Rumen-bypassed tributyrin alleviates heat stress by reducing the inflammatory responses of immune cells

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ABSTRACT Heat stress (**HS**) in summer will seriously affect the health and performance of dairy cows. To alleviate the injury to dairy cows caused by HS, we added the rumen-bypassed tributyrin to the feed. We determined whether cows were in a heat-stressed environment by testing the temperature humidity index in the morning, at noon, and in the evening. The detection of anal temperature and respiratory frequency further proved the HS state of the dairy cows. The quantificational real time PCR results showed that tributyrin could significantly reduce the relative expression of tumor necrosis factor α, interleukin 1β, and Interleukin 6. Western blot results showed that tributyrin could alleviate the lymphocyte inflammatory response by inhibiting the mitogen-activated protein kinase and nuclear factor-κB signaling pathways. To further detect the effect of tributyrin on HS in dairy cows, routine biochemical and blood tests were carried out. The results showed that the contents of aspartate aminotransferase, total bilirubin, creatinine, albumin, and globulin were significantly reduced by tributyrin. The results showed that tributyrin could significantly alleviate the liver and kidney injury induced by heat stress in dairy cows. Moreover, tributyrin could also significantly reduce the numbers of intermediate cells and increase the level of hemoglobin. Tributyrin could also improve the performance of dairy cows. These results suggested that tributylglycerol may have a positive effect on breast health of dairy cows. In conclusion, these results indicated that tributyrin could relieve HS and increase the production performance of dairy cows by reducing the inflammatory responses of lymphocytes.

Key words: heat stress, tributyrin, dairy cow, MAPK, NF-κB

2021 Poultry Science 100:348–356
https://doi.org/10.1016/j.psj.2020.10.006

INTRODUCTION

Heat stress (**HS**) causes nonspecific responses in humans or animals to exposure to high temperatures that exceed the thermoregulatory ability (Belhadj Slimen et al., 2016). In essence, HS in dairy cows results from a lack of heat dissipation, which leads to heat imbalance and the decline of production performance (Gantner et al., 2017), reproduction performance (Ross et al., 2017), and immunity (Dahl et al., 2016). The HS is one of the main reasons for the decline of milk production performance, reproductive performance, and immune ability in summer (Sullivan and Mader, 2018). Owing to global warming, the temperature in summer is increasing each year, and the intensity of HS is also increasing each year (Xu et al., 2018). At present, most of the cows used to produce milk are Holstein cows, which have strong cold resistance and weak heat resistance (Pennington et al., 1985; de Andrade Ferrazza et al., 2017), so they are very sensitive to HS. High temperatures not only reduce milk production but also reduce milk quality. The contents of milk fat, milk protein, lactose, and nonfat milk solids are decreased by high temperatures (Li et al., 2017; Liu et al., 2017). The HS will seriously affect the production and welfare of dairy cows (Polsky and von Keyserlingk, 2017). It may cause milk protein reduction in dairy cows (Gao et al., 2017). This may be because of a series of changes in the genes and proteins regulating milk protein synthesis, which is caused by HS in dairy cows (Santana et al., 2017). Therefore, HS will cause huge economic losses to the dairy industry every summer (Fodor et al., 2018).

Received March 3, 2020.
Accepted October 6, 2020.

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Effective methods for reducing the damage caused by HS to dairy cows is a hot research topic.

Tributyrin decomposes into butyric acid in the animal intestine, and butyrate will be absorbed and utilized by intestine (Cresci et al., 2017). Some studies have shown that butyrate can reduce the secretion of inflammatory mediators such as interleukin 6 (**IL-6**), interleukin 1β (**IL-1β**), and tumor necrosis factor α (**TNF-α**) in vivo (Bach Knudsen et al., 2018), thus reducing the inflammatory response (Sheng et al., 2017). Butyrate can also promote the differentiation of T cells into Treg cells and increase the expression of anti-inflammatory factors such as IL-10 (Schwarz et al., 2017; Wang et al., 2017). Studies have also shown that tributyrin can reduce the activities of alanine aminotransferase, aspartate transferase, and alkaline phosphatase in piglets (He et al., 2015). Therefore, tributyrin could alleviate animal stress. Moreover, tributyrin will decompose into butyrate in the intestine, thus providing energy for intestinal mucosal cells (Hamer et al., 2008), repairing the intestinal damage caused by HS (Abdelqader et al., 2017), and protecting intestinal health. However, the effects of tributyrin on HS in dairy cows have not been reported. We speculate that tributyrin could alleviate HS in dairy cows.

MATERIALS AND METHODS

Preparation of Tributyrin

The rumen-bypassed tributyrin used in this study (Hangzhou Dehong Biotech Co., Ltd., Zhejiang, China) contained 50% tributyrin on a per weight basis. Rumen protection technology improves the nutrient utilization ratio in ruminants, increases the intestinal absorption of nutrients, and limits the degradation of nutrients.

Animals and Housing

The study was conducted at Jilin University, Jilin, China. All animal experiments were conducted in strict accordance with the International Guiding Principles for Biomedical Research. Twenty multiparous lactating Holstein cows, with a parity number of 2 to 3, 140 ± 15 d of milk, an average body weight of 586 ± 49 kg, and an average milk yield of 45 ± 5 kg/d, were housed in individual tie stalls inside a barn of a commercial dairy farm in Changchun, China (43.88 N, 125.35 E, and an altitude of 219 m). The cows were fed in the stalls, had ad libitum access to clean water, and were allowed to roam freely after feeding. After each feeding, the weight of the remaining feed was weighed to calculate the daily intake of each cow. The barn was equipped with 2 juxtaposed hanging fans (750 W, 1.0 m diameter, and wind speed > 2.5 m/s) that cycled every 5 m and sprinklers (delivery rate of 2.5 L/min) at 2-m intervals along the feeding driveway. Fans were mounted at a height of approximately 2.1 m with a 16.5° tilt and were switched on at 22°C. The cows were cooled 8 times daily starting at 9:00 a.m. and ending at 5 p.m. Each cooling period lasted 30 min, and each cycle consisted of 60 s of showering and ventilation followed by 3 min of ventilation alone.

Table 1. Ingredient and nutrient composition of the diet.[1]

Item	Value
Comminuted maize	3.7
Premix[2]	1.03
Calcium fatty acid	0.38
Distiller-dried grains with solubles	0.6
Soybean meal	4
Cotton seed	1.1
Imported alfalfa	2.1
Imported oats	0.7
Pressed corn	2.2
Soybean hull	1.2
Brewer's grains	5
Silage	23
Molasses	1.8
Nutrient composition	
CP, %	25.17
NDF, %	29.269
ADF, %	17.1
NE_L[3], Mcal/kg	1.535
DM, %	46.81
Fat, %	1.977
Cl, %	0.468
Ca, %	0.464
P, %	0.533
K, %	1.325
Na, %	0.217
Mg, %	0.559
S, %	0.27

Abbreviations: ADF, acid detergent fiber; NDF, neutral detergent fiber.
[1]Diet DM average of 46.81% by weight.
[2]Contained 40 g of $CaHPO_4$; 280 g of $CaCO_3$; 220 g of $NaHCO_3$; 100 g of NaCl; 80 g of MgO; 10 g of adsorbent for mycotoxin; 50 g of K_2CO_3; and 60 g of vitamins and minerals (per kg mixture containing 1,200,000 IU vitamin A; 450,000 IU vitamin D; 6,100 mg vitamin E; 3,900 mg Fe; 1,000 mg Cu; 3,200 mg Mn; 9,000 mg Zn; 35 mg Co; 70 mg of Se; and 170 mg I).
[3]NE_L: net energy of lactation; estimated according to the NRC (Council, 2001) and energy value of the ingredients.

Cows were fed a total mixed ration (**TMR**) 3 times daily (07:00, 14:30, and 21:30 h) and were milked 3 times daily before feeding. The TMR was formulated (Table 1) based on NRC guidelines (Council, 2001) for a 586 kg lactating Holstein cow. Feed (1 kg) was collected after feeding and stored at −20°C. The TMR samples were dried at 55°C for 48 h in a forced-air oven, allowed to air-equilibrate, and ground in a mill (FZ102; Shanghai Hong Ji Instrument Co., Ltd., Shanghai, China, 2016) to a 1-mm mesh size. Processed samples were analyzed for DM, CP, and acid detergent fiber. The levels of Ca, P, and other minerals were measured. The Neutral detergent fiber was determined by the method reported by Goering and Van Soest (1970).

Experimental Design

The study had a completely randomized design and lasted 21 d (from July 15, 2019 to August 4, 2019). Based on the number of days of milk, parity, and previous milk yield per cow, the HS cows were randomly allocated into 2 groups, with 10 cows per group: a control group (fed a basal diet) and a treatment group (treated with rumen bypassed tributyrin, 37.5 g per cow/d). The rumen-

bypassed tributyrin was mixed into the upper quarter portion of the TMR offered in the morning feed.

Measurements and Sampling

The body condition score (1–5 scale) and body weight were determined by 3 technicians. The BCS of all dairy cow was 3 ± 0.5. Milk yield was recorded at day 0, day 11, and day 21, and individual milk samples were collected on days 0, 7, 14, and 21 from 3 daily milkings and were pooled, mixed, and stored at 4°C with bichromicum kalium (0.06% final concentration) as a preservative. Blood samples were collected before the morning feeding on days 0, 7, 14, and 21. The separation of the peripheral blood lymphocytes was carried out according to the instructions of the bovine peripheral blood lymphocyte separation kit (Tianjin Haoyang Biological Manufacture, Tianjin, China). Milk fat and daily protein levels were measured with a Milkoscan FT + analyser (FOSS Electric, Hillerod, Denmark, 2016), and the somatic cells were counted in a Fossomatic FC analyser (FOSS Electric) according to AOAC (1972) methods 972.16, 997, and 975.16. The ambient air temperature (**Ta, °C**) and relative humidity (**RH, %**) inside and outside the barn were recorded hourly every day using 3 hygrothermographs located 1.5 m from the ground. Temperature humidity index (**THI**) was calculated using the following equation: THI = (1.8 × Td + 32) – (0.55 – 0.55 × RH × 0.01) × (1.8 × T – 26).

Biochemical and Routine Blood Tests

On days 0, 7, 14, and 21, the peripheral blood of dairy cows was collected, and routine biochemical and blood tests were carried out. The biochemical test kit was purchased from Chengdu Pulitai Biotechnology Co., Ltd. The biochemical indicators were tested with an automatic biochemical analyzer (Seamaty, Chengdu, China). Routine blood indexes were detected using an automatic blood cell analyzer (Shenzhen Pukang Electronic Co., Ltd., China).

Quantificational Real Time PCR Analysis

Total RNA was isolated from cultured peripheral blood lymphocytes with TRIzol reagent (Invitrogen, Carlsbad, CA), and the gene levels of *TNF-α*, *IL-6*, and *IL-1β* were detected. The primer sequences are shown in Table 2.

Table 2. The primer sequences of TNF-α, IL-1β, IL-6, and β-actin.

Gene	Sequences (5′-3′)	Product length
TNF-α	(F) ACGGGCTTTACCTCATCTACTC (R) GCTCTTGATGGCAGACAGG	140
IL-6	(F) ATGCTTCCAATCTGGGTTC (R) TGAGGATAATCTTTGCGTTC	143
IL-1β	(F) AGGTGGTGTCGGTCATCGT (R) GCTCTCTGTCCTGGAGTTTGC	142
β-actin	(F) TCACCAACTGGGACGACA (R) GCATACAGGGACAGCACA	205

Abbreviations: IL-1β, interleukin 1β IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

Western Blot Analysis

Total proteins were isolated from peripheral blood lymphocytes with RIPA lysis buffer (Beyotime, Shanghai, China). The protein concentrations determined with a Pierce BCA Protein Assay Kit (Thermo Scientific, Shanghai, China). The Western blot assay was performed in accordance with the experimental methods that our group has previously used (Guo et al., 2019).

Statistical Analyses

All statistical analyses were performed with SAS statistical software. A total of 20 cows were included in the study. All data are presented as the mean ± SD. Significance was declared at $P \leq 0.05$. Western blot results were analyzed using a 2-samples t test, with tributyrin treatment as a discriminant factor. The quantificational real time PCR, biochemical and routine blood results were analyzed by using Repeated Measure ANOVA. Energy-corrected milk (**ECM**) was calculated by standardizing milk production to 3.5% milk fat and 3.2% daily protein using the formula ECM (kg) = 0.327 × milk yield (kg) + 12.95 × milk fat (kg) + 7.2 × protein yield (kg).

RESULTS

Intakes

Our study found that there was no significant difference in DM intake between the Control group and the Treatment group during the experiment (Table 3).

Environmental THI, Anal Temperature, and Respiratory Frequency of Dairy Cows

The HS state of dairy cows is closely related to the external environmental temperature. High temperature and high humidity are important factors leading to HS. The HS begins at THI = 68. The THI values > 72, 80, and 90 represent the potential for mid, high, and severe levels of HS, respectively (Zimbelman and Collier, 2011). Our results showed that the THI in the morning, at noon, and at night in

Table 3. Dry matter intake of dairy cows during the experiment.

DM (kg)				
Day	Control (kg)	Treatment (kg)	SEM	*P*-value
0 d	17.10	17.35	0.3908	0.9514
7 d	17.29	17.81	0.3771	0.5624
14 d	17.24	17.66	0.2953	0.5339
21 d	17.20	17.84	0.3429	0.2789

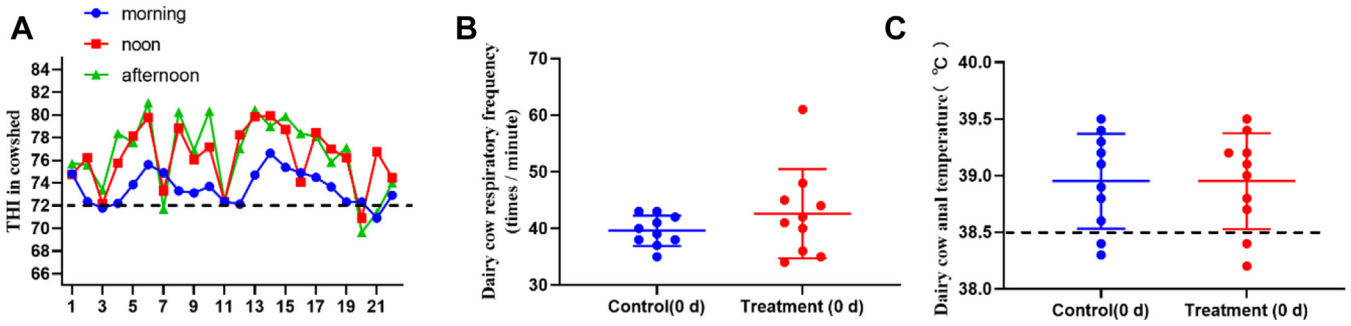

Figure 1. The THI in the cowshed and the respiratory frequency and anal temperature of dairy cows. (A) The THI of the cowshed in the morning, at noon and at night. (B) The respiratory frequency of dairy cows. (C) The anal temperature of dairy cows. All data are presented as the mean ± SD, n = 20. Abbreviation: THI, temperature humidity index.

the cowshed was higher than 72, which could have led to HS reactions in dairy cows (Figure 1A). To further determine whether the cows were in a HS state, we tested the anal temperatures and respiratory frequency of the dairy cows. We found that the respiratory frequency of dairy cows was approximately 2 times that observed in the normal state (Figure 1B), whereas the anal temperature was high; in some cows, it reached 39°C to 39.5°C (Figure 1C). These results showed that dairy cows were in the HS state.

The Alleviative Effect of Rumen-Bypassed Tributyrin on Peripheral Blood Lymphocyte Inflammation

During HS, lymphocytes may be stimulated to secrete a large number of proinflammatory mediators, which may lead to inflammatory damage in dairy cows. Our results showed that tributyrin can significantly reduce the expression of TNF-α, IL-6, and IL-1β in peripheral blood lymphocytes in dairy cows with the time (Table 4). As the feeding time increased, the anti-inflammatory effect of tributyrin was improved. To further study the anti-inflammatory mechanism of tributyrin, we measured the phosphorylation levels of ERK1/2, p38, JNK1/2, and p65. The results showed that tributyrin could inhibit the phosphorylation of these proteins and reduce the formation of proinflammatory mediators (Figures 2A–D). Finally, it could reduce the inflammatory damage caused by HS.

Effect of Rumen-Bypassing Tributyrin on the Biochemical Indexes of Dairy Cows

The HS may damage the functions of the liver and kidney, and the damage of these functions may affect the normal lactation function of dairy cows. Our study

Table 4. Effect of tributyrin on IL-6, IL-1β, and TNF-α levels.

Item	Time (d)	Treatment				*P*-value
		Control[1]	SEM	Tributyrin[1]	SEM	
IL-1β	0	1.182^{B}	0.105	1.668^{B}	0.150	0.7058
IL-1β	7	1.295^{B}	0.110	1.513^{B}	0.149	>0.9999
IL-1β	14	$2.211^{A,a}$	0.154	$1.325^{B,b}$	0.097	0.0169
IL-1β	21	$4.219^{C,a}$	0.212	$1.408^{B,b}$	0.096	<0.0001
IL-6	0	1.108^{B}	0.076	1.253^{D}	0.045	0.9733
IL-6	7	$1.370^{A,B}$	0.071	1.146^{D}	0.047	0.4399
IL-6	14	$1.398^{A,B,a}$	0.087	$1.076^{D,b}$	0.035	0.0405
IL-6	21	$1.665^{A,a}$	0.065	$1.015^{D,b}$	0.047	<0.0001
TNF-α	0	1.491^{B}	0.057	1.577^{B}	0.096	>0.9999
TNF-α	7	1.945^{B}	0.058	1.376^{B}	0.094	0.4182
TNF-α	14	2.895^{A}	0.229	3.215^{A}	0.171	0.9948
TNF-α	21	$4.833^{C,a}$	0.277	$2.757^{A,b}$	0.131	<0.0001

Data are presented as mean ± SEM (n = 10). Different uppercase letters represent significant differences ($P < 0.05$) within a treatment (control or tributyrin). Different lowercase letters represent differences between control and tributyrin group, and no lowercase letter means no differences. The *P*-value indicates the difference between control and Tributyrin.

Abbreviations: IL-1β, interleukin 1β IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

[1]Control means no supplemental rumen-bypassed tributyrin; Tributyrin means supplemented with rumen-bypassed tributyrin.

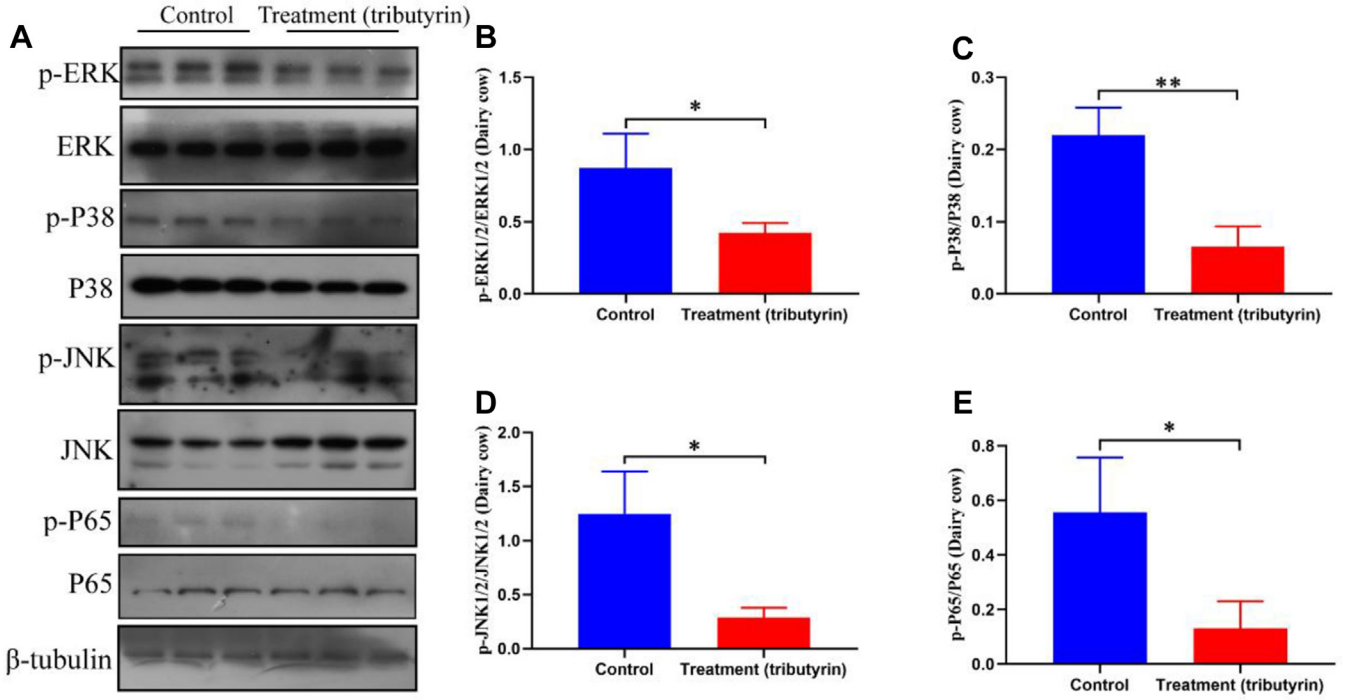

Figure 2. Effects of tributyrin on the mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB signaling pathways. (A–E) Tributyrin reduced the protein levels of p-ERK1/2, p-38, p-JNK1/2, and p-P65 in lymphocytes on day 21. All data are presented as the mean ± SD, n = 20. $^{*}P < 0.05$ vs. Control, $^{**}P < 0.01$ vs. Control.

found that the levels of ASL (Figure 3A), total bilirubin (Figure 3C), urea (Supplementary Figure 1C), and creatinine (Figure 3D) on day 21 in the treatment group were significantly lower than those in the HS group, and tributyrin and time showed an interaction. On the 21st d, the amounts of albumin (Figure 3E) and globulin (Figure 3F) in the treatment group were significantly decreased, but there was no

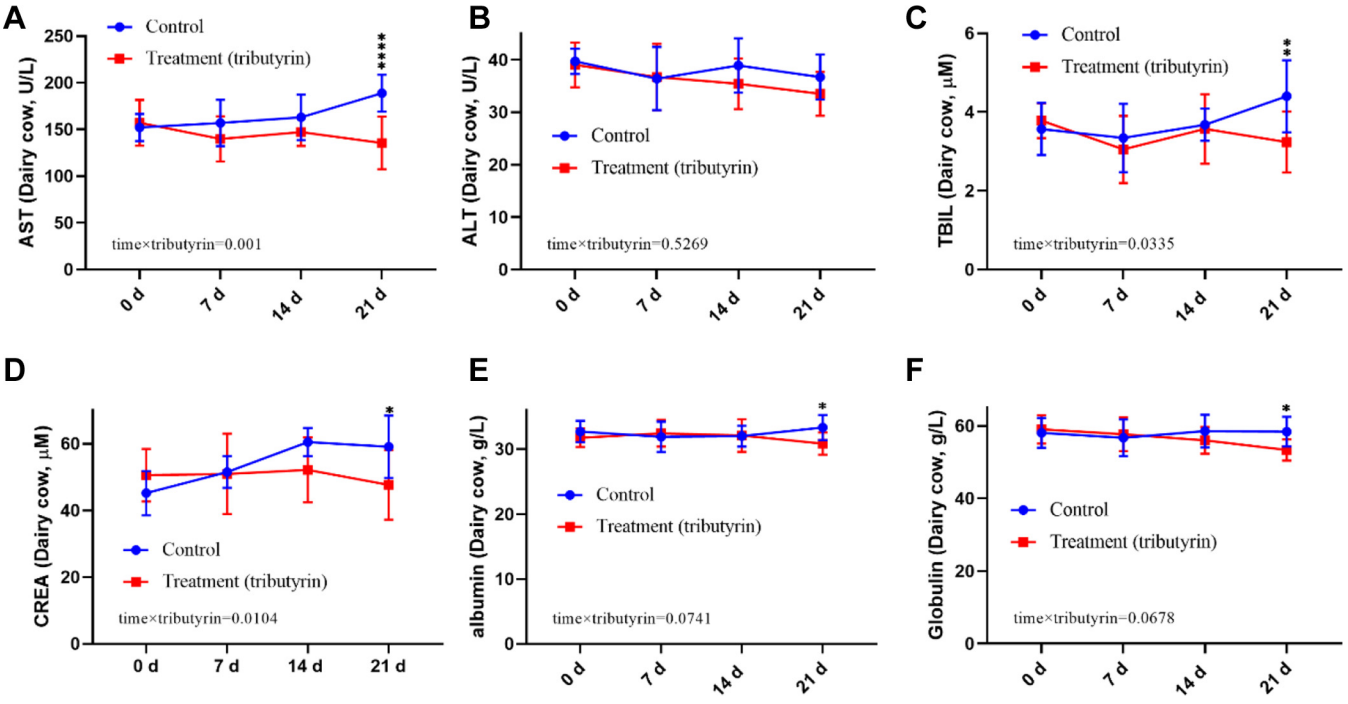

Figure 3. Effect of tributyrin on biochemical indexes. (A–F) Effect of tributyrin on aspartate aminotransferase (AST), ALT, total bilirubin (TBIL), creatinine (CREA), albumin, and globulin. All data are presented as the mean ± SD, n = 20. $^{*}P < 0.05$ vs. Control, $^{**}P < 0.01$ vs. Control, $^{****}P < 0.0001$ vs. Control.

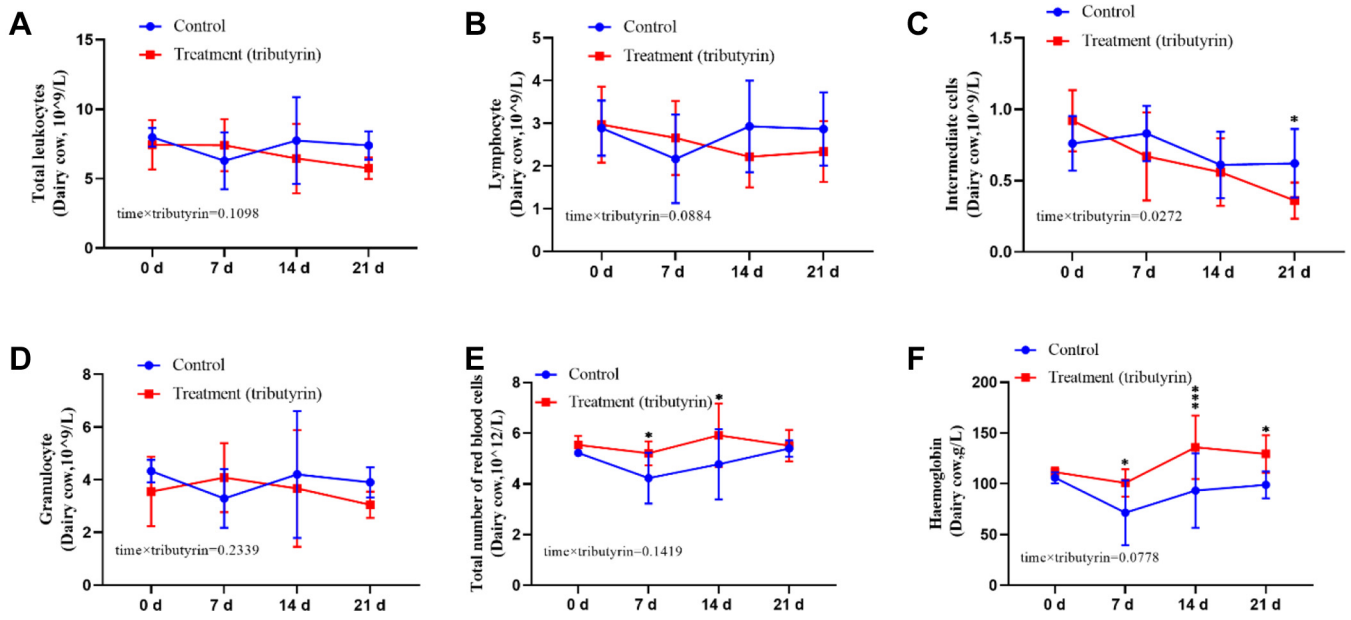

Figure 4. Effect of tributyrin on routine blood indexes. (A–F) Effect of tributyrin on total leukocytes, lymphocytes, intermediate cells, granulocytes, total number of red blood cells, and hemoglobin. All data are presented as the mean ± SD, n = 20. $^{*}P < 0.05$ vs. Control, $^{***}P < 0.001$ vs. Control.

interaction between tributyrin and time. However, the ALT (Figure 3B) content did not change significantly between the 2 groups, and it was also found that the decline in the curve of the treatment group was smooth according to the line chart, whereas the curve of the control group increased and then decreased. However, our results showed that there was no significant difference in the contents of total protein, amylase, GLU, CK, serum calcium, and serum phosphorus (Supplementary Figures 1A, 1B, 1D–G), and these indexes in the treatment group were decreased. These results showed that tributyrin could alleviate HS in dairy cows.

Effect of Rumen-Bypassed Tributyrin on Blood Routine Index of Dairy Cows

There were no significant changes in the number of total leukocytes, lymphocytes, granulocytes, and total red blood cells in dairy cows fed rumen-bypassed tributyrin (Figures 4A, 4B, 4D, 4E). However, we found a significant increase in hemoglobin levels on days 7, 14, and 21 (Figure 4F). The results showed that rumen-bypassing tributyrin could significantly increase the oxygen content in the blood of dairy cows, thus enhancing the energy supply of dairy cows and promoting energy consumption activities such as lactation. We also found that on the 21st d, the number of intermediate cells in the treatment group decreased significantly (Figure 4C), which indicated that rumen-bypassing tributyrin could alleviate the inflammatory response caused by HS.

Effect of Rumen-Bypassing Tributyrin on Performances in Heat-Stressed Dairy Cows

Studies have shown that HS can affect milk yield, milk fat, and milk protein. Our experimental results showed that the amounts of milk fat, milk protein, and ECM increased significantly (Figures 5A, 5B, 5F), and the amounts urea nitrogen and somatic cell count decreased significantly (Figures 5D, 5E), whereas lactose and daily milk production did not change significantly. However, the daily milk production of the treatment group was slightly higher than that of the control group (Figure 5G). For the indexes of milk fat and urea nitrogen, rumen-bypassing tributyrin had a significant interaction over time.

DISCUSSION

The HS will seriously affect the performance and immune levels of dairy cows (Akhavan-Salamat and Ghasemi, 2016). Our study showed that the milk yield and milk quality in the treatment group were significantly improved. In addition, the indexes of liver and kidney injury in the serum of dairy cows in the treatment group were significantly reduced. We also found that the mitogen-activated protein kinase (**MAPK**) and nuclear factor (**NF**)-κB signaling pathways in lymphocytes of heat-stressed dairy cows were significantly activated, and these signaling pathways were inhibited after feeding cows rumen-bypassing tributyrin. These results showed that tributyrin could significantly reduce the inflammatory state, relieve HS, and improve the performance of dairy cows.

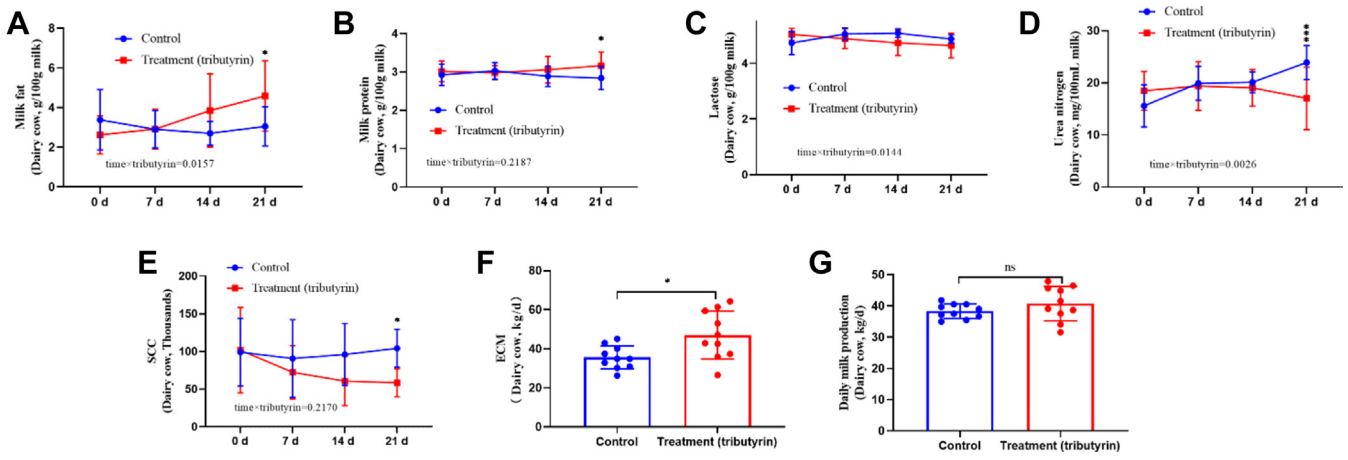

Figure 5. Tributyrin enhances the performance of heat-stressed dairy cows. (A–E) Effects of tributyrin on milk fat, milk protein, lactose, urea nitrogen, and somatic cell count (SCC). (F, G) Effects of tributyrin on ECM and daily milk production. All data are presented as the mean ± SD, n = 20. $^{*}P < 0.05$ vs. Control, $^{***}P < 0.001$ vs. Control. Abbreviation: ECM, energy-corrected milk.

Studies have shown that THI, respiratory frequency, and anal temperature could reflect the HS state of dairy cows (Ahmed et al., 2017). Our study found that the THI of the cowshed reached or exceeded 72 during the entire experimental stage (Carabano et al., 2016). We found that the respiratory frequency of dairy cows was 1.5- to 2-fold higher than that of normal cows, and the anal temperature was generally higher. These results showed that the dairy cows were in a state of HS. Studies have shown that HS can promote the expression of proinflammatory mediators in dairy cows (Min et al., 2016), thus affecting their performance (Brown et al., 2016). The MAPK signaling pathway and NF-κB signaling pathway are important inflammatory-related signaling pathways (Campillo-Gimenez et al., 2018). In our study, we found that tributyrin could significantly inhibit the phosphorylation of p38, ERK1/2, JNK1/2, and p65 in lymphocytes. Peripheral blood lymphocytes are important immune cells that play an important role in immune defence (Carroll and Isenman, 2012). Moreover, lymphocytes circulate in all organs of the body, so the inflammatory state of lymphocytes may directly affect the physiological state of dairy cows. Our study found that tributyrin can significantly reduce the gene levels of *IL-6*, *TNF-α*, and *IL-1β* in peripheral blood lymphocytes in dairy cows. This is achieved by inhibiting the activation of the MAPK and NF-κB signaling pathways. This may be due to the abnormal function of immune cells caused by heat stress. The changes of these immune cells may directly lead to liver and kidney damage in dairy cows.

When HS occurs in dairy cows, it will cause liver and kidney damage, resulting in a decline in production performance (Skibiel et al., 2018; Tang et al., 2018). Moreover, we speculate that the inflammatory responses of lymphocytes may also cause liver, kidney, and mammary gland injury. This damage will cause changes in the biochemical indexes of the liver, kidney, and other organs. In this experiment, we found that the total bilirubin, aspartate aminotransferase, and creatinine values of dairy cows in the control group increased significantly over time, whereas these indexes did not increase after feeding cows tributyrin. This may be related to the reduction of inflammatory status of immune cells and indicated that tributyrin could alleviate HS and relieve liver and kidney injury in dairy cows. In addition, we also performed routine blood tests on dairy cows, and the results showed that the number of intermediate cells in the treatment group were significantly reduced while the hemoglobin content was significantly increased. We speculated that tributyrin may also reduce the inflammatory responses of heat-stressed cows by reducing the number of intermediate cells and increasing the hemoglobin content. To reduce the injury to different tissues and organs, the HS of dairy cows was finally relieved.

Many studies have shown that HS could affect the performance of dairy cows (Collier et al., 2017). Most of the previous studies only focused on the effect of HS on the performance of dairy cows, but our experiments found that the increase in the inflammatory responses of peripheral immune cells caused by HS was also an important factor contributing to the damage of tissues and organs of dairy cows. Interestingly, we found that tributyrin could also increase the levels of ECM, milk protein, milk fat and reduce somatic cell count. This showed that tributyrin could improve the lactation performance of dairy cows by alleviating HS.

CONCLUSION

The above described experiments showed that tributyrin could alleviate the inflammatory responses of peripheral blood lymphocytes in dairy cows, thereby reducing liver and kidney damage and ultimately alleviating HS in dairy cows.

ACKNOWLEDGMENTS

The authors thank Guangze ecological animal husbandry Co., Ltd. for providing the experimental site and experimental cows and thank Ziyang Zhang for testing the biochemical indexes of dairy cows. This work was supported by the National Natural Science Foundation of China (Project No. 31702211, 31672509, and 31873004), Jilin Scientific and Technological Development Program (Project No. 20190103021JH and 20200201111JC), and JLU Science and Technology Innovative Research Team.

DISCLOSURES

The authors declare that they have no competing interests.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.10.006.

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