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Original Research Article

Pogostemon cablin essential oil as feed additive promotes the repair of the rumen epithelial barrier in heat-stressed beef cattle

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ABSTRACT

Pogostemon cablin essential oil (PEO), extracted from *P. cablin*, has anti-oxidant, anti-inflammatory, and anti-stress properties, as well as the ability to improve gastrointestinal digestion. This study aims to evaluate the effects of PEO on the performance, rumen epithelial morphology, and barrier function in heat-stressed beef cattle. Thirty-six male Jingjiang cattle at 18 months old were randomly assigned into four groups and fed a diet containing PEO at 0 (control), 50, 100, or 150 mg/kg in the feed concentrate ($n = 9$). All experimental cattle were fed under high temperature and humidity in summer for 60 days. The results indicated that 50 mg/kg of PEO treatment enhanced the average daily gain of beef cattle compared with the control group ($P = 0.032$). All PEO treatments reduced the diamine oxidase activity ($P = 0.004$) and malondialdehyde content ($P = 0.008$) in serum. In addition, the content of 70 kDa heat shock protein in the 100 mg/kg group was increased, and the activity of glutathione peroxidase and total antioxidant capacity in both 100 mg/kg and 150 mg/kg groups were enhanced compared to the control group ($P < 0.05$). More importantly, PEO treatment with 50 mg/kg enhanced the mRNA relative expressions of occludin in ruminal epithelia but decreased the mRNA relative expressions of c-Jun N-terminal kinase, P38 mitogen-activated protein kinases, caspase-3, Beclin1 ($P < 0.05$), and extremely significant declined the mRNA relative expressions of extracellular regulated protein kinases and ubiquitin-binding protein in contrast to the control group ($P < 0.01$). These findings indicated that dietary PEO supplementation might be favorable to improve growth performance and repairing damaged rumen epithelium of heat-stressed cattle by down-regulating the mitogen-activated protein kinase signaling pathway.

1. Introduction

Heat stress caused by the hot environment in summer is becoming a major concern for pastoralists with the elevation of mean global temperature, especially in tropical and subtropical areas (St-Pierre et al., 2003). Reportedly, heat stress has been linked to reduced feed intake, antioxidant capacity, and nutrient digestion and utilization in beef cattle (Mader et al., 2006). The rumen is critical for nutrient and electrolyte absorption in ruminants; however, heat stress reduces the blood flow in the gastrointestinal tract, which may be harmful to the integrity of the rumen epithelium (Bernabucci et al., 2009). Heat stress injury may lead to a decrease in nutrient absorption efficiency, and pathogenic microorganisms and their abnormal metabolites can enter the blood from the rumen and cause infection, suggesting a central role of the rumen in heat stress response in ruminants (Liu et al., 2013; Ma et al., 2021a,b; Zhai et al., 2019).

Pogostemon cablin essential oil (PEO) is an active ingredient derived from *P. cablin* (Blanco) Benth. (Labiatae) that is commonly

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used to treat vomiting, diarrhea, headache, and fever (Xu et al., 2021). *P. cablin* extract at 1×10^{-3} mg/mL can protect the follicular granulosa cells of hens from heat damage and maintain the normal secretory functions of the granulosa cells (Zhao et al., 2022). *P. cablin* essential oil plays a vital role in anti-oxidative and anti-inflammation processes (Wongsukkasem et al., 2018), and PEO by 90 μg/g DM in vitro culture may be responsible for modifying rumen fermentation ecology by improving ruminal feed degradability, microbial enzyme activities, and total protozoa counts (El-Zaiat and Abdalla, 2019). The main components of PEO include patchoulene, patchouliol, pogostone, etc. Patchouliol and pogostone are the active ingredients. Pogostone has shown anti-colorectal tumor effects in human colorectal carcinoma hct116 cells by inducing autophagy and apoptosis involving phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) axis (Cao et al., 2017). These results suggested that PEO has the potential to improve ruminal fermentation and maintain a stable rumen environment in ruminants. However, it is not clear what the effects of PEO are on ruminant heat stress and rumen epithelial barrier. We hypothesized that PEO might improve the growth performance by promoting the repair of the rumen epithelial barrier in heat-stressed beef cattle. Therefore, the present study was designed to test this hypothesis.

2. Materials and methods

2.1. Animal ethics statement

Jiangxi Agricultural University's Committee for the Care and Use of Experimental Animals approved this trial (JXAU-2020-40).

2.2. Preparation of PEO products

P. cablin essential oil (purity of 98%, contains 27.38% patchouliol, 19.25% α-patchoulene, 15.23% α-guaiene, etc.) was obtained from Jiangxi Zhongcheng Pharmaceutical Co., Ltd, Jiangxi province (China). The process of making the powder was completed by Hangzhou King Techina Feed Co., Ltd. (Hangzhou, China), and the contents of PEO in the final PEO products were 60%, and the remaining components are β-cyclodextrin inclusion agents.

2.3. Animals and experimental design

Thirty-six healthy male Jinjiang cattle of 18 months of age at a weight of 330 ± 21 kg were kept indoors in individual pens with concrete floors. Following a 10-day adaptation period, the feeding trial lasted 60 days (from July 11 to September 8 in 2022). Throughout the trial period, all cattle were allocated randomly to one of four treatments and dietary with 0 (control), 50, 100, and 150 mg/kg PEO products ($n = 9$). Table 1 shows the composition and nutrient levels of the basal diet. NE_{mf} was calculated according to the Chinese Feeding Standard of Beef Cattle (NY/T 815-2004). Dry matter (DM) content was detected by the National Standard of China (GB/T 6435-2014) using a constant temperature oven, and crude protein (CP) content was determined by standard GB/T 6432-2018 using an automatic Kjeldahl apparatus (HGK-50, Shanghai Heguan, China). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined by standards GB/T 20806-2022 and NY/T 1459-2022, respectively, using an automatic fiber analyzer (ANKOM 200, USA). Calcium content was determined using the potassium permanganate titration method (GB/T 6436-2018) and total phosphorus was determined by standard GB/T 6437-2018 using a dual beam UV visible spectrophotometer (TU-1901, Beijing Puxi, China). There were no antibiotics in the diets. The PEO products were premixed in the concentrate and then evenly mixed with brewer grains (wet, moisture 79.25%) and rice straw (3 to 5 cm length). Diets were supplied to the cattle twice daily at 06:00 and 17:00 and leftover materials collected 1 h after each feeding. Fresh water was available for ad libitum consumption throughout the study. The body weight of animals on an empty stomach was measured at 09:00 at the beginning and end of the trial, and the daily feed intakes were recorded during the experimental period. Based on these data, average daily dry matter intake (ADMI), average daily gain (ADG), and the ratio of feed and gain (F/G) were calculated.

Table 1
Composition and nutrient levels of the basal diet (air-dry basis, %).

Ingredients	Content	Nutrient levels[2]	Content
Brewers grains	20.0	DM	90.81
Rice straw	40.0	NE_{mf}, MJ/kg	7.25
Corn	21.5	CP	12.69
Soybean meal	15.0	NDF	44.30
Premix[1]	2.0	ADF	24.89
NaCl	0.5	Ca	1.00
$NaHCO_3$	1.0	TP	0.36
Total	100.0		

DM = dry matter; NEmf = combined net energy; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TP = total phosphorus.
[1] One kilogram of premix contained the following: vitamin A 190,000 IU, vitamin D_3 70,000 IU, vitamin E 2000 IU, Fe 2100 mg, Mn 3300 mg, Zn 3400 mg, Cu 460 mg, I 19 mg, Se 10 mg, Co 10 mg, Ca 130 g, P 30 g.
[2] NE_{mf} was a calculated value, while the others were measured values.

2.4. Detection of the temperature-humidity index (THI)

The temperature and humidity index was calculated from air temperature and relative humidity according to the method of Herbut et al. (2018). The air temperature and relative humidity in the cowshed at 08:00, 14:00 and 20:00 each day were determined using a thermohygrometer according to methods described by a previous study (Peng et al., 2021).

2.5. Serum parameters analysis

On day 60, blood sampling (15 mL each) was obtained from cattle by jugular vein puncture, and then serum was obtained by centrifugation (3000 × *g*, 10 min, 4 °C) and then stored at −20 °C for assay. The diamine oxidase (DAO) activity, 70 kDa heat shock protein (HSP70) content, and lipopolysaccharide (LPS) content in serum were measured following the manufacturer's recommendations when utilizing Enzyme linked immunosorbent assay (ELISA) kits from Solarbio Science & Technology (Beijing, China). The total antioxidant capacity (T-AOC), activities of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA) in serum were measured using spectrophotometric kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The contents of interleukin-6 (IL-6), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) in serum were analyzed using ELISA kits (Nanjing Jiancheng Bioengineering Institute, China).

2.6. Rumen epithelial tissue sample collection and preparation

Eight cattle from the control and 50 mg/kg PEO groups (four individuals per therapy with medium body weight) were chosen as experimental cattle for rumen epithelial tissue sample collection based on the performance and serum indices results. At the end of this trial, these cattle were transported to a slaughterhouse approximately 500 m away and slaughtered according to standard procedures. All contents were quickly removed from the rumen,

and one rumen wall piece measuring 2 cm × 2 cm was cut using surgical scissors from the abdominal sac, and then they were rinsed in ice-cold sterile phosphate buffer solution (pH 7.4). The rumen tissue samples were divided into three pieces: one of the samples was chopped into smaller pieces (approximately 1 g) and frozen in liquid nitrogen for quantitative real-time PCR, and the other two pieces for histomorphological examination were cut into 1 cm × 1 cm and 0.5 cm × 0.5 cm pieces and put in 4% polyformaldehyde and 2.5% glutaraldehyde solutions for scanning electron microscopy and paraffin sections, respectively.

2.7. Pre-staining and electron microscopy of rumen epithelial tissue sections

Rumen epithelial tissue sections for light microscopy and electron microscopy analysis were prepared using methods previously described by Liu et al. (2013). The 40 × objective lens was used to measure each stratum, and five photos were recorded per papilla for a total of 10 replicates per measurement per animal. The thickness of total epithelium (TE), stratum corneum (SC), stratum granulosum (SG), and stratum spinosum and basal (SSB) in the rumen wall were measured by Image-Pro Plus software (version 6.0, Media Cybernetics Inc., Bethesda, MD, USA). The papillae were dried to a critical point using liquid CO_2 as a medium, then mounted and gold-coated for scanning electron microscopy. The samples were observed with scanning electron microscopy (Hitachi Model S-3000N, Hitachi Technologies, Tokyo, Japan).

2.8. Quantitative real-time PCR (RT-qPCR)

Quantitative real-time PCR was used to quantify the relative expression of tight junction proteins, apoptosis and autophagy, mitogen-activated protein kinase (MAPK) signaling pathway-related genes at the mRNA relative expression levels. The ruminal samples were extracted for total RNA, and the cDNA was reverse-transcribed using the cDNA Synthesis Supermix Kit (TransGen Biotech, Beijing, China). Quantitative real-time PCR was conducted via the Green qPCR SuperMix Kit (TransGen Biotech, Beijing, China) and CFX96 Touch Real-Time PCR System (Bio-Rad Inc., Hercules, CA, USA) according to the following specifications: 95 °C for 10 min, 45 cycles at 95 °C for 15 s, 60 °C for 60 s, and extension at 95 °C for 15 s, which were the RT-qPCR profiles. All samples were examined three times. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expressions of these genes, with β-actin serving as the housekeeping gene. Primer 5.0 software was used to design amplification primers for all genes, displayed in Table 2.

2.9. Statistical analysis

The data are presented as mean and standard deviation (SEM). The SPSS 19.0 statistical software's one-way ANOVA method was used to analyze growth performance and serum parameters (Windows; SPSS, Chicago, USA). The ruminal morphological parameters and protein variables (mRNA relative expression levels) between the control and 50 mg/kg groups were compared using the SPSS 23.0 independent sample *t*-test. $P < 0.05$ was used as the significance level, and a trend was set at $0.05 < P < 0.10$.

3. Results

3.1. Temperature humidity index, growth performance

As shown in Fig. 1, the values of average daily THI during the whole trial period were higher than 79, of which 35 days were above 84. Moreover, the data in Table 3 showed PEO treatment with 50 mg/kg increased the ADG of beef cattle ($P = 0.032$), and tended to enhance their ADMI compared with the control group ($P = 0.081$). As the PEO levels increased, ADG ($P = 0.047$) and ADMI ($P = 0.042$) increased quadratically.

Table 2
Primers used for quantitative real-time PCR of cattle in this study.

Genes	Primer sequence (5′ to 3′)	Accession number
β-actin	F: CCCTGGAGAAGAGCTACGAG R: CAGGAAGGAAGGCTGGAAGA	NM_173979.3
Claudin-1	F: GGCAGATCCAGTGCAAAGTC R: ATGCCAATCACCATCAAGGC	XM_027538401.1
Occludin	F: AGATGCACGTTCGACCAATG R: ATTACTCCGGGAGGAGAGGT	XM-027520117.1
ZO-1	F: GCAGCCTGCATACAGATACG R: GGTAAGGCTGCTTGTCATCG	XM_019983400.1
CAS	F: GAACGTCCCTTTCTGCCATC R: TGCAAAGGATGGATGAGGGT	XM_010820245.3
BAX	F: CCCGAGTTGATCAGGACCAT R: GTGGGTGTCCCAAAGTAGGA	XM_027515208.1
BCL	F: ATGACCGAGTACCTGAACCG R: GCCATACAGCTCCACAAAGG	XM_027526256.1
LC3	F: GTCCGACTTATCCGAGAGCA R: TGAGCTGTAAGCGCCTTCTT	XM_027513856.1
P62	F: CTCCGGAAGCTGAAACATGG R: ACTGGGATCTTCCGATGGAC	XM_027548457.1
BEC	F: ACTGGACACGAGCTTCAAGA R: GGCTGTGGCAAGTAATGGAG	NM_001033627.2
JNK	F: CGCTACTACAGAGCACCTGA R: GCACCCAACTGACCAAATGT	NM_001192974.2
ERK	F: TTCAGATTCTGCTGCGCTTC R: AGACATCCCTCATGGCTTCC	XM_027526589.1
P38	F: CTTTGACCCAGATGCCGAAG R: GTCGTCAGGATCGTGGTACT	XM_024983652.1

F = forward; R = reverse; *ZO-1* = zonula occludens-1; *CAS* = caspase-3; *BAX* = BCL2-associated X; *BCL* = B-cell lymphoma-2; *LC3* = microtubule-associated protein 1 light chain 3; *P62* = ubiquitin-binding protein; *BEC* = Beclin1; *JNK* = c-Jun N-terminal kinase; *ERK* = extracellular regulated protein kinases; *P38* = P38 mitogen-activated protein kinases.

3.2. Blood index

As presented in Table 4, PEO treatment with 100 mg/kg enhanced the content of HSP70 ($P = 0.010$), PEO at 50 mg/kg tended to decrease the content of IL-1β ($P = 0.090$), and all treatments reduced the DAO activity and MDA content in serum ($P < 0.05$). Also, there was an increasing trend for the activity of T-SOD in the 50 mg/kg group ($P = 0.065$) and GSH-Px activity and T-AOC were enhanced in the 100 and 150 mg/kg groups when compared to the 0 mg/kg (control) group ($P < 0.05$). With an increase in PEO level, HSP70 content ($P = 0.012$) and GSH-Px activity ($P = 0.006$) and T-AOC ($P = 0.007$) increased linearly, however, the DAO activity ($P = 0.017$; $P = 0.007$) and MDA content ($P = 0.029$; $P = 0.006$) declined linearly and quadratically, respectively.

3.3. Histomorphological parameters

P. cablin essential oil treatment at 50 mg/kg decreased the ruminal SC's thickness compared with the control group ($P = 0.007$, Table 5). Moreover, the rumen papillae of the control group showed a state of desquamation, cracking, and excessive keratinization, whereas in the 50 mg/kg group, the surface of the papillae was repaired and keratinization was not obvious (Fig. 2).

3.4. The mRNA expression of tight junction proteins in the ruminal epithelium

In Fig. 3A, 50 mg/kg dietary supplementation with PEO increased the mRNA expression of occludin in ruminal epithelium compared with the control group ($P = 0.035$).

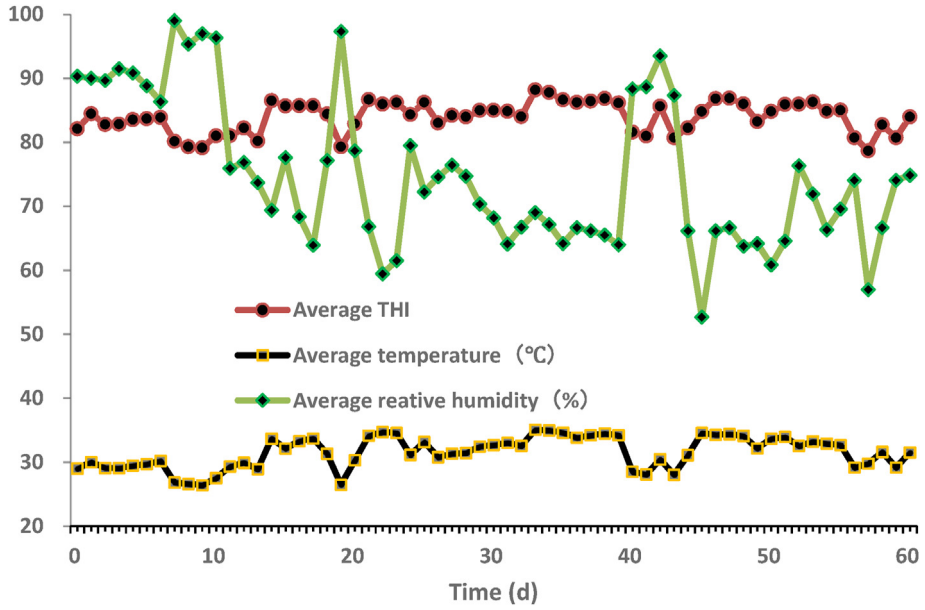

Fig. 1. Daily changes in daily average temperature, daily average relative humidity, daily average THI values at different days. No stress: THI ≤ 74; mild stress: 74 < THI < 79; high stress: 79 ≤ THI < 84; severe stress: THI ≥ 84. THI = temperature and humidity index.

Table 3
Effects of *Pogostemon cablin* essential oil on growth performance of beef cattle under heat stress ($n = 9$).

Item	Supplemental PEO level, mg/kg				SEM	*P*-value		
	0 (control)	50	100	150		ANOVA	Linear	Quadratic
ADG, kg/d	0.52[b]	0.68[a]	0.59[ab]	0.61[ab]	0.021	0.032	0.247	0.047
ADMI, kg/d	6.69	7.45	7.04	6.96	0.112	0.081	0.628	0.042
F/G	12.89	11.14	12.08	11.79	0.479	0.696	0.621	0.502

PEO = *Pogostemon cablin* essential oil; ADG = average daily gain; ADMI = average dry matter intake; F/G = feed/gain.
[a,b]Means in a row not sharing a common letter are significantly different ($P < 0.05$).

Table 4
Effects of *Pogostemon cablin* essential oil on parameters of serum of beef cattle under heat stress ($n = 6$).

Item	Supplemental PEO level, mg/kg				SEM	*P*-value		
	0 (control)	50	100	150		ANOVA	Linear	Quadratic
HSP70, pg/mL	170.00[b]	181.22[b]	215.96[a]	195.84[ab]	5.412	0.010	0.012	0.100
IFN-γ, pg/mL	150.68	145.10	149.54	149.61	3.443	0.951	0.970	0.704
TNF-α, pg/mL	34.12	31.57	34.01	33.22	0.904	0.761	0.974	0.645
IL-6, pg/mL	35.43	32.93	34.78	33.78	1.089	0.875	0.764	0.748
IL-1β, pg/mL	26.34	21.56	22.67	24.66	0.734	0.090	0.520	0.020
LPS, EU/L	165.61	163.29	154.77	160.07	2.639	0.526	0.307	0.486
DAO, U/L	57.03[a]	42.91[b]	45.51[b]	46.43[b]	1.623	0.004	0.017	0.007
GSH-Px, U/mL	104.71[c]	112.37[bc]	124.35[a]	117.57[ab]	2.289	0.010	0.006	0.066
T-AOC, U/mL	0.28[b]	0.31[ab]	0.33[a]	0.34[a]	0.009	0.045	0.007	0.485
T-SOD, U/mL	17.74	20.10	18.36	18.26	0.341	0.065	0.952	0.058
MDA, nmol/mL	3.65[a]	3.13[b]	3.11[b]	3.27[b]	0.101	0.008	0.029	0.006

PEO = *Pogostemon cablin* essential oil; HSP70 = 70 kDa heat shock protein; IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α; IL-6 = interleukin-6; IL-1β = interleukin-1β; LPS = lipopolysaccharide; DAO = diamine oxidase; GSH-Px = glutathione peroxidase; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase; MDA = malondialdehyde.
[a–c]Means in a row not sharing a common letter are significantly different ($P < 0.05$).

3.5. Effects of PEO on apoptosis and autophagy in heat-stressed beef cattle ruminal epithelium

As presented in Fig. 3B and C, compared to the control group, 50 mg/kg dietary supplementation with PEO decreased the mRNA expression of caspase-3 (*CAS*, $P = 0.029$), ubiquitin-binding protein (*P62*, $P < 0.001$) and Beclin1 (*BEC*, $P = 0.033$) in ruminal epithelium compared with the control group. Meanwhile, the 50 mg/kg PEO group reduced the average optical density of CAS ($P = 0.010$) and P26 ($P = 0.029$) as shown in Table 6.

3.6. The mRNA expression of MAPK signaling pathway-related genes in the ruminal epithelium

In Fig. 3D, 50 mg/kg dietary supplementation with PEO decreased the mRNA relative expressions of c-Jun N-terminal

Table 5
Effects of *Pogostemon cablin* essential oil on ruminal morphological indexes in heat-stressed beef cattle.

Item	Supplemental PEO level, mg/kg		SEM	*P*-value
	0 (control)	50		
Stratum corneum, μm	12.06[a]	8.71[b]	0.820	0.007
Stratum granulosum, μm	13.13	14.63	1.570	0.376
Stratum spinosum and basale, μm	51.28	50.59	4.950	0.894
Total epithelia, μm	76.46	73.93	4.900	0.624

PEO = *Pogostemon cablin* essential oil.
[a,b]Means in a row not sharing a common letter are significantly different ($P < 0.05$).

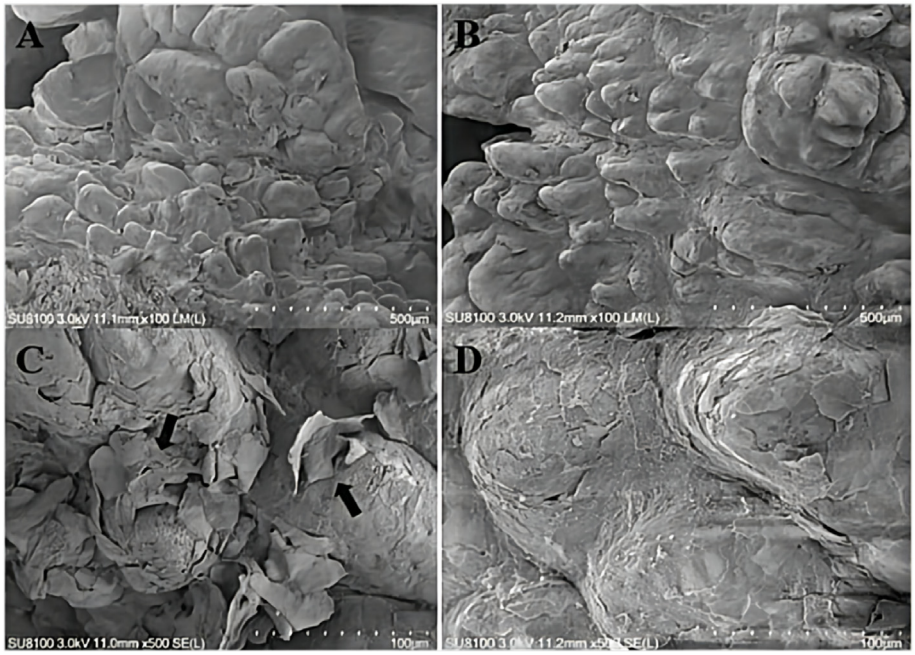

Fig. 2. Scanning electron microscopy of ruminal epithelial surface of 0 mg/kg PEO group (A, scale bar = 500 μm; C, scale bar = 100 μm) and 50 mg/kg PEO group (B, scale bar = 500 μm; D, scale bar = 100 μm). Arrow denotes excessive keratinization and desquamation of the ruminal epithelial surface.

kinase (*JNK*, $P = 0.017$), extracellular regulated protein kinases (*ERK*, $P < 0.001$), and P38 mitogen-activated protein kinases (*P38*, $P = 0.013$) in ruminal epithelium compared with the control group.

4. Discussion

The THI is used to evaluate the occurrence of heat stress among cattle. The critical values for minimum, mean, and maximum THI were $74 \leq \text{THI} \leq 79$, $79 \leq \text{THI} \leq 84$, and ≥ 84, respectively (Herbut et al., 2018). In this study, the THI was above 79 during the whole period, which indicated that the experimental cattle were in a state of heat stress and as a consequence the feed intake and growth performance were decreased (Mader et al., 2006). The current results indicated that dietary supplementation with 50 mg/kg PEO increased the ADG and tended to increase ADMI, and ADG and ADMI increased quadratically with the increase of the PEO level. These results suggested that dietary with PEO alleviated the heat stress response of beef cattle. This may be related to the effect of *P. cablin*, the source of PEO, commonly used to treat heat stroke. Consistently, our previous reports showed dietary supplements with Chinese medicine preparations containing *P. cablin* could increase the ADG and DMI of beef cattle (Chen et al., 2020). Animal heat tolerance was positively correlated with the concentration of HSP70, which is sensitive to temperature, is involved in cell development, and can protect animals from heat stress (Bagatell et al., 2000; Wang et al., 2017). The current results indicated that the content of HSP70 in serum increased linearly, suggesting PEO may contribute to the heat tolerance of beef cattle.

Although little research exists on the effect of patchouli oil against heat stress of animals, numerous studies have shown that patchouliol and pogostone could improve anti-oxidative and anti-inflammatory properties (Lin et al., 2014; Dechayont et al., 2017). In this investigation, PEO treatment linearly enhanced the T-AOC and GSH-Px activity in serum and decreased the content of MDA in serum linearly and quadratically, which suggested that PEO was important in anti-oxidative stress. High ambient temperature can induce High environmental temperatures can induce oxidative stress in farm animals (Bernabucci et al., 2014). A similar result in vitro showed that patchouli alcohol, the main component of PEO, modulated the morphology of IEC-6 cells and reduced their oxidative stress responses to heat shock (42 °C for 3 h) (Liu et al., 2016). Another study has demonstrated that supplementing with patchouli oil enhanced the activities of glutathione reductase (GR), GSH, and SOD in liver of animals under oxidative stress, which was in line with our results (Huang et al., 2018).

Additionally, heat stress can cause dysfunction or inflammation in animals, such as increased serum inflammatory cytokines and white blood cell count, and intensify inflammation (Tao et al., 2012). In this study, the serum content of IL-1β in the 50 mg/kg group tended to decrease. These results suggested that PEO

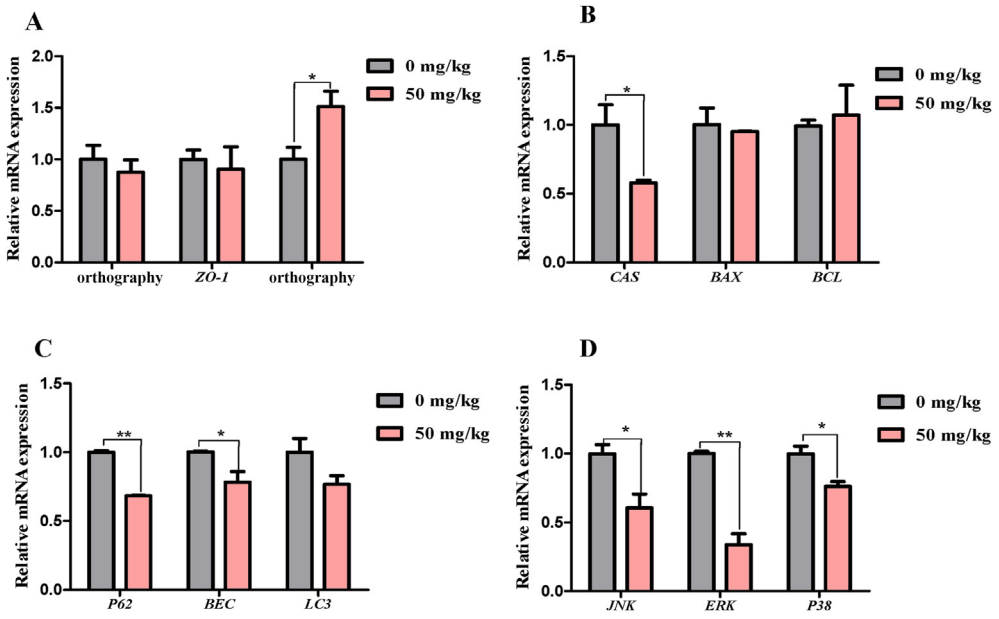

Fig. 3. Effects of *Pogostemon cablin* essential oil (PEO) on mRNA expression of tight junction proteins (A, claudin-1, *ZO*-1, and occludin), apoptotic genes (B, *CAS, BCL, BAX*), autophagy (C, *P62, BEC,* and *LC3*), MAPK signaling pathway-related genes (D, *JNK, ERK* and *P38*) in rumen of heat-stressed cattle. *ZO*-1 = zonula occludens-1; *CAS* = caspase-3; *BCL* = B-cell lymphoma-2; *BAX* = BCL2-associated X; *P62* = ubiquitin-binding protein; *BEC* = Beclin1; *LC3* = microtubule-associated protein 1 light chain 3; *JNK* = c-Jun N-terminal kinase; *ERK* = extracellular regulated protein kinases; *P38* = P38 mitogen-activated protein kinases. *$P < 0.05$; **$P < 0.01$.

Table 6
Effects of *Pogostemon cablin* essential oil on the average optical density of autophagy and apoptotic proteins in rumen tissues of heat-stressed beef cattle.

Item	Supplemental PEO level, mg/kg		SEM	*P*-value
	0 (control)	50		
BCL	0.0207	0.0169	0.00600	0.441
BAX	0.0196	0.0164	0.00290	0.311
CAS	0.0315[a]	0.0238[b]	0.00190	0.010
P62	0.0224[a]	0.0181[b]	0.00130	0.029
BEC	0.0143	0.0132	0.00090	0.248
LC3	0.0135	0.0124	0.00140	0.478

PEO = *Pogostemon cablin* essential oil; BCL = B-cell lymphoma-2; BAX = BCL2-associated X; CAS = caspase-3; P62 = ubiquitin-binding protein; BEC = Beclin1; LC3 = microtubule-associated protein 1 light chain 3.
[a,b]Means in a row not sharing a common letter are significantly different ($P < 0.05$).

inhibited the inflammatory response, which may be due to a single or synergistic effect of the essential oil's main components (including patchoulol, a-bulnesene and a-guaiene) or other minor constituents (such as seychellene and a-patchoulene) (Silva-Filho et al., 2016). A previous study on rats has shown that PEO and its derived compounds can reduce the concentration of inflammatory cytokines (Leong et al., 2019).

The rumen epithelium, which is made up of the stratum corneum, granular layer, spinous process layer, and basal layer, serves as a primary physical barrier to keep toxic substances in the rumen from infecting the rumen epithelium cells (Kleen et al., 2003). A previous study has demonstrated that oxidative stress induces inflammation and apoptosis of keratinized cells, leading to injury of the rumen epithelium (Ma et al., 2021a,b). In this study, supplementation with PEO reduced the thickness of SC in the rumen, suggesting PEO as a feed additive could repair damaged rumen epithelium of heat-stressed cattle. Scanning electron microscopy results consistently showed that PEO treatment improved rumen epithelial abscission and hyperkeratosis. Moreover, the current study showed that PEO decreased the activity of serum DAO linearly and quadratically. Diamine oxidase, which is found in the epithelial villus, may be released into blood vessels as the gastrointestinal epithelium's integrity deteriorates (Wu et al., 2018). The preceding outcomes indicated that adding PEO repaired the damaged rumen epithelium and improved the integrity of the rumen epithelium in heat-stressed beef cattle. Similarly, several studies on rats have shown that supplementing with PEO can improve gastrointestinal tract injury and protect the integrity of the gastrointestinal tract epithelial barrier (Chen et al., 2014; Liu et al., 2017).

A network of tight junctions and junction-associated proteins maintains the rumen epithelial barrier (Aschenbach et al., 2019). Tight junction proteins such as Claudin, ZO-1, and Occludin are essential in rumen epithelial barrier integrity. A previous finding showed that stress caused a decrease in mRNA expression of tight junction proteins, resulting in the disruption of tight junction structures (Hashimoto et al., 2008). The current results showed that supplementation with PEO increased the levels of *Occludin* mRNA relative expression. The above results indicated that diets with PEO could repair the damage to the rumen epithelial barrier caused by heat stress, which is consistent with the results of antioxidant and anti-inflammatory PEO treatment. A similar finding showed that PEO significantly enhanced the mRNA relative expression of *ZO*-1 and *Occludin*, and improved intestinal barrier function in rats (Leong et al., 2019).

Heat stress usually induces the production of ROS, and oxidative stress occurs when intracellular ROS cannot be eliminated as quickly as possible (Houston et al., 2018). Some studies have found that ROS can activate MAPK pathways, resulting in inflammation, apoptosis and cell death, then giving rise to tissue damage (McCubrey et al., 2006; Torres and Forman, 2003; Cargnello and Roux, 2011). In general, cell apoptosis is controlled by genes to maintain a stable internal environment, with key genes being *BEC* and BCL2-associated X (*BAX*). Previous studies have reported that

various toxic agents cause apoptosis in multiple tissues, including the liver and kidney, by increasing cytochrome c, BAX, and CAS levels and decreasing BEC levels, resulting in severe damage to tissues (Küçükler et al., 2021; Thangarajan et al., 2018). Autophagy is another physiological event induced by oxidative stress; moreover, BEC, microtubule-associated protein 1 light chain 3 (LC3) and P26 are involved in the autophagic process, which are essential markers used to determine the degree of autophagy. Furthermore, a previous study showed that autophagy activation protects against multiple tissue injuries under various stress conditions (Mo et al., 2018). It has been reported that natural antioxidant resveratrol reduced the abnormal reactive oxygen species accumulation, reducing autophagy and apoptosis in ovarian tissues (Jiao et al., 2022). A previous study determined that RUT (rutin, a flavone derivative) treatment protected against liver and kidney damage by attenuating VPA-induced (valproic acid) oxidative stress, endoplasmic reticulum stress, inflammation, apoptosis and autophagy (Kandemir et al., 2022).In the presented study, RT-qPCR and immunofluorescence results showed that PEO addition decreased the mRNA relative expression levels of *CAS*, *P26* and *BEC*, which inferred that PEO protected against apoptosis and autophagy, and it is consistent with previous results from scanning electron microscopy and paraffin sections.

The MAPK are a highly conserved family of serine/threonine kinases that play a central role in the range of fundamental cellular processes like cell growth, proliferation, death, and differentiation, including JNK, ERK1/2, and P38. Thus, ERK1/2 activation regulates cell proliferation. In the meantime, JNK and P38 are stress-responsive proteins that participate in inflammatory and apoptotic responses, respectively (Cargnello and Roux, 2011; Zhou et al., 2021). A previous study has confirmed that platycodon grandiflorum saponins (PGS) treatment protected against heat stress-induced oxidative injury in testis tissues by decreasing phosphorylation of JNK, ERK, and P38 MAPK (Leng et al., 2019). Another report showed that PIF_9 (pterostilbene derivative, a homolog of natural polyphenolic product of resveratrol) could block LPS-induced phosphorylation of ERK, JNK, and P38, which relieves oxidative stress (Fang et al., 2021). However, in our research, the 50 mg/kg PEO treatment group decreased the mRNA relative expressions of MAPK signaling pathway-related genes and the content of MDA. Still, it tended to increase the GSH-Px activity, T-AOC, SOD activity compared with the control group, which indicates that PEO treatment protected against heat stress-induced oxidative injury in the rumen epithelial tissue by reducing the expression levels of JNK, ERK, and P38 MAPK, which was consistent with the previous rumen epithelial tissue sections results. Nevertheless, the specific molecular mechanisms will need to be investigated further.

5. Conclusion

In conclusion, dietary supplements with PEO alleviated heat stress response, improved antioxidant status and immunity, and PEO at 50 mg/kg promoted the repair of the rumen epithelial barrier and improved beef cattle growth performance in hot conditions.

Author contributions

Huan Chen: Methodology, Writing – Original draft preparation. **Mingrui Yang:** Formal evaluation, Writing – Review & Editing. **Xianglong Shang:** Data curation, Writing – Original draft preparation. **Hao Chen:** Validation. **Yi Li:** Investigation, Visualization. **Yanjiao Li:** Supervision. **Lin Li:** Resources. **Mingren Qu:** Project administration. **Xiaozhen Song:** Work design, funding acquisition, and manuscript critical revision.

Data availability statement

There was no official repository for the data. However, the data supporting the findings are available if needed from the corresponding author with permission from Bovbjerg Økologi, Denmark.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Aschenbach JR, Zebeli Q, Patra AK, Greco G, Amasheh S, Penner D. Symposium review: the importance of the ruminal epithelial barrier for a healthy and productive cow. J Dairy Sci 2019;102(2):1866–82.
Bagatell R, Paine-Murrieta GD, Taylor CW, Pulcini EJ. Whitesell. Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of Hsp90-binding agents. Clin Cancer Res 2000;6(8):3312–8.
Bernabucci U, Biffani S, Buggiotti L, Vitali A, Lacetera N, Nardone A. The effects of heat stress in Italian Holstein dairy cattle. J Dairy Sci 2014;97(1):471–86.
Bernabucci U, Lacetera N, Danieli PP, Bani P, Nardone A, Ronchi B. Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. Int J Biometeorol 2009;53(5):387–95.
Cao ZX, Yang YT, Yu S, Li YZ, Wang WW, Peng C, et al. Pogostone induces autophagy and apoptosis involving pi3k/akt/mtor axis in human colorectal carcinoma hct116 cells. J Ethnopharmacol 2017;202:20–7.
Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 2011;75(1):50–83.
Chen H, Liao H, Liu Y, Zheng Y, Su Z. Protective effects of pogostone from Pogostemonis Herba against ethanol-induced gastric ulcer in rats. Fitoterapia 2014;100:110–7.
Chen J, Guo K, Song XZ, Lan LT, Liu SQ, Hu RM, et al. The anti-heat stress effects of Chinese herbal medicine prescriptions and rumen-protected γ-aminobutyric acid on growth performance, apparent nutrient digestibility, and health status in beef cattle. Anim Sci J 2020;91(1):e13361.
Dechayont B, Ruamdee P, Poonnaimuang S, Mokmued K, Chunthorng-Orn J. Antioxidant and antimicrobial activities of *pogostemon cablin* (blanco) benth. J Bot 2017;(4):1–6.
El-Zaiat HM, Abdalla AL. Potentials of patchouli (*Pogostemon cablin*) essential oil on ruminal methanogenesis, feed degradability, and enzyme activities in vitro. Environ Sci Pollut Res 2019;26(29):30220–8.
Fang M, Zou T, Yang X, Zhang Z, Cao P, Han J, et al. Discovery of novel pterostilbene derivatives that might treat sepsis by attenuating oxidative stress and inflammation through modulation of MAPKs/NF-κB signaling pathways. Antioxidants 2021;10(9):1333.
Hashimoto K, Oshima T, Tomita T, Kim Y, Matsumoto T, Yongmin K, et al. Oxidative stress induces gastric epithelial permeability through claudin-3. Biochem Biophys Res Commun 2008;376(1):154–7.
Herbut P, Angrecka S, Walczak J. Environmental parameters to assessing of heat stress in dairy cattle-a review. Int J Biometeorol 2018;62(12):2089–97.
Houston BJ, Nixon B, Martin JH, De Iuliis GN, Trigg NA, Bromfield EG, et al. Heat exposure induces oxidative stress and DNA damage in the male germ line. Biol Reprod 2018;98(4):593–606.
Huang Q, Wu X, Chen X, Wu J, Su Z, Liang J, et al. Patchouli oil isolated from the leaves of *pogostemon cablin* ameliorates ethanol-induced acute liver injury in rats via inhibition of oxidative stress and lipid accumulation. RSC Adv 2018;(8).

Jiao JH, Gao L, Yong WL, Kou ZY, Ren ZQ, Cai R, et al. Resveratrol improves estrus disorder induced by bisphenol A through attenuating oxidative stress, autophagy, and apoptosis. J Biochem Mol Toxicol 2022;36(9):e23120.
Kandemir FM, Ileriturk M, Gur C. Rutin protects rat liver and kidney from sodium valproate-induce damage by attenuating oxidative stress, ER stress, inflammation, apoptosis and autophagy. Mol Biol Rep 2022;49(7):6063–74.
Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP. Subacute ruminal acidosis (SARA): a review. J Vet Med A 2003;50(8):406–14.
Küçükler S, Çomaklı S, Özdemir S, Çağlayan C, Kandemir FM. Hesperidin protects against the chlorpyrifos-induced chronic hepato-renal toxicity in rats associated with oxidative stress, inflammation, apoptosis, autophagy, and up-regulation of PARP-1/VEGF. Environ Toxicol 2021;36(8):1600–17.
Leng J, Hou J, Fu CL, Ren S, Jiang S, Wang YP, et al. Platycodon grandiflorum Saponins attenuate scrotal heat-induced spermatogenic damage via inhibition of oxidative stress and apoptosis in mice. J Funct Foods 2019;54:479–88.
Leong W, Huang GX, Khan I, Xia W, Li YC, Liu YH, et al. Patchouli essential oil and its derived compounds revealed prebiotic-like effects in C57BL/6J mice. Front Pharmacol 2019;10(10):1229.
Lin RF, Feng XX, Li CW, Zhang XJ, Yu XT, Zhou JY, et al. Prevention of UV radiation-induced cutaneous photoaging in mice by topical administration of patchouli oil. J Ethnopharmacol 2014;154(2):408–18.
Liu JH, Xu TT, Liu YJ, Zhu WY, Mao SY. A high-grain diet causes massive disruption of ruminal epithelial tight junctions in goats. Am J Physiol Regul Integr Comp Physiol 2013;305(3):232–41.
Liu XX, Jiang LS, Liu FH, Chen YP, Xu L, Li DY, et al. Effect of patchouli alcohol on the regulation of heat shock-induced oxidative stress in IEC-6 cells. Int J Hyperth 2016;32(5):474–82.
Liu Y, Liang J, Wu J, Chen H, Li Y. Transformation of patchouli alcohol to β-patchoulene by gastric juice: β-patchoulene is more effective in preventing ethanol-induced gastric injury. Sci Rep 2017;7(1):5591.
Ma J, Shah AM, Wang ZS, Hu R, Zou HW, Wang XY, et al. Dietary supplementation with glutamine improves gastrointestinal barrier function and promotes compensatory growth of growth-retarded yaks. Animal 2021a;15(2):100108.
Ma Y, Zhang Y, Zhang H, Wang HR. Thiamine alleviates high-concentrate-diet-induced oxidative stress, apoptosis, and protects the rumen epithelial barrier function in goats. Front Vet Sci 2021b;8:663698.
Mader TL, Davis MS, Brownbrandl T. Environmental factors influencing heat stress in feedlot cattle. J Anim Sci 2006;84(3):712–9.
McCubrey JA, LaHair MM, Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. Antioxid Redox Sign 2006;8(9–10):1775–89.
Mo R, Lai R, Lu J, Zhuang Y, Zhou T, Jiang S, et al. Enhanced autophagy contributes to protective effects of IL-22 against acetaminophen-induced liver injury. Theranostics 2018;8(15):4170.
Peng T, Shang HL, Yang MR, Li YJ, Luo JR, Qu MR, et al. Puerarin improved growth performance and postmortem meat quality by regulating lipid metabolism of cattle under hot environment. Anim Sci J 2021;92(1):e13543.
Silva-Filho SE, Wiirzler L, Cavalcante H, Uchi D, Maria S, Cardia G, et al. Effect of patchouli (*Pogostemon cablin*) essential oil on in vitro and in vivo leukocytes behavior in acute inflammatory response. Biomed Pharmacother 2016;84(1):1697–704.
St-Pierre NR, Cobanov B, Schnitkey G. Economic losses from heat stress by US Livestock Industries. J Dairy Sci 2003;86(supp-S):52–77.
Tao S, Monteiro A, Thompson IM, Hayen MJ, Dahl GE. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci 2012;95(12):7128–36.
Thangarajan S, Vedagiri A, Somasundaram S, Sakthimanogaran R, Murugesan M. Neuroprotective effect of morin on lead acetate-induced apoptosis by preventing cytochrome c translocation via regulation of Bax/Bcl-2 ratio. Neurotoxicol Teratol 2018;66:35–45.
Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. Biofactors 2003;17(1–4):287–96.
Wang X, Xie H, Liu F, Wang Y. Production performance, immunity, and heat stress resistance in Jersey cattle fed a concentrate fermented with probiotics in the presence of a Chinese herbal combination. Anim Feed Sci Technol 2017;228:59–65.
Wongsukkasem N, Soynark O, Suthakitmanus M, Chongdiloet E, Chairattanapituk C, Vattanikitsiri P, et al. Antiacne-causing bacteria, antioxidant, anti-tyrosinase, anti-elastase and anti-collagenase activities of blend essential oil comprising rose, bergamot and patchouli oils. Nat Prod Commun 2018;13(5):639–42.
Wu QJ, Liu N, Wu XH, Wang GY, Lin L. Glutamine alleviates heat stress-induced impairment of intestinal morphology, intestinal inflammatory response, and barrier integrity in broilers. Poult Sci 2018;97(8):2675–83.
Xu Y, Ding L, Wang Z, Wang Y, Sun L. Virtual screening of cablin patchouli herb as a treatment for heat stress: a study based on network pharmacology, molecular docking, and experimental verification. Evid Based Complement Altern Med 2021;(3):1–14.
Zhai RN, Dong XS, Feng L, Li SL, Hu ZY. The effect of heat stress on autophagy and apoptosis of rumen, abomasum, duodenum, liver and kidney cells in calves. Animals 2019;9(10). 854–854.
Zhao QH, Xue WH, Zhang S, Guo Y, Li YR, Wu XJ, et al. The functions of Patchouli and Elsholtzia in the repair of hen follicular granular cells after heat stress. Poult Sci 2022;101(2):101306.
Zhou C, Gao J, Ji H, Li W, Xing X, Liu D, et al. Benzoylaconine modulates LPS-induced responses through inhibition of toll-like receptor-mediated NF-κB and MAPK signaling in RAW264. 7 cells. Inflammation 2021;44(5):2018–32.