Effect of *Lonicera japonica* extract on lactation performance, antioxidant status, and endocrine and immune function in heat-stressed mid-lactation dairy cows

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ABSTRACT

Here, we examined the effects of *Lonicera japonica* extract (LJE) on lactation performance, antioxidant status, and endocrine and immune function in heat-stressed mid-lactation dairy cows. Twenty-four healthy Chinese Holstein mid-lactation dairy cows, all with similar milk yield (30.0 ± 1.0 kg/d), parity (2.5 ± 0.3), and days in milk (105 ± 5 d) were allocated to 4 groups using a randomized complete block design: a negative control group (without LJE supplementation; CON) and groups that received LJE at 14, 28, and 56 g/d. The experiment lasted 10 wk over a hot summer, with a pre-feeding period of 2 wk. Cows were exposed to heat stress, as the average temperature-humidity index was greater than 72. The results showed that LJE had no effect on respiration rate; however, it reduced the rectal temperature of dairy cows experiencing heat stress in both a linear and quadratic manner; the lowest (39.03°C) was recorded for the LJE-28 group, lower than the CON group. Supplementation with LJE did not affect dry matter intake, milk yield, or milk composition. The majority of biochemical parameters in serum were unaffected by supplementation with different amounts of LJE; the exception was creatinine, which was reduced quadratically. Compared with the CON group, serum triiodothyronine concentrations increased significantly in the LJE-28 group. Addition of LJE to the diet increased thyroxine concentrations quadratically; values peaked at 18.62 ng/mL in the LJE-28 group. Furthermore, supplementation with increasing amounts of LJE quadratically increased the activity of glutathione peroxidase and total antioxidant capacity in serum but decreased concentration of malondialdehyde. Although we detected no differences in the concentrations of IgA, IgM, or cytokines, dairy cows in the LJE-28 group had higher IgG and IL-4 concentrations than

did cows in the CON group. Supplementation with LJE increased concentrations of IgG and IL-4 in the serum quadratically but decreased that of IL-2. Finally, heat shock protein 72 concentrations in the serum tended to fall quadratically as the amount of LJE increased. In summary, LJE had no negative effects on lactation performance but helped to alleviate heat stress by improving antioxidant status and promoting endocrine and immune functions. Supplementation with LJE at 28 g/d is recommended for lactating dairy cows experiencing heat stress during hot summers.

Key words: *Lonicera japonica* extract, heat stress, antioxidant status, endocrine and immune function, dairy cow

INTRODUCTION

Heat stress jeopardizes the welfare and health of dairy cows, decreases feed intake and milk yield, and causes severe economic losses (Wrinkle et al., 2012); as such, it is one of the most important factors that poses a danger to the dairy industry (Collier et al., 2017). The optimal ambient temperature for Holstein dairy cows is between 0 and 20°C (Liu et al., 2019). When temperatures exceed 25°C and the temperature-humidity index (THI) is greater than 68, dairy cows may suffer from heat stress (Collier et al., 2017). When the THI exceeds 72, dairy cows enter a mild state of heat stress, which reduces nutrient metabolism and absorption (Cowley et al., 2015). Simultaneously, heat stress induces oxidative stress (Sun et al., 2019); in such a state, production of reactive oxygen species (ROS) overwhelms the capacity of antioxidant defense systems, particularly in lactating dairy cows, which require large amounts of energy for synthesis of large quantities of milk components (Sun et al., 2017). In addition to evaporative cooling (using soakers and fans) inside barns, supplementation with feed or pharmaceutical additives is an effective strategy for alleviating heat stress (Liu et al., 2013; Pan et al., 2014).

Chinese herbal additives have nutritional and medical value and few toxic side effects; as such, they have been

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widely used to improve livestock production during high ambient temperatures in hot summers (Wang et al., 2008; Dong et al., 2012; Pan et al., 2014). *Lonicera japonica*, also known as jinyinhua, ren dong, and Japanese honeysuckle, belongs to the Caprifoliaceae family (Shang et al., 2011). It grows in China, Poland, Russia, and Japan (Jang et al., 2008; Frejnagel and Wroblewska, 2010) and is used as a pharmaceutical and healthy food ingredient (Bai et al., 2020). *Lonicera japonica* contains essential oils, organic acids, flavones, saponins, iridoids, and inorganic elements (Zhao et al., 2015). Studies show that honeysuckle has antibacterial, anti-inflammatory, and anticarcinogenic effects in rats (Tzeng et al., 2014). *Lonicera japonica* is a good source of polyphenolic compounds and has strong antioxidant properties (Salah et al., 1995; Gladine et al., 2007; Lee et al., 2019). Many studies have examined the antioxidant properties of *Lonicera japonica* and its extract (Jang et al., 2008; Wu et al., 2015). Therefore, we hypothesized that *Lonicera japonica* extract (**LJE**) might alleviate oxidative stress induced by heat stress in lactating dairy cows. In the present study, we examined the effects of different doses of LJE on lactation performance, serum biochemical and antioxidant indicators, and endocrine and immune factors in heat-stressed mid-lactation dairy cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The study was conducted at the Junyuan Dairy Farm (Shijiazhuang, Hebei Province, China). All animals were raised and cared for according to standards established by the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). The experimental design and procedures were approved by the Chinese Academy of Agricultural Sciences Animal Care and Use Committee (IAS 2019-10). The dairy cows were exposed to heat stress before the trial: THI and ambient temperature averaged 74.6 and 27.4°C, respectively, for 2 wk before the trial commenced. Rectal temperature (**RT**) and respiration rate (**RR**) averaged 39.4°C and 77 breaths/min, respectively, in the week before the experiment.

Twenty-four multiparous lactating Holstein dairy cows were blocked according to parity (2.5 ± 0.3), average daily milk yield (30.0 ± 1.0 kg/d), and DIM (105 ± 5 d), and a randomized complete block design was used to assign cows to 1 of 4 treatment groups: a negative control group (without LJE supplementation, **CON**) or groups that received LJE at 14, 28, and 56 g/d (**LJE-14**, **LJE-28**, and **LJE-56**). Shanxi Zhongxin Bio-Tech Co. Ltd. (Xi'an, Shanxi Province,

China) provided LJE, which was identified according to the standard of the Pharmacopeia of the People's Republic of China. The dairy cows were individually fed with the same TMR (Table 1), which was formulated to meet the nutrient requirements stipulated by the NRC (2001). The TMR was offered to individual cows 3 times per day after each milking (0630, 1330, and 2030 h); the refusal rate was 5 to 10%. Refusals were removed and weighed every morning. The LJE was top-dressed on the TMR during the morning feeding. The experiment lasted for 10 wk, with a pre-feeding period of 2 wk. All cows were housed in a mechanically ventilated tiestall barn, divided into 4 plots with 6 cows each, and had free access to water throughout the experiment.

Table 1. Ingredients and chemical composition of the basal diet (% of as-fed DM)

Item	%
Ingredient	
Alfalfa hay	11.33
Oat hay	4.37
Corn silage	33.76
Molasses	1.33
Ground corn	18.82
Soybean meal	11.43
DDGS ¹	0.44
Flaked corn	4.07
Sugar beet pulp	0.67
Whole cottonseed	7.04
Yeast culture ²	0.70
CaHPO ₄	0.51
NaHCO ₃	0.79
Limestone	1.16
NaCl	0.50
MgO	0.14
K ₂ CO ₃	0.19
Fat powder ³	1.27
Premix ⁴	1.48
Nutrient composition ⁵	
EE ⁶	5.10
CP	16.93
NDF	28.42
ADF	14.54
Ca	0.82
P	0.27
Ash	8.20
NE _L ⁷ (Mcal/kg)	1.56

¹DDGS = distillers dried grains with solubles.

²Diamond V XP yeast culture supplement (Diamond V, Cedar Rapids, IA).

³Bergafat, a saturated free fatty acid supplement (Berg+Schmidt GmbH & Co. KG, Hamburg, Germany).

⁴Premix provided 200,000 IU/kg vitamin A; 320,000 mg/kg vitamin B; 40,000 IU/kg vitamin D; 5,000 IU/kg vitamin E; 100 mg/kg D-biotin; 95,200 mg/kg Mg; 1,000 mg/kg Zn; and 50 mg/kg Co.

⁵Analyzed value.

⁶EE = ether extract.

⁷Calculated value (based on China NY/t34, 2004).

Sampling and Analysis

Ambient temperature (**T**) and relative humidity in the barn were recorded 3 times per day (at 0600, 1400, and 2200 h) using thermometers (Beijing Yaguang Equipment Co. Ltd., Beijing, China) hung 1.5 m from the ground. The THI was calculated using the equation reported by Dikmen and Hansen (2009): $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$, where **T** is the ambient temperature (°C) and **RH** is the relative humidity (%). Cows' RT was monitored daily, at 0730, 1430, and 2130 h, using a glass mercury thermometer (Nasco, Ft. Atkinson, WI), and the RR was also measured 3 times (at 0730, 1430, and 2130 h) every day, by counting flank movements for 1 min.

Dry matter intake for individual cows was calculated daily by subtracting refusals from the TMR offered, on a DM basis. Samples of TMR were collected consecutively for 3 d/wk during the treatment period to measure the DM content. All collected samples were mixed and dried for 48 h in an oven at 65°C. Next, samples were ground to 1 mm with a fodder grinder. The DM content of the feed was determined by drying in an oven for 4 h at 105°C to achieve a constant weight (AOAC International, 2005; method 930.15). Crude protein (AOAC International, 2000; method 976.05) and ether extract (AOAC International, 2003; method 4.5.05) were measured using standard procedures according to AOAC International. The NDF and ADF contents were measured as described by Van Soest et al. (1991). Calcium and phosphorus content were determined by atomic absorption spectroscopy (AOAC International, 1990; method 985.35) and spectrophotometry (AOAC International, 1990; method 986.24), respectively. The composition and nutrient levels of TMR are listed in Table 1.

The cows were milked 3 times (0530, 1230, and 1930 h) each day, and individual milk yield was recorded at each milking. Milk samples from each cow were collected from 3 consecutive milkings and combined weekly (at a ratio of 4:3:3, Sun et al., 2017). Preservative (bronopol tablet, D&F Control System, San Ranmon Inc., Dublin, ON, Canada) was added to milk samples, which were stored at 4°C for analysis of milk components (fat, protein, lactose, TS, and SNF) using infrared analysis (Foss MilkoScan 2000, Foss Food Technology Corp., Eden Prairie, MN).

Before morning feeding at the end of the experiment (at 0600 h on d 57), blood samples were collected from the coccygeal vein of all animals using Vacutainer tubes (BD Biosciences, San Jose, CA), and centrifuged at $3,000 \times g$ for 15 min at 4°C. Serum was collected, ali-

quoted, and stored at -20°C until analysis. The concentrations of creatinine (**CREA**), glucose, total protein, uric acid, and urea nitrogen in serum were measured using an automated biochemical analyzer (Hitachi 7080; Hitachi Valve Ltd., Tokyo, Japan), as reported previously (Sun et al., 2017). The intra- and interassay coefficients of variation (CV) for CREA, total protein, uric acid, glucose, and urea nitrogen were respectively 1.98 and 2.32, 1.34 and 1.93, 3.15 and 4.09, 1.46 and 2.07, and 1.61 and 2.13%. Serum triiodothyronine (**T₃**), thyroxine (**T₄**), glucagon, and prolactin concentrations were determined in a radioimmunoassay, as described previously (Herbein et al., 1985; Miller et al., 1999; Vicari et al., 2008). The intra- and interassay CV for **T₃**, **T₄**, glucagon, and prolactin were, respectively, 6.3 and 8.4; 7.1 and 9.5; 3.9 and 5.2; and 7.9 and 13.8%. The activity of total antioxidant capacity (**T-AOC**, catalog number A015), superoxide dismutase (**SOD**, catalog number A001-3), and glutathione peroxidase (**GSH-Px**, catalog number A005), and the concentration of malondialdehyde (**MDA**, catalog number A003-1) in serum, were analyzed using commercial assay kits (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions (Sun et al., 2017). Serum levels of IgG (catalog number E11-118), IgA (catalog number E11-131), and IgM (catalog number E11-101) were measured using enzyme-linked immunosorbent assay kits (Bethyl Laboratories, Montgomery, TX), as were the concentrations of serum cortisol (catalog number MBS701325), insulin (catalog number MBS4500450), IL-1 β (catalog number MBS2609338), TNF- α (catalog number MBS2609886), and heat shock protein (**HSP**)-72 (catalog number MBS2609980; MyBioSource Inc., San Diego, CA).

Statistical Analysis

The data were checked for normality using the UNIVARIATE procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). All data were analyzed using the MIXED procedure in SAS. The repeated measures model contained fixed effects of week and treatment and the random effect of cow. Contrasts were constructed to examine treatment effects and LJE supplementation levels, with orthogonal polynomials accounting for unequal spacing of LJE dosage levels. Tukey's multiple range test was used for the evaluation of differences between the groups. Data are expressed as least squares means and standard errors of the mean. Differences with $P < 0.05$ were considered statistically significant, and $0.05 \leq P < 0.1$ was referenced as a tendency.

Table 2. Effects of *Lonicera japonica* extract (LJE) on rectal temperature and respiratory rate in heat-stressed dairy cows

Item	Treatment mean ¹				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
RT, ² °C	39.40 ^a	39.12 ^b	39.03 ^b	39.06 ^b	0.05	<0.01	<0.01	<0.01
RR, ³ breaths/min	81.61	80.33	79.44	80.94	1.70	0.83	0.83	0.38

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

²Rectal temperature.

³Respiratory rate.

RESULTS

Measurement of THI and Heat Stress

The average daily ambient temperatures in the barn at 0600, 1400, and 2200 h were 26.9, 33.6, and 29.0°C, respectively. As seen in Figure 1, the average THI during the experimental period was >72, and the average THI from d 11 to d 25 and from d 30 to d 56 was >78. Therefore, the dairy cows were in a heat-stressed environment. Table 2 shows the RT and RR of the dairy cows. Supplementation with LJE did not affect RR but reduced RT compared with the CON group ($P < 0.05$). Furthermore, cows' RT decreased linearly and quadratically; the lowest temperature (39.03°C) was recorded for the LJE-28 group ($P < 0.05$).

Lactation Performance

The effects of LJE supplementation on lactation performance and DMI of heat-stressed dairy cows are presented in Table 3. Supplementation with different amounts of LJE had no effect on DMI, milk yield, or milk composition. The exception was a trend toward an effect on SNF, which tended to fall linearly as the dose of LJE increased ($P = 0.07$).

Biochemical and Hormone Parameters in Serum

Supplementation with different doses of LJE did not affect the concentrations of glucose, total protein, uric acid, and urea nitrogen in the serum of dairy cows under heat stress (Table 4). However, LJE supplementation significantly reduced serum CREA concentrations compared with the CON group ($P < 0.01$).

As shown in Table 5, the concentrations of T₃ in the serum were higher in the LJE-28 group than in the CON group ($P < 0.05$). Simultaneously, the concentrations of T₄ increased quadratically, peaking at 18.62 ng/mL in the LJE-28 group ($P < 0.05$). No differences were observed in serum cortisol, glucagon, insulin, or prolactin between groups.

Antioxidant, Immune Indicators, and HSP-72 in Serum

The effects of different amounts of LJE on antioxidants, immune indicators, and HSP-72 are shown in Tables 6 and 7. Supplementation with LJE at 28 g/d increased serum T-AOC activity compared with the CON group ($P < 0.05$). Increasing amounts of LJE quadratically increased the activity of GSH-Px and T-AOC but reduced that of MDA in the serum of heat-

Figure 1. Average temperature-humidity index (THI) pattern in the barn during the experimental period.

Table 3. Effects of *Lonicera japonica* extract (LJE) on DMI, milk yield, and milk composition in heat-stressed dairy cows

Item	Treatment mean ¹				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
DMI, kg/d	20.09	20.22	21.00	20.76	0.69	0.76	0.44	0.67
Milk yield, kg/d	27.46	28.19	28.34	27.92	1.03	0.93	0.82	0.55
Fat, %	3.93	4.07	3.98	3.87	0.06	0.14	0.18	0.10
Protein, %	3.49	3.12	3.31	3.23	0.11	0.15	0.31	0.25
Lactose, %	4.57	4.67	4.61	4.69	0.09	0.76	0.42	0.90
SNF, %	8.82	8.74	8.56	8.54	0.11	0.25	0.07	0.49
TS, %	12.75	12.49	12.60	12.69	1.14	0.57	0.95	0.27

¹Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

Table 4. Effects of *Lonicera japonica* extract (LJE) on blood biochemical index in heat-stressed dairy cows

Item	Treatment mean ¹				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
Creatinine, $\mu\text{mol/L}$	118.90 ^a	78.57 ^b	80.67 ^b	82.47 ^b	7.76	<0.01	0.01	<0.01
Glucose, mmol/L	3.18	3.26	3.50	3.37	0.11	0.23	0.19	0.18
Total protein, g/L	76.14	71.36	74.51	72.08	3.66	0.78	0.59	0.80
Uric acid, $\mu\text{mol/L}$	69.62	73.56	65.99	70.27	5.29	0.79	0.88	0.80
Urea nitrogen, mmol/L	2.31	2.07	2.88	2.77	0.40	0.46	0.27	0.80

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

Table 5. Effects of *Lonicera japonica* extract (LJE) on serum hormone indices in heat-stressed dairy cows

Item ¹	Treatment mean ²				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
T ₄ , ng/mL	16.66 ^{ab}	18.00 ^a	18.62 ^a	14.75 ^b	0.94	0.04	0.10	0.02
T ₃ , pg/mL	1,507.25 ^b	1,608.79 ^{ab}	1,793.53 ^a	1,753.43 ^{ab}	71.26	0.04	0.45	0.38
Cortisol, ng/mL	88.70	84.61	83.72	84.95	6.06	0.59	0.89	0.67
Glucagon, pg/mL	201.53	204.09	218.67	206.45	15.11	0.86	0.77	0.52
Insulin, mU/L	34.52	26.73	31.62	33.35	2.66	0.21	0.76	0.16
Prolactin, pg/mL	280.87	305.53	387.72	335.74	32.65	0.15	0.19	0.11

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹T₄ = thyroxine; T₃ = triiodothyronine.

²Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

Table 6. Effects of *Lonicera japonica* extract (LJE) on serum antioxidant indices in heat-stressed dairy cows

Item	Treatment mean ¹				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
Glutathione peroxidase, U/mL	97.77	97.20	102.51	107.07	3.69	0.23	0.98	<0.05
Total antioxidant capacity, U/mL	4.74 ^b	4.99 ^{ab}	6.58 ^a	5.34 ^{ab}	0.39	0.02	0.18	0.02
Superoxide dismutase, U/mL	95.37	108.00	122.27	121.67	9.62	0.19	0.06	0.27
Malondialdehyde, nmol/mL	3.46	2.70	2.41	3.14	0.34	0.16	0.68	0.03

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

Table 7. Effects of *Lonicera japonica* extract (LJE) on serum immune indices and heat shock protein (HSP)-72 levels in heat-stressed dairy cows

Item	Treatment mean ¹				SEM	P-value		
	Control	LJE-14	LJE-28	LJE-56		Treatment	Linear	Quadratic
IgA, mg/mL	0.32	0.40	0.47	0.37	0.07	0.51	0.67	0.16
IgM, mg/mL	4.21	4.89	2.89	3.89	0.78	0.36	0.51	0.53
IgG, mg/mL	91.49 ^b	94.72 ^{ab}	108.96 ^a	102.66 ^a	4.43	0.04	0.23	0.02
TNF- α , pg/mL	219.04	203.91	215.61	220.98	36.08	1.00	0.90	0.86
IL-1 β , pg/mL	46.33	40.04	46.21	44.00	3.42	0.54	0.96	0.74
IL-2, ng/mL	4.51	2.34	3.01	5.69	0.99	0.11	0.20	0.04
IL-4, ng/mL	7.68 ^b	7.45 ^b	9.77 ^a	7.81 ^b	0.53	0.04	0.56	0.04
HSP-72, ng/mL	10.26	9.95	7.23	8.66	0.82	0.06	0.11	0.10

^{a,b}Mean values within treatment with different superscripts are significantly different ($P < 0.05$).

¹Treatments: control = 0 g of LJE/d (no LJE supplementation); LJE-14 = 14 g of LJE/d; LJE-28 = 28 g of LJE/d; LJE-56 = 56 g of LJE/d; n = 6 per group.

stressed dairy cows ($P < 0.05$). Furthermore, a linear trend appeared in the activity of SOD as the LJE dose increased ($P = 0.06$).

No differences occurred in the concentrations of serum IgA or IgM after supplementation with LJE. However, addition of increasing doses of LJE quadratically increased the concentration of IgG; values in the LJE-28 group peaked at 108.96 mg/mL ($P < 0.05$). Concentrations of IgG in the LJE-28 and LJE-56 groups were higher than in the CON group ($P < 0.05$). In addition, supplementation with differing amounts of LJE quadratically increased the concentration of IL-4 but decreased that of IL-2 in the serum of heat-stressed dairy cows. Concentrations of HSP-72 in the serum tended to fall quadratically as the amount of LJE increased ($P = 0.10$). However, we detected no differences in the levels of TNF- α or IL-1 β between groups.

DISCUSSION

Environmental heat stress during summers induces hyperthermia of dairy cows. During our experimental period, we found that the ambient temperature exceeded 25°C, the average THI was continuously above 72, and the RR and RT of CON dairy cows were 39.40°C and 81.61 breaths/min, respectively, suggesting that dairy cows were subjected to heat stress conditions.

Rectal temperature is a representative indicator of core temperature and reflects thermal balance in the body (Marai et al., 2007; Liu et al., 2019). Even subtle alterations in RT have profound effects on tissues, organs, and endocrine function, which may result in reduced growth and lactation (McDowell et al., 1976). Dairy cows suffer heat stress when the RT exceeds 38.5°C (Collier et al., 2017). Respiration rate is another indicator of heat stress in dairy cattle; often, this exceeds 60 breaths/min when cows are experiencing heat stress (Staples and Thatcher, 2011). Although RR was

not affected by supplementation with increasing doses of LJE, RT fell, suggesting that feeding LJE reduced core temperature.

Although LJE supplementation had no effects on DMI or on milk yield and composition, it did reduce RT, which might help to improve cows' overall health. A breakdown product of creatine phosphate from muscle and protein metabolism, CREA is an important metabolic indicator that reflects kidney function. Accumulation of serum CREA implies damage to nephrons, suggesting impaired excretion and renal failure (Perrone et al., 1992; Bellomo et al., 2012). It is well established that heat stress has direct effects on protein metabolism in lactating dairy cows, including decreasing milk protein content and yield, which might be partly due to increased protein turnover and degradation (Bequette and Backwell, 1997; Gao et al., 2017). Cowley et al. (2015) found that CREA concentrations in the plasma of dairy cows increase under heat stress conditions. Tzeng et al. (2014) demonstrated that an ethanol extract of Japanese honeysuckle improves renal function in diabetic rats by increasing the rate of CREA clearance from serum. Consistent with these reports, we found that serum CREA concentration fell significantly after LJE supplementation.

As mentioned, upward shifts in core temperature during hyperthermia have profound effects on endocrine function (McDowell et al., 1976). During heat stress, animals mobilize almost all organs and tissues in the body through neuro-endocrine mechanisms to fight high-temperature stimulation; this occurs mainly through the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes (Afsal et al., 2018). High temperatures inhibit proliferation and secretion by thyroid cells, thereby reducing thyroid mass and secretory activity (Bernabucci et al., 2010), and reducing the concentrations of T₃ and T₄ in serum (Saber et al., 2006), as these are secreted by the thyroid gland and

its peripheral tissues. In the present study, serum T₄ concentration increased quadratically, peaking in the LJE-28 group; this indicates that supplementation with LJE alleviates heat stress.

Dairy cows encounter a variety of situations (calving, lactating, stress) during which ROS accumulate in the body (Sun et al., 2019). As previously mentioned, oxidative stress induced by heat stress results from imbalances between oxidant and antioxidant systems, in which production of ROS exceeds the capacity of antioxidant defense systems; MDA levels increase, but SOD, GSH, and T-AOC are suppressed (Pal et al., 2020). Because it catalyzes superoxide anion radical disproportionation to produce oxygen and hydrogen peroxide, SOD is an important marker reflecting antioxidant status (Gong et al., 2014). An important antioxidant enzyme, GSH-Px protects against harmful effects of ROS accumulation (Gong and Xiao, 2016). The T-AOC is an indicator for evaluating the body's ability to resist oxidation (Kampa et al., 2002), and MDA is a product of lipid peroxidation, the levels of which are related to the degree of lipid peroxidation in tissue cells (Samsonowicz and Regulska, 2017). Under heat stress conditions, the activity of antioxidant enzymes in serum, including GSH-Px and SOD, decreases, but serum MDA levels increase, resulting in oxidative damage to tissue cells (Gilgun-Sherki et al., 2001; Colakoglu et al., 2017). As expected, supplementation with LJE had antioxidant effects in heat-stressed dairy cows. Activity of GSH-Px and T-AOC increased, but concentrations of MDA fell after dietary supplementation with increasing doses of LJE; this effect was most noticeable in the LJE-28 group. Similar results were obtained by Wang and Huang (2004), who suggested that removal of excess free radicals was attributable to the potent antioxidant effects of polyphenols contained in LJE.

Many previous studies have demonstrated that LJE inhibits inflammatory responses by reducing inflammatory factors, which in turn supports immune function (Bai et al., 2020). T-helper cells comprise T-helper 1 (Th1) and T-helper 2 (Th2) subsets, categorized according to their cytokine secretion patterns (Carter and Dutton, 1996). IL-2 is a representative Th1-type cytokine that is vital for cell-mediated immunity; it also stimulates proliferation and differentiation of B cells to boost humeral immunity (He et al., 2005). IL-4, produced by Th2 cells, induces strong antibody responses that generate IgG (Sun et al., 2008). Heat stress has a negative effect on the immune system by altering the Th1:Th2 ratio and inhibiting production of cytokines; thus, appropriate amelioration strategies via nutritional interventions can improve immune competency of dairy cows (Bagath et al., 2019). We found that dietary supplementation with LJE increased con-

centrations of serum IL-4 and IgG, but decreased that of IL-2, particularly in the LJE-28 group, indicating that LJE helps to maintain the balance of Th1 and Th2 and improves immune function in heat-stressed dairy cows.

CONCLUSIONS

The present study showed that supplementation with different doses of LJE reduced RT of heat-stressed dairy cows. Although dietary addition of LJE did not affect DMI, milk yield, or milk composition, it did decrease serum CREA levels and increase T₃ and T₄ concentrations, and led to increased antioxidant effects and immune function of heat-stressed dairy cows. These findings proved that LJE effectively alleviated heat stress of dairy cows during a hot summer, and the optimum amount of LJE required is 28 g/d under the conditions described herein.

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