHS = non-heat-stressed

*Same measure was significantly different ($P < 0.05$) with in the same column

those in the HS group decreased by 0.86% and 0.97%, respectively, on day 61 compared with day 1.

Oxidative and antioxidative indices

As shown in Figure 4, the levels of superoxide dismutase and GSH-Px in the serum of the heat-stressed group were significantly lower than those in the non-heat-stressed group during the experimental period ($P < 0.05$), whereas malondialdehyde levels were higher in the heat-stressed group ($P < 0.05$). However, no significant alterations were found in serum oxidative indices with a reduction in temperature-humidity index ($P > 0.05$).

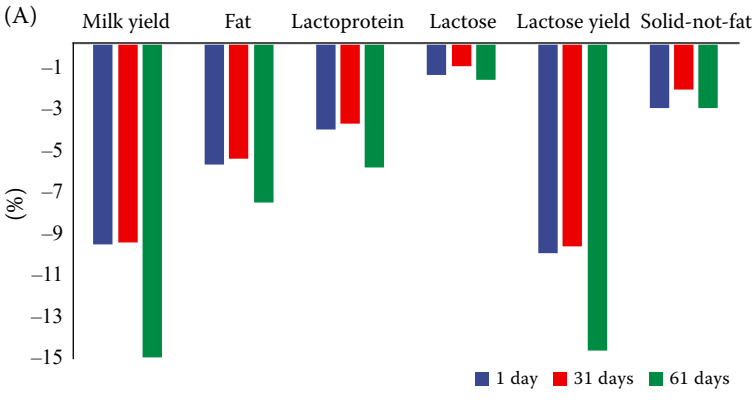

Figure 2. Reduction rate in milk yield and composition of the heat-stressed group compared with the non-heat-stressed group

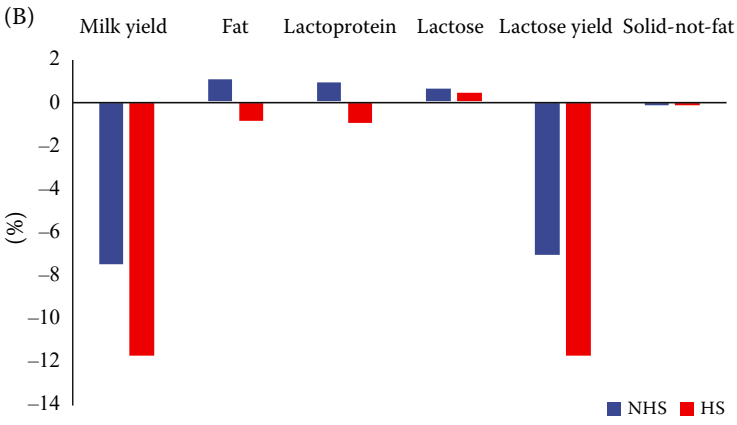

Figure 3. Changes of milk yield and composition in NHS and HS groups on day 61 compared to day 1

HS = heat-stressed; NHS = non-heat-stressed

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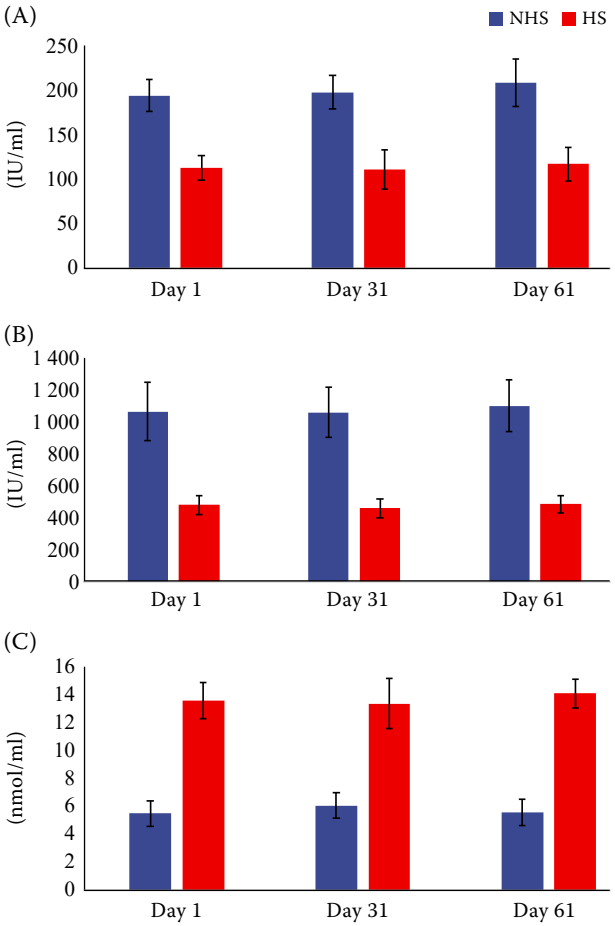

Figure 4. Effect of heat stress on serum oxidative indices of dairy cows during mid-lactation

(A) Superoxide dismutase (IU/ml); (B) glutathione peroxidase (IU/ml); (C) malondialdehyde (nmol/ml)

HS = heat-stressed; NHS = non-heat-stressed

*Indicates that the same measure was significantly different ($P < 0.05$) within the same time

Serum and milk metabolites

The PCA score plots showed clearly separated clusters with 95% confidence in the serum and water/chloroform layers of the milk samples between the HS and NHS groups (Figure 5). Each dot represents one sample and samples outside the 95% confidence interval were removed. Ultimately, 19 serum samples from the HS group, 18 serum samples from the NHS group, and 12 milk samples each from the HS and NHS groups were analysed. The serum and water/chloroform layers of milk samples were significantly separated in the PCA score plots.

Fourteen and 11 significantly altered metabolites (VIP > 1, $P < 0.05$) were detected in the serum and milk of the HS and NHS groups, respectively.

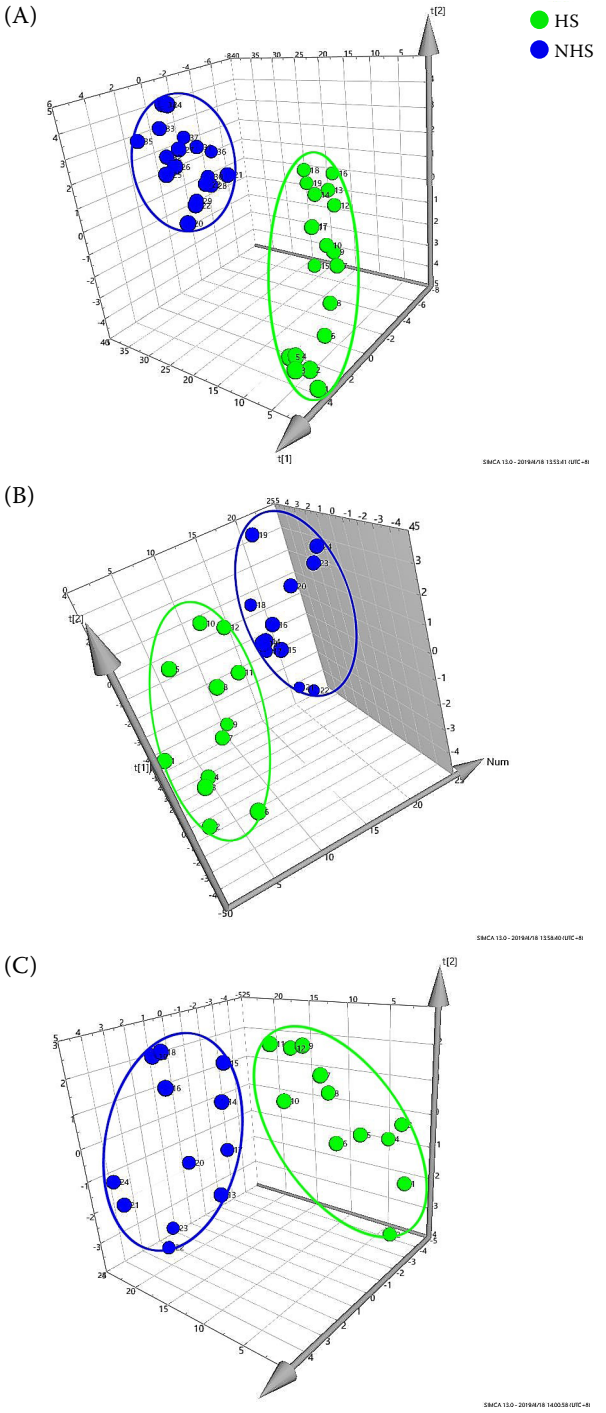

Figure 5. Principal component analysis (PCA) score plots of serum and milk samples serum between heat-stressed (HS) and non-heat-stressed (NHS) groups

(A) Serum; (B) water layer in milk; (C) chloroform layer in milk

As shown in Figure 6, three serum metabolic biomarkers (D-glucose, D-lactose, and 9,12-octadecadienoic acid), were significantly increased in the NHS group ($P < 0.05$), whereas, the other ten metabolic

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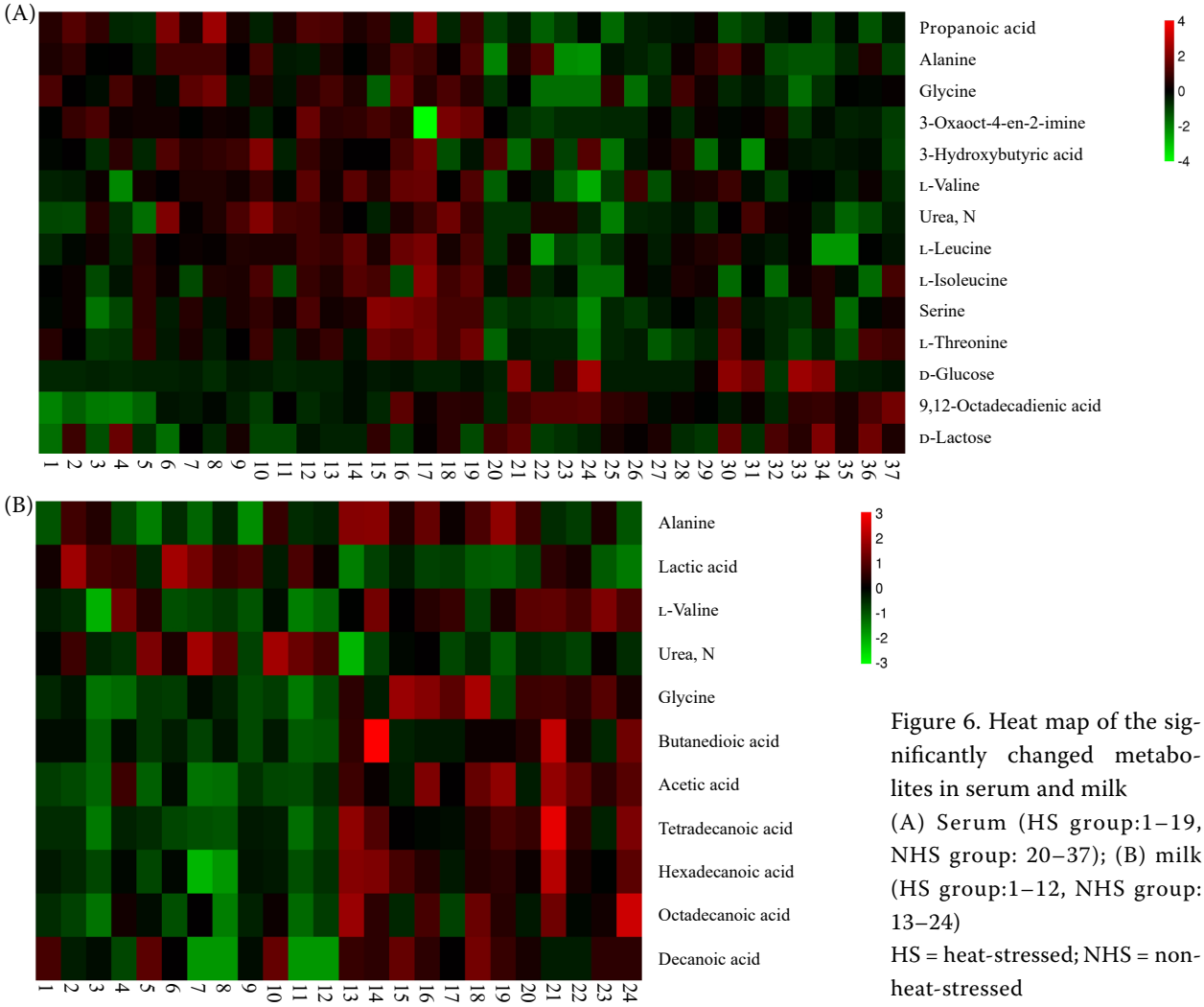

Figure 6. Heat map of the significantly changed metabolites in serum and milk
(A) Serum (HS group:1–19, NHS group: 20–37); (B) milk (HS group:1–12, NHS group: 13–24)
HS = heat-stressed; NHS = non-heat-stressed

biomarkers were significantly decreased in the HS group ($P < 0.05$). Except for lactic acid and urea nitrogen, which were significantly lower in milk, other metabolic metabolites were significantly higher in the NHS group than in the HS group ($P < 0.05$).

The metabolites in the serum and milk revealed the enrichment of eight and five pathways ($P < 0.05$, and an impact value > 0), respectively (Figure 7). Metabolic parameters in the serum were principally related to five KEGG pathways: translation, amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, and lipid metabolism. Those in milk were primarily involved in four KEGG pathways: lipid metabolism, translation, carbohydrate metabolism, and amino acid metabolism (Table 3).

The metabolic network involved in the typical KEGG pathway in the serum of HS cows is shown in (Figure 8). The metabolites in red indicate an increasing abundance of metabolites and green indicates a decrease in their abundance. Purple arrows indicate promotion of metabolic pathways and yellow arrows indicate restraint of metabolic pathways. The results of metabolic pathway analysis showed that HS promoted glycolysis and lipid metabolism provided energy to satisfy the maintenance requirements of cows, and increased the conversion of amino acids to glucose via gluconeogenesis, except for milk protein synthesis.

Correlation analysis

According to Spearman's correlation coefficients, the serum metabolites appeared to be significantly correlated with oxidative and antioxidative indices ($P < 0.05$, Figure 9A). MDA was negatively correlated with D-glucose, 9,12-octadecadienoic acid, and D-lactose levels, and positively correlated with other serum metabolites. The correlation between the met-

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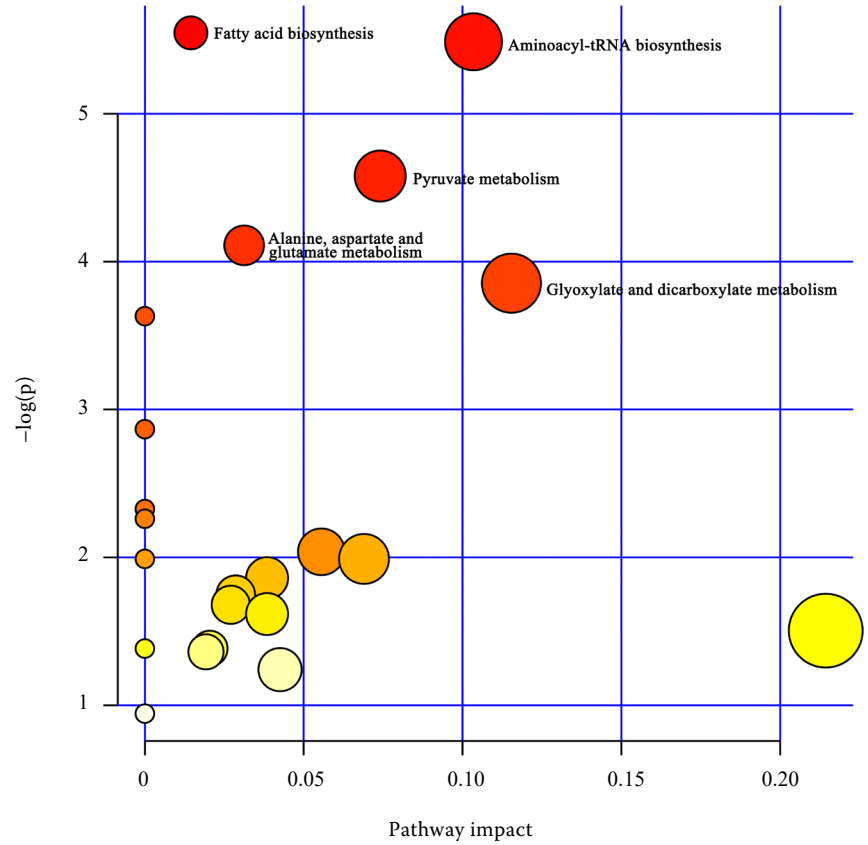
(A)



(B)

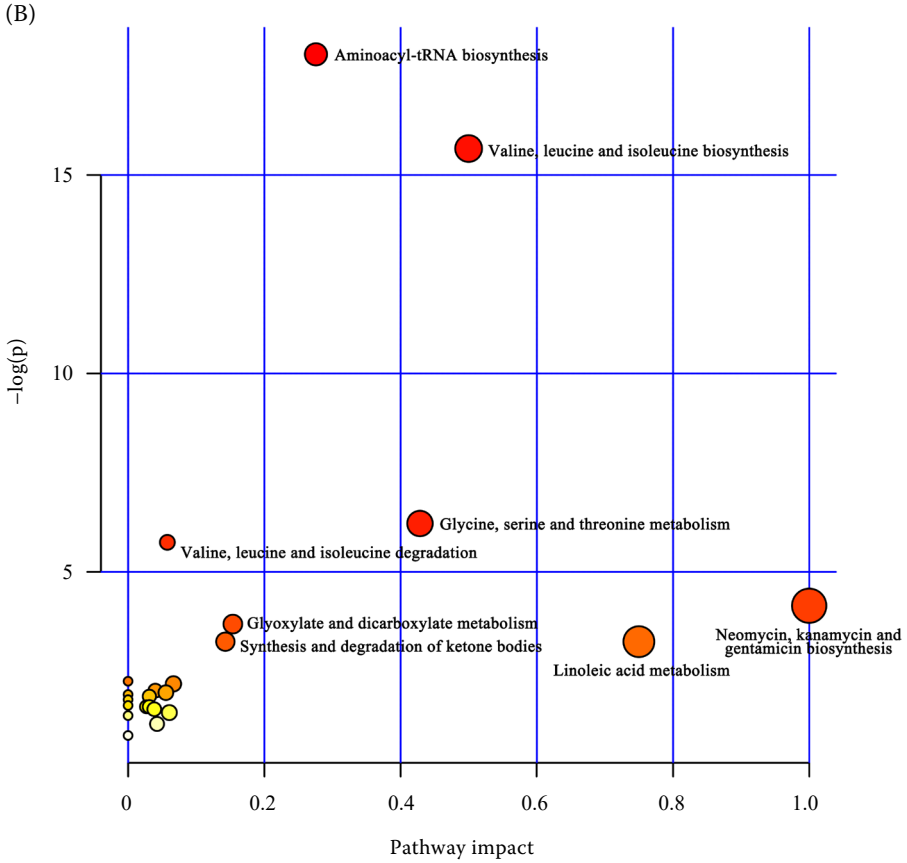


Figure 7. Pathway-topology analysis related to serum and milk
(A) Serum; (B) milk

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Table 3. Affected metabolic pathways based upon KEGG classification

Class	Pathway name	Metabolites	*P*-value	Impact value
Serum				
Translation	aminoacyl-tRNA biosynthesis	glycine; serine; L-valine; alanine; L-isoleucine; L-leucine; L-threonine	< 0.01	0.28
Amino acid metabolism	valine, leucine, and isoleucine biosynthesis	L-threonine; L-leucine; L-isoleucine; L-valine	< 0.01	0.50
	glycine, serine and threonine metabolism	serine; glycine; L-threonine	< 0.01	0.43
	valine, leucine and isoleucine degradation	L-valine; L-isoleucine; L-leucine	< 0.01	0.06
Biosynthesis of other secondary metabolites	neomycin, kanamycin and gentamicin biosynthesis	D-glucose	< 0.05	1.00
Carbohydrate metabolism	glyoxylate and dicarboxylate metabolism	serine; glycine	< 0.05	0.15
Lipid metabolism	synthesis and degradation of ketone bodies	3-hydroxybutanoate	< 0.05	0.14
	linoleic acid metabolism	9,12-octadecadienoic acid	< 0.05	0.75
Milk				
Lip metabolism	fatty acid biosynthesis	hexadecanoic acid; tetradecanoic acid; decanoic acid	< 0.01	0.01
Translation	aminoacyl-tRNA biosynthesis	glycine; L-valine; alanine	< 0.01	0.10
Carbohydrate metabolism	pyruvate metabolism	(R)-lactate; acetic acid	< 0.05	0.07
	glyoxylate and dicarboxylate metabolism	glycine; acetic acid	< 0.05	0.12
Amino acid metabolism	alanine, aspartate and glutamate metabolism	L-alanine; butanedioic acid	< 0.05	0.03

abolic biomarkers and GSH-Px and SOD was the opposite of that between MDA and its metabolites. As shown in Figure 9B, all milk metabolic parameters were significantly correlated with milk fat content ($P < 0.05$). Correlation analysis revealed that all milk metabolites, except lactic acid and urea, were positively correlated with milk composition ($P < 0.05$). Lactoprotein and lactose yields were positively correlated with milk metabolites, except for alanine and lactic acid ($P < 0.05$). Lactose positively correlated with butanedioic acid, acetic acid, tetradecanoic acid, hexadecanoic acid and octadecanoic acid ($P < 0.05$). MSNF positively correlated with L-valine and butanedioic acid, acetic acid, tetradecanoic acid, hexadecanoic acid, and octadecanoic acid ($P < 0.05$).

DISCUSSION

Effects of HS on milk yield and composition

Ravagnolo et al. (2000) reported that milk yield decreased by 0.2 kg per unit increase in the THI when THI was ≥ 72. Consistent with this, the present study found that milk yield decreased in the HS group during the experimental period. There are many explanations for the decline in milk production caused by HS, including the traditional view that it is due to a reduction in dry matter intake (DMI), or an increase in respiratory and sweating rates when cows are exposed to hot weather. In addition, peripheral blood flow in cows increases to enhance cooling, and mammary blood flow is reduced, both of which contribute to a lower milk yield (West 2003). Furthermore, HS results in reduced pH and increased lactate levels in the rumen fluid, which induces sub-acute ruminal acidosis and suppresses milk production (Zhao et al. 2019). Tao et al. (2018) indicated that HS affect the function of mammary gland cells, leading to a reduction in milk yield.

Declines in the percentages of milk fat, MSNF, and milk protein are associated with HS environments (Kadzere et al. 2002). Bernabucci et al. (2015) demonstrated a significant drop in milk protein levels in summer, especially in casein in mid-lactation Holstein cows. The decrease in milk composition may be due to the effect of HS on milk-component synthesis, either directly or indirectly, by influencing

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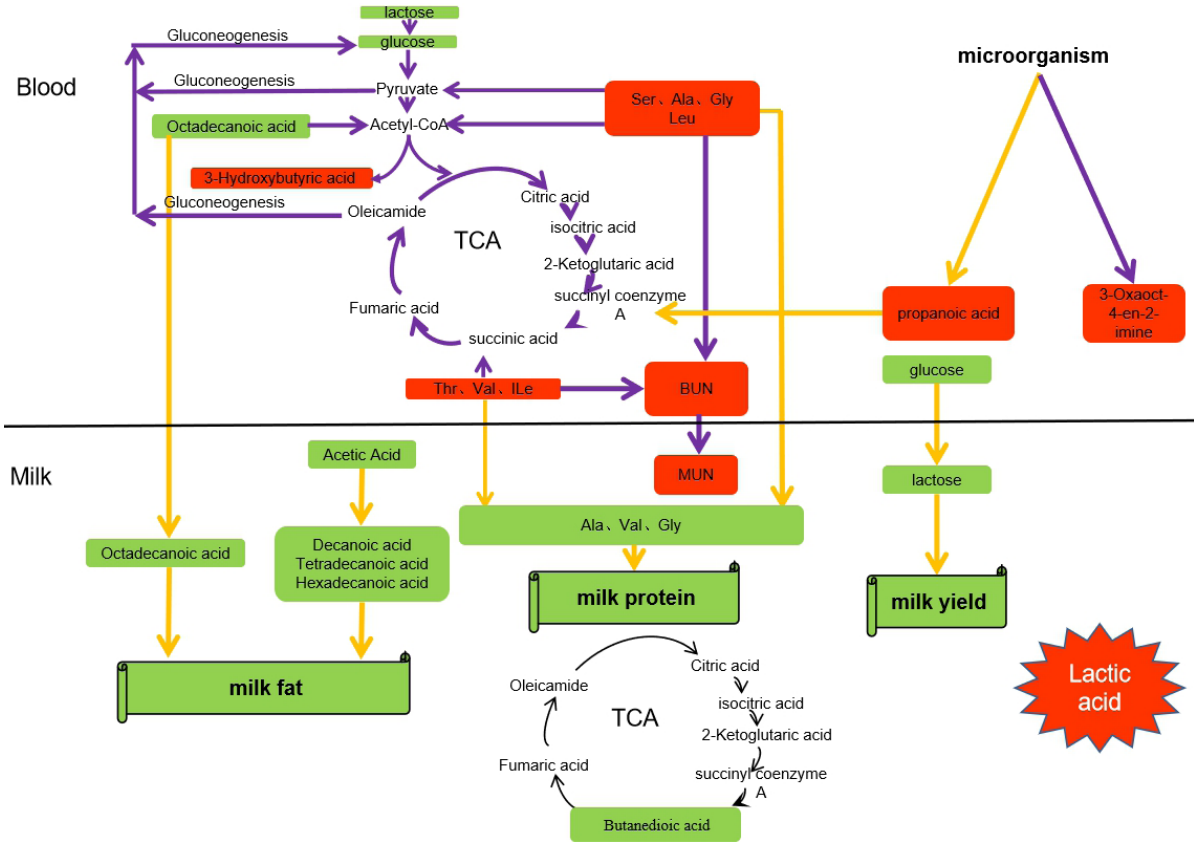

Figure 8. KEGG pathways linked together manually as reflected in blood and milk of dairy cows under heat stress

the delivery of the component precursors (Shwartz et al. 2009). Hu et al. (2016) reported that HS lowered the synthesis of total casein via the major milk protein genes. Lactose is the major carbohydrate present in milk and controls milk yield by maintaining milk osmolarity (Zhao and Keating 2007). However, when the hot season ended, milk fat, milk protein, non-fat solids, and total lactose content in the HS group did not improve significantly during the experimental period. In contrast, the decrease in milk yield and milk composition, such as milk fat composition, lactoprotein, and lactose yield in the HS group were more obvious than those in the NHS group. These results indicate that the effect of HS on the lactation performance of dairy cows was delayed and could not recover in a short time.

Effects of HS on serum oxidative and antioxidative indices

MDA is the end product of lipid peroxidation, and its content can indicate the oxidative status of dairy cows (Sharma et al. 2011). Similarly, increased oxidative stress, as indicated by increased MDA levels in HS cows, has been reported by Safa et al. (2019). As GSH-Px is an important antioxidant enzyme that protects against oxidative stress (Zhang et al. 2006), and as SOD can scavenge the superoxide radical ($\bullet O_2^-$) (Bernabucci et al. 2002), Guo et al. (2018) reported that HS decreased the levels of GSH-Px and SOD while increasing the level of MDA, which was similar to what we observed in the present study. Furthermore, increased MDA concentrations and decreased antioxidant enzymes in the HS group indicated an imbalance between oxidants and antioxidants, and the cows exhibited an increased risk of oxidative damage. However, we did not observe a decrease in MDA or an increase in the antioxidant index concomitant with a decreasing THI, which is consistent with our results regarding milk yield and composition.

Effects of HS on serum and milk metabolites

Amino acids are the building blocks of proteins that control milk-protein synthesis. In addition,

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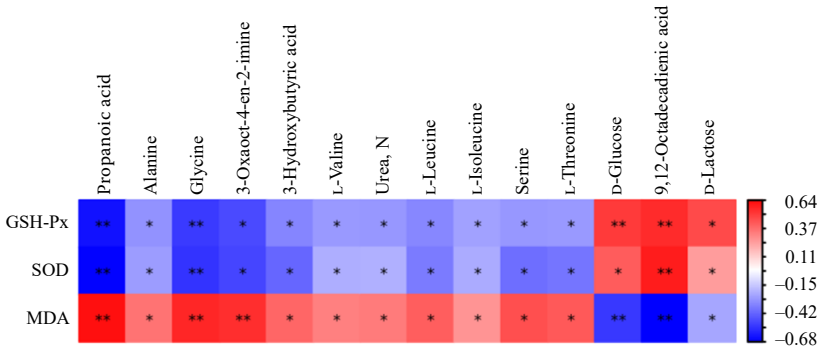
(A)



(B)

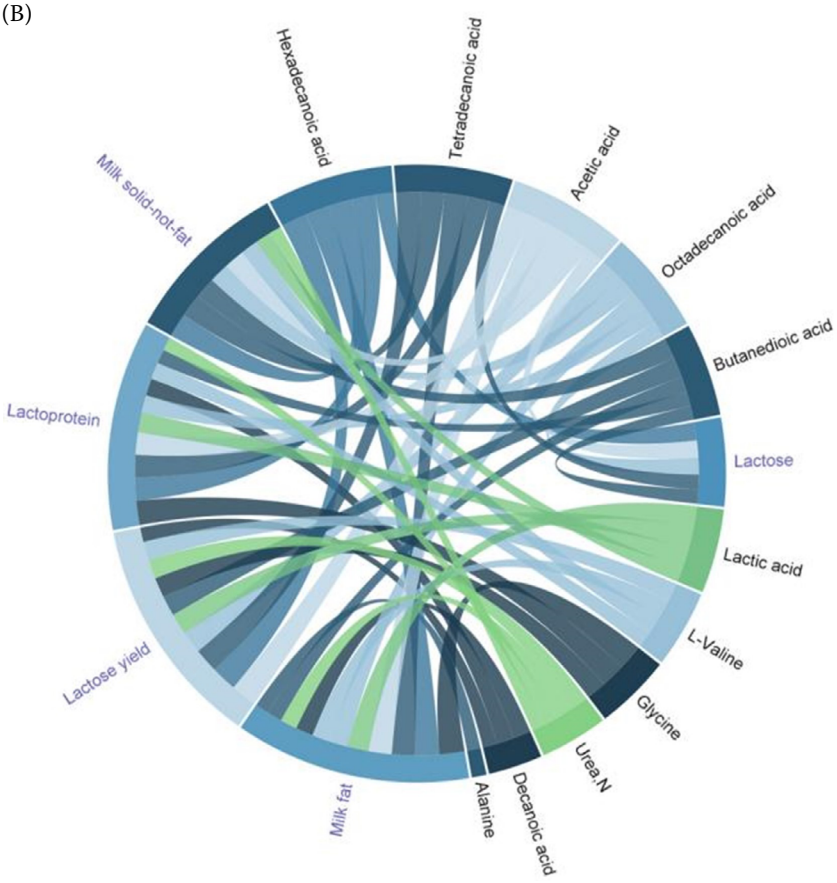


Figure 9. Correlation analysis
(A) Spearman correlation analysis between serum metabolic candidates and serum oxidative and antioxidative index affected by heat stress. (B) Circos showed the significant Spearman correlation between the milk metabolic candidates and milk composition affected by heat stress
*Significant correlation ($P < 0.05$)
**Significant correlation ($P < 0.01$)
The green curves mean a negative Spearman's correlation ($P < 0.05$), and other connected curves mean a positive Spearman's correlation ($P < 0.05$)

25% of the plasma flux is partitioned into the mammary glands (Bequette et al. 1997). In the present study, the levels of alanine, L-valine, and glycine in milk decreased in the HS group, which may be due to an increase in the amino acids in the blood that participate in gluconeogenesis; and a smaller supply of amino acids routed to the glands, all of which contribute to the losses of milk protein in dairy cows exposed to HS. Cowley et al. (2015) indicated that HS augmented milk urea nitrogen, which is consistent with the results of the current study. The increase in milk urea may be due to a decrease in DMI and an increase in energy maintenance requirements in cows exposed to HS (Muroya et al. 1997). Free fatty acids (FFAs), such as tetradecanoic acid, hexadecanoic acid, octadecanoic acid, and decanoic acid are the predominant FFAs in milk (Guler 2007), and the decrease in the levels of FFAs observed in the HS group may be due to the inhibition of fat metabolic pathways. The decrease in amino acids and FFAs in the HS group was consistent with the milk composition results. Glucose is the primary precursor for mammary gland lactose synthesis, and the mammary tissues of dairy cows extract approximately 20% of glucose from the blood (Osorio et al. 2016).

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Under HS, the decline in blood glucose and lactose concentrations resulted in an abatement of milk lactose content in our study. Furthermore, the decrease in serum glucose and lactose levels in the HS group may be due to the cows consuming more energy by promoting glycolysis for thermoregulation via panting and sweating (Beede and Collier 1986). Moreover, the concentrations of blood serine, alanine, glycine, and L-leucine associated with gluconeogenesis increased in the HS group, similar to the results reported by Guo et al. (2018). Koch et al. (2016) reported more intensive utilization of amino acids as precursors for gluconeogenesis at high ambient temperatures. Increased blood urea nitrogen in the serum might be the result of inefficient incorporation of rumen ammonia into microbial proteins or hepatic deamination of amino acids mobilized from skeletal muscles (Wheelock et al. 2010).

CONCLUSION

In summary, the current study indicates that HS reduces milk yield and composition of Holstein cows and destroys the oxidation balance. HS promotes glycolysis and lipid metabolism and provides energy for dairy cows maintenance. In addition, more amino acids were converted to glucose via gluconeogenesis, except during milk protein synthesis. In addition, the metabolites in milk and serum could be used as biomarkers to precisely monitor the HS status in mid-lactation cows.

Conflict of interest

The authors declare no conflict of interest.

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