



Original Research Article

Hydroxy-selenomethionine helps cows to overcome heat stress by enhancing antioxidant capacity and alleviating blood-milk barrier damage

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ABSTRACT

Heat stress can lead to decreased feed intake, apoptosis of mammary epithelial cells, and decreased milk yield and quality. Selenium is an important element in the composition of at least 25 selenoproteins. Hydroxy-selenomethionine (HMSeBA) is a novel organic selenium that has been shown to have a better deposition effect. However, whether HMSeBA alleviates damage to the mammary gland blood-milk barrier caused by heat stress and how this affects the performance of dairy cows remain largely unexplored. Therefore, 32 healthy Holstein cows with similar gestation days (150.41 ± 20.07 d), milk yield (36.15 ± 3.02 kg) and parity (3.25 ± 0.51) were selected and randomly divided into two total mixed rations with different selenium (Se) sources: sodium selenite (SSe) and HMSeBA. This study evaluated the outcomes of HMSeBA on antioxidant capacity, immunity, and blood-milk barrier damage in dairy cows during heat stress by collecting the samples of blood, rumen fluid and mammary gland biopsy. The experiment was conducted over 35 d, including a 5-day pre-feeding period and a 30-day experimental period. The temperature and humidity index (THI) were all above 80 throughout the experiment period. The results showed that HMSeBA decreased the respiratory rate ($P < 0.001$) and the content of inflammatory cytokines in the serum and increased the content of immune factors and antioxidant capacity ($P < 0.05$). In addition, HMSeBA reduced the expression of inflammatory cytokines and heat shock proteins in mammary gland ($P < 0.05$). Hematoxylin-eosin-stained pathological sections showed massive thickening of acinar walls and severe destruction of glandular structures in the SSe group, but the structure of the acinar mammary gland in the HMSeBA group was intact. Furthermore, HMSeBA promoted the expression of the phosphatidylinositol 3-kinase (*PI3K*, $P < 0.001$)/protein kinase B (*AKT*, $P = 0.011$)/mammalian target of rapamycin (*mTOR*, $P = 0.008$) pathway and improved the expression of zonula occludens-1 (*ZO-1*, $P = 0.014$) and occluding (*OCLN*, $P = 0.012$) in the mammary gland, suggesting less damage caused by heat stress to the blood-milk barrier. Our results demonstrated that HMSeBA can improve the antioxidant capacity and immunity of dairy cows and the expression of tight junction proteins in mammary gland to help alleviate the blood-milk barrier damage by heat stress, which could reduce the damage of heat stress on milk yield.

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1. Introduction

Dairy cows have dense hair and good thermal insulation; therefore, they are cold-resistant but not heat-resistant. The environmental temperature-humidity index (THI) is usually used to evaluate the extent to which animals suffer from heat stress. Cows are considered to be in heat stress condition when THI exceeds 72 (Ravagnolo et al., 2000). In dairy cows, heat stress can decrease feed intake and immunity and cause the apoptosis of mammary

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epithelial cells (MEC) (Tao et al., 2018), leading to decreased milk yield and quality (Zheng et al., 2021b). The mammary gland is the lactating organ and is key for ensuring production performance. Mammary epithelial cells are the milk secretory cells in the cow mammary gland (Boutinaud et al., 2015), and tight junctions between MECs separate the mammary compartment of the gland from the surrounding extracellular fluid. This tight junction structure prevents uncontrolled exchange of blood and milk components (Nguyen and Neville, 1998; Stelwagen and Singh, 2014). Therefore, the integrality of the blood-milk barrier is a premise for ensuring the normal function of the mammary gland in dairy cows. When the THI exceeds 72, dairy cows suffering from heat stress have decreased local immune function in the mammary gland and lead to temporary leakage of the mammary epithelial barrier (Tao et al., 2018). The effects of heat stress on cow performance have been reported in previous studies; milk yield of cows decreases with the increase of THI (St-Pierre et al., 2003; Tao et al., 2018). Becker et al., (2020) suggested that heat stress not only affects milk yield by reducing dry matter intake (DMI) but may also directly affect the distribution of energy in the whole body and lead to a decrease in milk yield. Therefore, we hypothesized that the decrease in milk yield caused by heat stress is associated with the damage to the blood-milk barrier induced by heat stress.

The biological function of Selenium (Se) is primarily mediated by selenoproteins containing selenocysteine (Hoffmann, 2007). Selenocysteine is the primary structural ingredient of 25 selenoproteins, including glutathione peroxidase (GSH-Px), thioxin reductase, and deiodinase, and can participate in regulating biological processes such as antioxidants, reproduction, and DNA synthesis in the body (Mehdi et al., 2013). Previous research had found that Se plays an important role in alleviating heat stress in animals. For example, Se can enhance feed efficiency by increasing nutrient metabolism (Stapleton, 2000), enhance antioxidant capacity of animals, decrease oxidative stress result from heat stress, and improve the thermoregulation ability of animals (Alhidary et al., 2012). Meanwhile, Se has also been observed to alleviate the inflammatory damage induced by heat stress through immunoreaction (Beck, 2007) and damage to the intestinal epithelial barrier and blood–brain barrier (Canter et al., 2021; Qiao et al., 2022). Hydroxy-selenomethionine (HMSeBA), as a novel organic Se, is proven to have a better deposition effect than other Se sources (Zheng et al., 2022b). Previous reports observed that HMSeBA can effectively mitigate the damage caused by heat stress in broilers and pigs (Liu et al., 2021; Sun et al., 2021), promote antioxidant status in cows (Sun et al., 2017), and improve Se concentration in milk and plasma (Juniper et al., 2019). Therefore, we hypothesized that HMSeBA could reduce the damage of heat stress on milk yield in cows by enhancing antioxidant capacity and immunity and alleviating damage to blood-milk barrier. To investigate this hypothesis, we used sodium selenite (SSe) as a control to evaluate the outcomes of HMSeBA on antioxidant capacity, immunity, and blood-milk barrier damage in dairy cows with heat stress.

2. Materials and methods

2.1. Animal ethics statement

All procedures were approved by the Ethics Committee of the College of Animal Science and Technology of the China Agricultural University (AW61902202-1-1), Beijing, China.

2.2. Experimental design and diets

This experiment was conducted at the Aomei Animal Husbandry Company in Xinxiang City, Henan Province, China. Thirty-two

healthy Holstein cows with days in milk (150.41 ± 20.07 d), milk yield (36.15 ± 3.02 kg/d) and parity (3.25 ± 0.51) were selected and randomly divided into two groups. During the experimental period, cows were provided the same total mixed ration (TMR, Table 1), except for the different source of Se, either SSe or HMSeBA (Seliseo, Adisseo France S. A. S.). In the HMSeBA group, we replaced an equal amount of SSe in the premix with HMSeBA, and the Se addition in the premix of both groups was 35 mg per kilogram. The diet was in accordance with NRC (2001). The experiment was conducted in summer; the environmental temperature and humidity of the cowshed were measured twice daily at 09:00 and 14:00 throughout the entire experiment period. The cowherd was divided into six points on average, and the temperature and humidity were measured at each point during each determination. Temperature and humidity index was calculated by the following formula (Hammami et al., 2013): $THI = (0.8 \times T) + [RH \times (T - 14.3)] + 46.4$, where T refers to ambient temperature ($^{\circ}\text{C}$) and RH refers to relative humidity (%). The average THI daily during the whole experiment period was shown in Fig. 1. Cows were group-fed and ad libitum twice daily at 07:00 and 19:00 with free access to water. All cows were milked three times daily at 06:30, 13:00 and 18:30. The experiment was conducted over 35 d, including a 5-d pre-feeding period and a 30-d experimental period. The diet for the pre-feeding period was the same as that of the experimental period.

Table 1
Ingredients and nutrient composition of the experimental diets (dry matter basis, %).

Item	Groups ¹	
	SSe	HMSeBA
Ingredients		
Corn silage	31.60	31.60
Alfalfa hays	10.89	10.89
Oat hays	1.76	1.76
Whole cottonseeds	2.87	2.87
Beet pulp	1.80	1.80
Corn flour	7.63	7.63
Steam-flaked corn	9.72	9.72
Soybean meal	13.32	13.32
Rapeseed meal	2.47	2.47
Expanded soybean	2.14	2.14
Grain silage	5.87	5.87
Molasses	4.07	4.07
Sodium bicarbonate	0.80	0.80
Premix 1 ²	2.59	–
Premix 2 ³	–	2.59
Fat powder	1.60	1.60
Yeast culture	0.68	0.68
Adsorbent	0.20	0.20
Total	100.00	100.00
Nutrient levels		
DM	48.14	48.41
CP	16.50	16.32
NDF	26.45	26.86
ADF	14.34	14.59
EE	5.14	5.08
Ash	6.97	7.29
Total selenium, mg/kg of DM	0.74	0.75

DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract.

¹ SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix.

² Additives per kilogram of premix 1: 450,000 IU, vitamin A, 150,000 IU, vitamin D₃, 3500 IU, vitamin E, 1600 mg nicotinamide, 250 g Ca, 40 g P, 200 g NaCl, 1200 mg Cu, 4000 mg Zn, 5000 mg Mn, 60 mg Co, 150 mg I, 90 mg β-Carotene, 35 mg sodium selenite.

³ Additives per kilogram of premix 2: 450,000 IU, vitamin A, 150,000 IU, vitamin D₃, 3500 IU, vitamin E, 1600 mg nicotinamide, 250 g Ca, 40 g P, 200 g NaCl, 1200 mg Cu, 4000 mg Zn, 5000 mg Mn, 60 mg Co, 150 mg I, 90 mg β-Carotene, 35 mg hydroxy-selenomethionine.

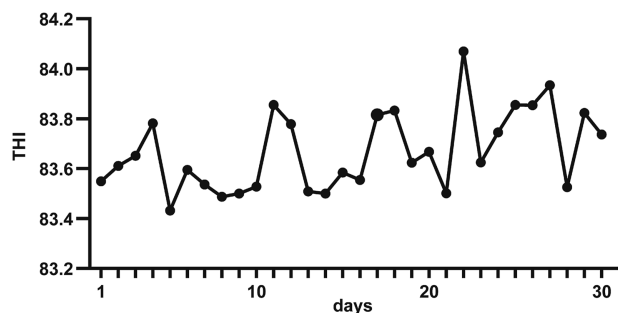


Fig. 1. Heat stress related indexes in SSe and HMSeBA cows. SSe: 35 mg sodium selenite group per kilogram of premix; HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix. THI = temperature and humidity index.

2.3. Performance, milk sampling and analysis

In order to estimate feed intake of cows after HMSeBA action, the feed intake was recorded on the last five days of the trial, on a group basis. The average daily feed intakes of the SSe and HMSeBA groups were 20.35 and 20.01 kg/d, respectively. Samples of feed were collected twice weekly and dried at 55 °C in a forced-draft oven for 72 h to determine the dry matter (DM) content and calculate the DMI. Dried TMR were smashed using a feedstuff mill for nutritional analysis. According to the methods 924.05, 984.12, and 920.39 (AOAC, 1995), the ash, crude protein (CP) and ether extract (EE) in TMR were determined, respectively. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed according to Van Soest et al., (1991). Total selenium content in the diet was determined according to the method described in Heard et al., (2007). Milk production was recorded daily from d 24 to 30 during the last week of the trial and the week prior to the start of the experiment. Milk samples were gathered at 30 d and stored at 4 °C with bronopol as a preservative. Milk protein, fat, and lactose were analyzed using an automated near-infrared milk analyzer (CombiFoss FT+; Foss Electric, Hillerød, Denmark). Selenium content in the milk was analyzed using atomic absorption spectroscopy (PerkinElmer Inc., Waltham, MA, USA). Rectal temperature, and respiratory rate were measured twice daily at 09:00 and 14:00, and the respiratory rate was determined by recording the number of breaths for each cow in 1 min.

2.4. Rumen fluid sample and analysis

On d 30 of the experiment, rumen fluids were collected before morning feeding by oral cannula and divided into three 2 mL cryopreservation tubes. One tube was stored at –80 °C for rumen 16S RNA determination, and the other samples were stored at –20 °C for the determination of subsequent fermentation parameters. Rumen volatile fatty acids (VFA) were measured by gas chromatography (6890N; Agilent Technologies, Santa Clara, CA, USA) (Zhang and Yang, 2011). Ammonia nitrogen (NH₃-N) content was measured using spectrophotometry, as previously described (Verdouw et al., 1978).

Ten cows were randomly selected from each group (milk yield = 28.32 ± 3.85 kg/d, Se in milk = 0.017 ± 0.0056 mg/kg in SSe group, *n* = 10; milk yield = 33.18 ± 2.85 kg/d, Se in milk = 0.035 ± 0.0007 mg/kg in HMSeBA group, *n* = 10) for 16S rRNA determination. Total microbial genomic DNA were extracted, detected and quality-controlled according to methods described by Yang et al., (2022). The Illumina MiSeq PE300 platform was used to sequence the amplification products, and the raw data were stored in FASTQ format. The raw data were demultiplexed and sliced by

FLASH version 1.2.7 and underwent quality control using FASTP version 0.19.6, as previously described (Yang et al., 2022). The sequence noise reduction method DADA2 (Callahan et al., 2016) was used to remove sequence errors from the optimized data to obtain the true sequence in the samples. The analysis of sequencing data and calculation of α -diversity index and β -diversity were performed through the Majorbio Cloud platform (<https://cloud.majorbio.com>).

2.5. Blood sample and analysis

On day 30 of the experiment, blood samples were collected before morning feeding from the caudal root vein for blood biochemical indices. The blood samples were collected in two 10-mL test tubes (one without additives, one with heparin) by venipuncture of the coccygeal vessels. The two tubes were centrifuged at 3000 × *g* for 15 min in a refrigerated centrifuge at 4 °C to obtain serum and plasma. Serum tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), lipopolysaccharide (LPS), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), nonesterified fatty acid (NEFA), β -Hydroxybutyric acid (BHBA), hydrogen peroxide (H₂O₂), nitric oxide (NO), cortisol (COR), serum amyloid A (SAA) and lipopolysaccharide binding protein (LBP) were measured by ELISA kits (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China).

Levels of superoxide dismutase (SOD), total antioxidant capacity (T-AOC), malondialdehyde (MDA), GSH-Px, blood urea nitrogen (BUN), total protein (TP), albumin (ALB), globulin (GLB), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine aminotransferase (ALT), cholinesterase (CHE), creatine kinase (CK), and creatinine (CRE), glucose (GLU) and uric acid (UA) in serum were determined by a biochemical analyzer (Hitachi Co., Tokyo, Japan). Plasma selenium content was measured using atomic absorption spectroscopy (PerkinElmer Inc., Shanghai, China).

2.6. Mammary gland biopsy and analysis

Mammary gland tissue was obtained by biopsy according to Farr et al., (1996). Briefly, five cows were randomly selected from each group (*n* = 5) to collect mammary gland tissue at 15:00 on the last day of the trial. The cows were mildly sedated with an intravenous injection of xylazine hydrochloride (0.05 mg/kg body weight; 2% Seractal; Bayer, Leverkusen, Germany). Approximately 10 square centimeters of skin from the left posterior gland was washed with 75% alcohol and iodine. The biopsy site was selected at the midpoint of the left posterior quarter, and 3 mL of lidocaine hydrochloride was subcutaneously injected (20 mg/mL; Lopaine, Wuhan, China). A 1 to 2 cm incision was made on the skin and gland capsule, and the core of the mammary gland was cut using a biopsy instrument rotated with an electric motor. Once the core was cut, the retractable blade was extended, and a 3- or 4-s rotation was used to cut off the inner end of the core. When the blade was extended, the instrument was withdrawn from the tissue with the sample. Pressure was applied to the puncture hole to reduce blood accumulation under the skin immediately after the biopsy, and the incision was sutured with absorbable suture (#2 Supramid). After sampling, procaine benzylpenicillin was administered intramuscularly for 3 d. The collected samples were rinsed with saline solution for analysis using immunofluorescence, transmission electron microscopy, hematoxylin and eosin staining, and relative messenger ribonucleic acid (mRNA) expression.

Hematoxylin and eosin staining of mammary gland samples was performed as described by Zheng et al., (2022b). According to the method described by Liu et al., (2019), histological scoring was performed. Referring to the methods of Zheng et al., (2021a),

transmission electron microscopy of tight junction structure, immunofluorescence detection of zonula occludens-1 (*ZO-1*), claudin 1 (*CLDN1*), and occluding (*OCLN*) were performed. Ribonucleic acid was extracted by TRIzol reagent (TaKaRa Bio, Kusatsu, Japan) from mammary gland samples, and a NanoDrop ND-2000 spectrophotometer was used to measure RNA concentration and purity (Zheng et al., 2021a). The primers of each target gene used in this study are listed in Table 2, with glyceraldehyde-3-phosphate dehydrogenase (*GADPH*) as the housekeeping gene. The relative mRNA expression levels were analyzed by real-time quantitative PCR (Livak and Schmittgen, 2001).

2.7. Statistical analysis

According to the results of the power analysis, each group was expected to have a power of 95.79% with a 0.05 significance level (α) based on the milk yield from dairy cows. The means of data were analyzed using a t-test in SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA). The model for the above data was the following equation: $Y_{ij} = \mu + Tr_{ti} + e_{ij}$, where μ is the overall mean, Y_{ij} is the dependent variable, Tr_{ti} is the fixed effect of treatment, and e_{ij} is the random error. The normal distribution of all the means was evaluated through the Shapiro–Wilk test. Histograms of rectal temperature, respiratory rate, performance, serum indexes, rumen fermentation parameters, and the relative mRNA expression were visualized by GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ indicated significant difference.

Wilcoxon rank-sum test was used to compare the differential genera of rumen bacteria, and false discovery rate (FDR) adjusted P -value < 0.05 was considered significant difference. The correlation of rumen dominant genera (TOP 30), performance, and blood indices were performed by Spearman's correlation test, and $|r| > 0.6$ and $P < 0.05$ was considered significant difference. The histograms of alpha diversity and differential bacteria, principal coordinate

analysis, circo diagram of the microbial community, and correlation heat map were visualized using the Majorbio Cloud platform (<https://cloud.majorbio.com>).

3. Result

3.1. Rectal temperature, respiratory rate and performance

Table 3 shows the rectal temperature of cows between the SSe and HMSeBA groups have no differences at 09:00 ($P = 0.817$) and 14:00 ($P = 0.369$), respectively. However, the respiratory rate of dairy cows in the HMSeBA group was significantly lower than the SSe group at both 09:00 ($P < 0.001$) and 14:00 ($P < 0.001$).

Table 4 shows the performance parameters of SSe and HMSeBA cows. Prior to the experiment, milk yield between the two groups was not different ($P = 0.842$), but the milk yield of the HMSeBA cows was higher compared with the SSe cows after the experiment ($P = 0.037$). Compared to the SSe group, the milk protein in the HMSeBA group was lower ($P = 0.006$). In contrast, the Se content of milk in HMSeBA cows was higher ($P < 0.001$) than the SSe group.

3.2. Serum indicators

Blood inflammatory cytokines, immune indices, and antioxidant levels are shown in Table 5. The Se content of plasma in the HMSeBA group was higher than the SSe group ($P < 0.001$). The concentrations of IL-1 β ($P = 0.001$), IL-6 ($P < 0.001$), SAA ($P = 0.028$) and LPS ($P < 0.001$) were lower, whereas the concentrations of IL-1 β ($P = 0.001$), IgA ($P = 0.009$), IgG ($P = 0.002$), and IgM ($P = 0.006$) were higher in the HMSeBA group compared to SSe group. The levels of T-AOC ($P = 0.007$), SOD ($P = 0.005$), and GSH-Px ($P = 0.001$) in the HMSeBA group were higher than the SSe group, however, the MDA ($P = 0.013$) levels were lower. As shown in Table S1, the levels of UA ($P < 0.001$) and NEFA ($P < 0.001$) in the HMSeBA group were higher compared to the SSe group, whereas the content of ALP ($P = 0.029$) and GLU ($P = 0.033$) in the HMSeBA group were lower than the SSe group.

3.3. Rumen fermentation parameter and bacteria

Table 6 shows that the content of isobutyrate ($P = 0.003$) and isovalerate ($P = 0.017$) in the HMSeBA group were lower than the SSe group. The concentrations of total volatile fatty acids (TVFA, $P = 0.257$), acetate ($P = 0.182$), propionate ($P = 0.945$), butyrate ($P = 0.159$), valerate ($P = 0.678$), acetate/propionate ($P = 0.081$) and $\text{NH}_3\text{-N}$ ($P = 0.872$) were not different between the two groups.

The values of the alpha diversity indices were not different ($P > 0.05$) between the SSe and HMSeBA groups. At the genus level, the beta diversity results by permutational multivariate analysis of

Table 2
Primer sequences for qPCR.¹

Gene symbol	Primer sequence (5' to 3')	Accession number
<i>HSF1</i>	F: ATTCCGACAGGACAGTGTACCAAG R: GCCTCGTTCTCGTTCATGG	NM_001076809.1
<i>HSP90</i>	F: ACTGCTCTCTGCTGCTCTCG R: TCCTTGGCGTACCTCATCTTC	NM_001079637.1
<i>CLDN1</i>	F: CCCGTGCCITGATGGTATTGG R: CATCTTCTGCTCGTCTGCTTC	NM_001001854.2
<i>OCLN</i>	F: GCCTGTGTGCTCCACTCTTG R: CCATAGCCATAACCGTAGCCATAGC	NM_001082433.2
<i>ZO-1</i>	F: GCATGATGATCGTCTGCTACCTG R: CCGCCTTCTGTCTGTCTCTTC	NC_037348.1
<i>IL-6</i>	F: GCCTTCACTCCATTCGCTGTCTC R: AAGTAGTCTGCTGGGTGGTG	NM_173923.2
<i>IL-1β</i>	F: CACTACAGGCTCCGAGATGAACAAC R: TGTCGGTCTGGTTCCTTGTAC	NM_008361.4
<i>TNF-α</i>	F: GCTGACGGGCTTACCTCATCTAC R: GGCTCTTGATGGCAGACAGGATG	NM_173966.3
<i>PI3K</i>	F: AGGTGTTGGTGAAGGATTCAGTTGG R: AGGTAGCATCCGAAGTCCAGAC	NM_001206047.2
<i>AKT</i>	F: GCAGGACGTGACGAGAAGAAGC R: GTCTGGTCAGGTGGCGTAATGG	NM_173986.2
<i>mTOR</i>	F: TCACCCTTGCTCTCCAACTCTC R: GTCTTGGTCAGGTGGCGTAATGG	XM_002694043.7
<i>GADPH</i>	F: CGGCACAGTCAAGGCAGAGAAC R: CCACATACTCAGCACCAGCATCAC	NM_001034034.2

HSF1 = heat shock transcription factor 1; *HSP90* = heat shock protein 90; *CLDN1* = claudin 1; *OCLN*, occluding; *ZO-1* = zonula occludens-1; *IL-6* = interleukin-6; *IL-1 β* = interleukin-1 β ; *TNF- α* = tumor necrosis factor- α ; *PI3K* = Phosphatidylinositol 3-kinase; *AKT* = protein kinase; *mTOR* = mammalian target of rapamycin; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase; F = forward; R = reverse.

¹ Primers were synthesized by Biotech (Shanghai) Co., Ltd.

Table 3
Rectal temperature and respiratory rate.

Item	Groups ¹		SEM	P -value
	SSe	HMSeBA		
09:00				
Rectal temperature, °C	38.70	38.22	0.039	0.817
Respiratory rate, times/min	80.84 ^a	67.53 ^b	1.484	<0.001
14:00				
Rectal temperature, °C	38.78	38.86	0.043	0.369
Respiratory rate, times/min	80.17 ^a	73.7 ^b	0.930	<0.001

SEM = standard error of the mean.

^{a,b} Within the same row, values with different small letter superscripts mean significant difference ($P < 0.05$).

¹ SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix.

Table 4
Milk yield and milk composition.

Item	Groups ¹		SEM	P-value
	SSe	HMSeBA		
Milk yield, kg/d				
MY-pre ²	36.26	36.04	0.534	0.842
MY-post ³	29.46 ^b	32.47 ^a	0.878	0.037
4% FCM ⁴	30.83	32.99	1.013	0.309
ECM ⁵	33.70	35.45	0.976	0.394
Milk composition				
Fat, %	4.18	4.00	0.179	0.632
Protein, %	3.81 ^a	3.40 ^b	0.078	0.006
Lactose, %	5.15	5.06	0.056	0.440
Fat yield, kg/d	1.26	1.32	0.058	0.623
Protein yield, kg/d	1.11	1.10	0.029	0.892
Lactose yield, kg/d	1.50	1.64	0.043	0.094
Se in milk, mg/kg	0.017 ^b	0.034 ^a	0.002	<0.001

MY = milk yield; FCM = fat corrected milk; ECM = energy corrected milk; SEM = standard error of the mean.

^{a, b} Within the same row, values with different small letter superscripts mean significant difference ($P < 0.05$).

¹ SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix.

² MY-pre: milk yield at pre-experiment.

³ MY-post: milk yield at post-experiment.

⁴ 4% FCM = $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$.

⁵ ECM = $12.96 \times \text{fat yield} + 7.04 \times \text{protein yield} + 0.3246 \times \text{milk yield}$.

Table 5
The blood inflammatory cytokines, indexes of immunity and antioxidant.

Item	Groups ¹		SEM	P-value
	SSe	HMSeBA		
Se in plasma, mg/L	0.10 ^b	0.12 ^a	0.016	<0.001
Inflammatory cytokines				
IL-1 β , ng/L	45.21 ^a	40.60 ^b	0.768	0.001
IL-6, ng/L	526.60 ^a	467.90 ^b	9.109	<0.001
TNF- α , ng/L	168.80	164.70	2.052	0.327
LPS, U/L	50.66 ^a	44.54 ^b	0.941	<0.001
SAA, $\mu\text{g/mL}$	30.73 ^a	27.73 ^b	0.693	0.028
Immune indexes, mg/mL				
IgA	551.70 ^b	622.40 ^a	14.053	0.009
IgG	16.11 ^b	18.22 ^a	0.109	0.002
IgM	4.26 ^b	4.83 ^a	0.365	0.006
Antioxidant indexes				
T-AOC, U/mL	9.41 ^b	9.95 ^a	0.104	0.007
GSH-Px, $\mu\text{mol/L}$	18.17 ^b	19.13 ^a	0.159	0.001
LBP, $\mu\text{mol/L}$	41.21	41.97	0.490	0.502
COR, ng/L	7.54	6.86	0.613	0.587
MDA, mmol/mL	1.59 ^a	1.44 ^b	0.031	0.013
SOD, U/mL	51.62 ^b	56.63 ^a	0.943	0.005
H ₂ O ₂ , mmol/L	38.25	38.21	0.776	0.977
NO, $\mu\text{mol/L}$	26.46	20.95	2.231	0.224

IL-1 β = interleukin-1 β ; IL-6 = interleukin-6; TNF- α = tumor necrosis factor- α ; LPS = lipopolysaccharide; SAA = serum amyloid A; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; LBP = lipopolysaccharide-binding protein; COR = cortisol; MDA = malondialdehyde; SOD = superoxide dismutase; H₂O₂ = hydrogen peroxide; NO = nitric oxide; SEM = standard error of the mean.

^{a, b} Within the same row, values with different small letter superscripts mean significant difference ($P < 0.05$).

¹ SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix.

variance (PERMANOVA) showed that the Curtis distances were distinct between the SSe and HMSeBA groups (Fig. 2B, PERMANOVA, $P = 0.006$). *Prevotella*, *Lachnospiraceae_NK3A20_group*, *Ruminococcus*, and *NK4A214_group* were the predominant genera in both the groups (Fig. 2C). In Fig. 2D, the relative abundance of *Ruminococcus* ($P = 0.001$), *Prevotellaceae_UCG_003* ($P = 0.021$), *Treponema* ($P = 0.031$), *Bifidobacterium* ($P = 0.032$), *CAG_352* ($P < 0.001$), and *U29_B03* ($P = 0.006$) in the HMSeBA group were

Table 6
Volatile fatty acids in the rumen.

Item	Groups ¹		SEM	P-value
	SSe	HMSeBA		
TVFA, mmol/L	142.86	131.81	4.780	0.257
Acetate, mmol/L	73.66	66.46	2.570	0.182
Propionate, mmol/L	37.63	37.83	1.440	0.945
Iso-butyrate, mmol/L	1.78 ^a	1.24 ^b	0.095	0.003
Butyrate, mmol/L	24.95	22.43	0.881	0.159
Iso-valerate, mmol/L	3.3 ^a	2.52 ^b	0.168	0.017
Valerate, mmol/L	3.64	3.53	0.131	0.678
Acetate to propionate ratio	2.01	1.77	0.070	0.081
N-NH ₃ , mg/dL	32.85	32.29	1.662	0.872

TVFA = total volatile fatty acids; N-NH₃ = N-ammonia; SEM = standard error of the mean.

^{a, b} Within the same row, values with different small letter superscripts mean significant difference ($P < 0.05$).

¹ SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine per kilogram of premix.

lower compared to the SSe group, but the relative abundance of *Syntrophococcus* ($P = 0.001$), *Erysipelotrichaceae_UCG_002* ($P = 0.025$), *Shuttleworthia* ($P < 0.001$), *Dialister* ($P = 0.023$), *Lachnospira* ($P = 0.031$), *Erysipelotrichaceae_UCG_007* ($P = 0.030$), *Pesudoscovicia* ($P = 0.005$), *Lachnospiraceae_NK4A136_group* ($P = 0.013$), and *Desulfovibrio* ($P = 0.004$) were higher. Spearman's correlation revealed that milk protein percentage had a negative relationship with *Dialister*, *Shuttleworthia*, *Syntrophococcus*, and *norank_f_norank_o_Clostridia_UCG-014* (Fig. 2E, $|r| > 0.6$, $P < 0.05$) but had a positive relationship with *Ruminococcus* and *NK4A214_group* (Fig. 2E, $|r| > 0.6$ and $P < 0.05$). The content of Se in milk had a positive relationship with *Shuttleworthia* and *Syntrophococcus* (Fig. 2E, $|r| > 0.6$, $P < 0.05$) but had a negative relationship with *Ruminococcus* and *norank_f_F082* (Fig. 2E, $|r| > 0.6$ and $P < 0.05$). The 09:00 respiratory rate had a negative relationship with *Shuttleworthia*, *Erysipelotrichaceae_UCG-002*, *Syntrophococcus*, *Ruminococcus_gauvreauii_group*, and *unclassified_f_Lachnospiraceae* (Fig. 2E, $|r| > 0.6$, and $P < 0.05$) but had a positive relationship with *Ruminococcus* and *Prevotellaceae_UCG-003* (Fig. 2E, $|r| > 0.6$ and $P < 0.05$). The 14:00 respiratory rate had a positive relationship with *Ruminococcus* and *norank_f_F082* (Fig. 2E, $|r| > 0.6$ and $P < 0.05$).

Fig. S1 shows the correlation among the dominant genera, immunity, and blood antioxidant indices. The results showed that *Ruminococcus* had a negative relationship with SOD, IgA, IgG, and IgM but a positive relationship with IL-1 β and IL-6 (Fig. S1, $|r| > 0.6$ and $P < 0.05$). There was a close relationship between *Prevotellaceae_UCG_003* and SOD (Fig. S1, negative, $|r| > 0.6$ and $P < 0.05$) and SAA (Fig. S1, positive, $|r| > 0.6$ and $P < 0.05$). *Syntrophococcus* had a positive relationship with T-AOC and SOD but a negative relationship with SAA (Fig. S1, $|r| > 0.6$ and $P < 0.05$). There was a close relationship between *Erysipelotrichaceae_UCG-002* and T-AOC (Fig. S1, positive, $|r| > 0.6$ and $P < 0.05$) and SAA (Fig. S1, negative, $|r| > 0.6$ and $P < 0.05$).

3.4. Blood-milk barrier injury, heat shock proteins and inflammatory cytokines

Hematoxylin and eosin staining (Fig. 3A) showed massive thickening of acinar walls and severe destruction of glandular structures in the mammary gland of the SSe group, however, the structure of the acinar mammary gland in the HMSeBA group was intact and there were no significant pathological changes. Figure 3B shows that the histological score of the mammary gland in HMSeBA group was lower than the SSe group ($P < 0.001$). In addition, the

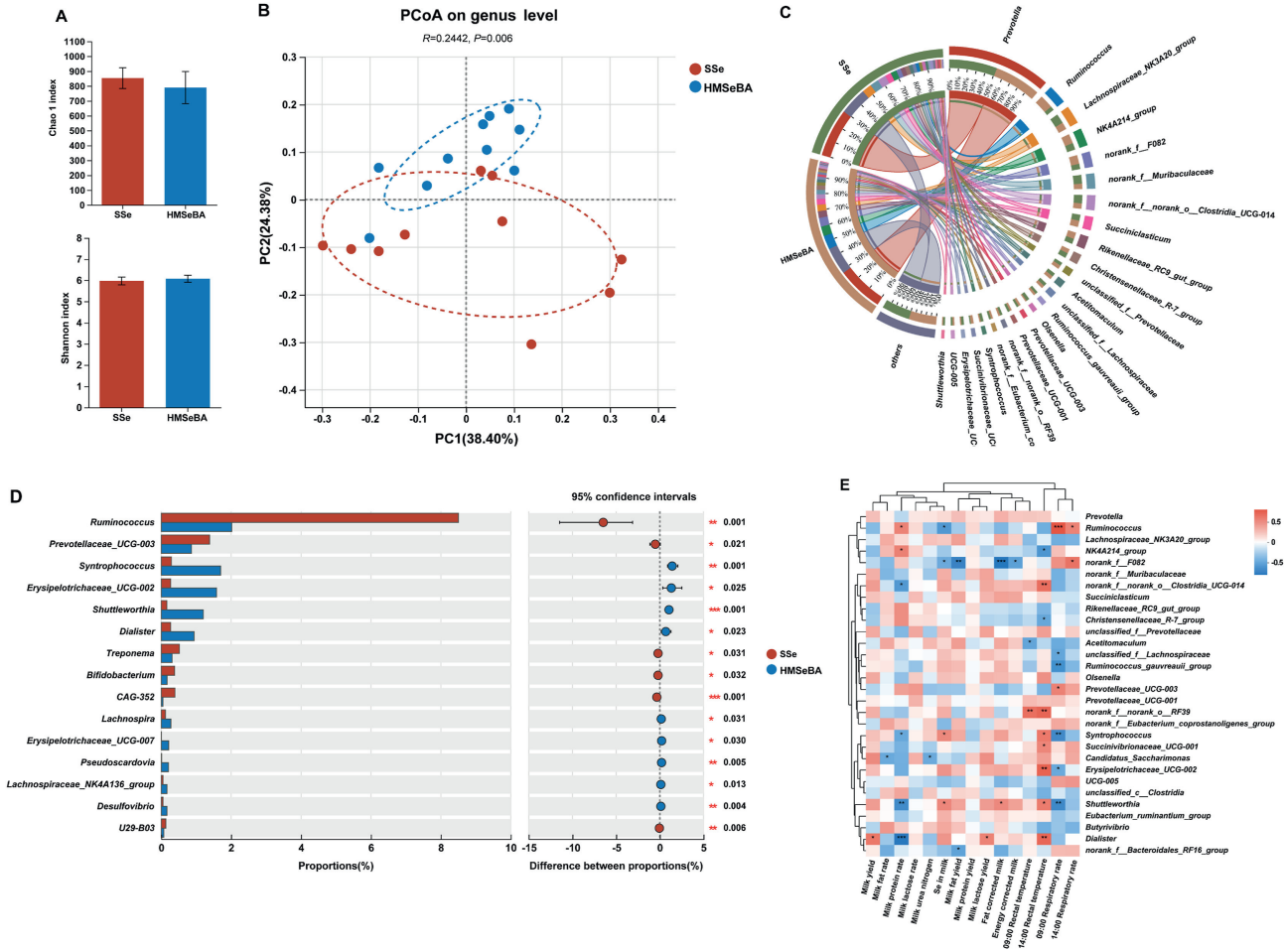


Fig. 2. Different bacteria communities in SSe and HMSeBA cows. (A) Alpha diversity; (B) Principal coordinate analysis (PCoA) profile of ruminal bacterial community based on unweighted UniFrac dissimilarity matrix at the taxa (ASVs) level (PERMANOVA, $P = 0.006$, $R = 0.2442$); (C) Dominant bacteria genus; (D) Differential bacterial genus (TOP 15); (E) The spearman's correlation of heat-stress related indexes and performance with bacteria community of genus level (TOP 30), $|r| > 0.6$ and $P < 0.05$ was considered significant difference. SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine group per kilogram of premix. Significance was tested using independent two-group Wilcoxon rank-sum tests with the FDR adjusted P -value, * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$.

acinar area in the HMSeBA group was larger than the SSe group ($P < 0.001$).

The mRNA expressions of *IL-6* (Fig. 3C, $P = 0.003$) and *TNF- α* (Fig. 3C, $P = 0.029$) in the HMSeBA group were significantly decreased compared with the SSe group, however, the *IL-1 β* (Fig. 3C, $P = 0.663$) mRNA expression were not different between the two groups. The mRNA expression of Heat Shock Transcription Factor 1 (*HSF1*, Fig. 3D, $P < 0.001$) and Heat shock protein 90 (*HSP90*, Fig. 3D, $P = 0.035$) in the HMSeBA group were significantly lower than the SSe group.

3.5. The tight junction structure, proteins and PI3K/AKT/mTOR signaling pathway

Electron microscopy was used to observe tight junctions (Fig. 4A). The tight junction structure of the mammary gland in the SSe group was seriously damaged and the structure was indistinct under an electron microscope, whereas the HMSeBA group was visibly improved. The mammary gland mRNA expressions of *ZO-1* (Fig. 4B, $P = 0.014$) and *OCLN* (Fig. 4B, $P = 0.012$) of the HMSeBA group were higher than in the SSe group. The immunofluorescence results for tight junction proteins showed visible expression in the mammary gland of the HMSeBA group, with a complete and

defined acinar structure (Fig. 4C). In the SSe group, the three tight junction proteins (*ZO-1*, *CLDN1*, *OCLN*) were severely damaged, and the acinar structure was blurred. As shown in Fig. 4D, the mammary gland mRNA expressions of the signal molecules phosphatidylinositol 3-kinase (*PI3K*) ($P < 0.001$), protein kinase B (*AKT*) ($P = 0.011$) and mammalian target of rapamycin (*mTOR*) ($P = 0.008$) in the HMSeBA group were higher than the SSe group.

4. Discussion

Heat stress is defined as a combination of internal and external forces that cause an animal to raise its body temperature and evoke physiological responses (Becker et al., 2020). Heat stress poses various hazards to cows such as raised body temperature, decreased appetite and milk yield (Brown-Brandl et al., 2005; Mader et al., 2006). Temperature-humidity indices are used to determine the severity of heat stress in cows (Bohmanova et al., 2007), and when $THI \geq 72$, cows are considered to be in a state of heat stress. It has been reported that when the surrounding temperature rises from 24 to 39 °C, the respiratory rate will increase from 2.8 to 3.3 times/min for each 1 °C rise in surrounding temperature (Gaughan et al., 2000). In this study, the THI was greater than 80 throughout the whole experiment (Fig. 1), illustrating that

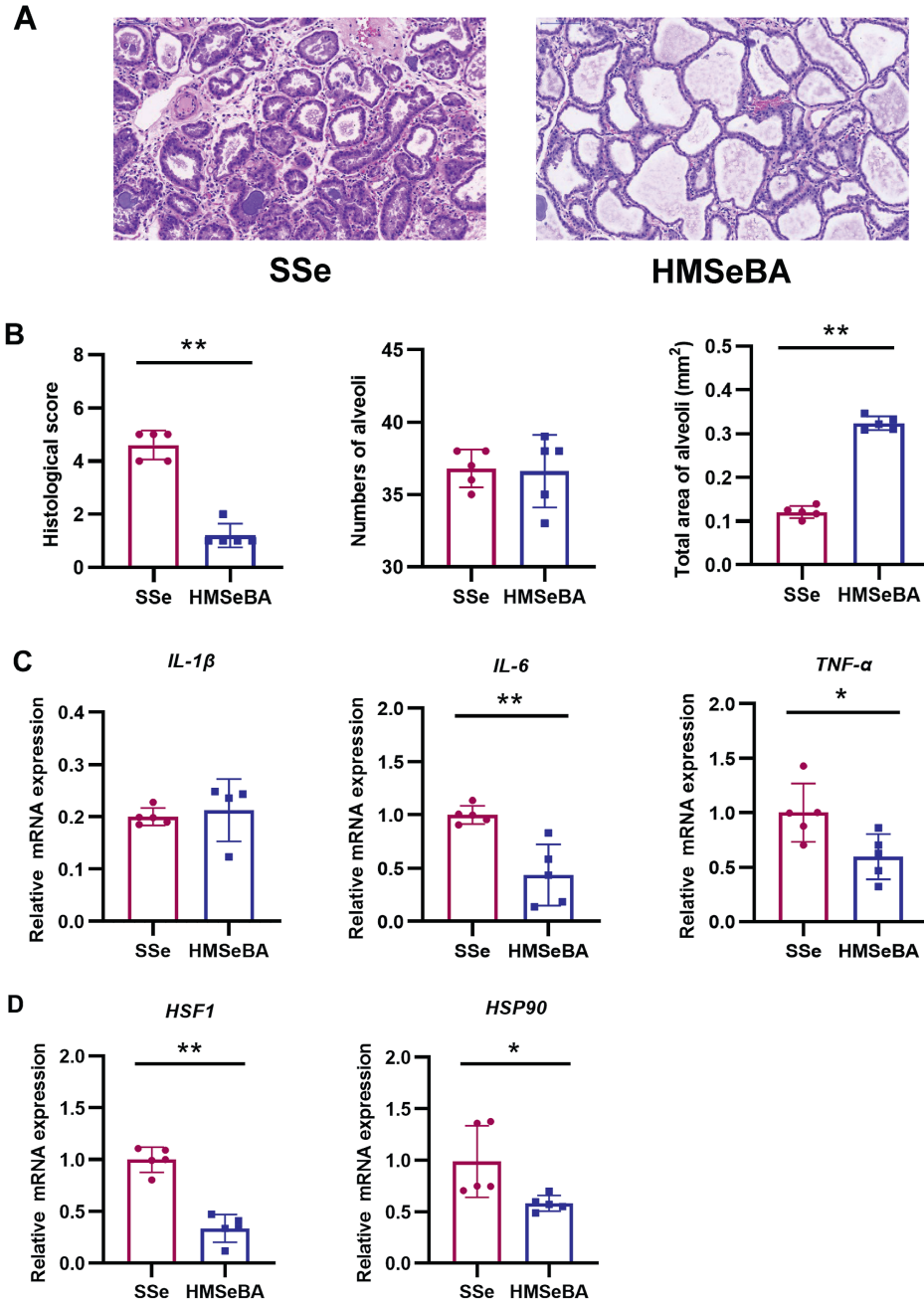


Fig. 3. The blood-milk barrier injury, the mRNA expression of heat shock proteins and inflammatory cytokines of mammary gland tissue in sodium selenite (SSe) and hydroxy-selenomethionine (HMSeBA) cows. (A) Hematoxylin-eosin-stained pathological sections; (B) Histological score, number of alveoli and total area of alveoli; (C) The mRNA expression of inflammatory cytokines; (D) The mRNA expression of heat shock proteins. SSe: 35 mg sodium selenite group per kilogram of premix, HMSeBA: 35 mg hydroxy-selenomethionine group per kilogram of premix. Significance was tested using *t*-test. Data with error bars are expressed as mean \pm standard deviation (SD). **P* < 0.05, ***P* < 0.01.

cows were subjected to severe heat stress. The respiratory rate of HMSeBA cows was lower than the SSe group, indicating that the heat stress state of cows in the HMSeBA was alleviated to a certain extent. Some studies have reported that heat stress could reduce the milk yield of cows (de Vries and Risco, 2005; St-Pierre et al., 2003; Tao et al., 2018), whereas selenium supplementation can alleviate the damage of milk yield by heat stress (Wang et al., 2009). Prior to the start of the experiment, milk yield did not differ between the two groups, however, the milk yield of the HMSeBA group was higher than the SSe group after the experiment. This may be due to HMSeBA improved the resistance of cows to heat stress. Dairy cows require a lot of GLU for lactation. When the GLU

content in the blood of cows cannot support their lactating needs, dairy cows draw on body energy reserves to support lactating demands, which will lead to the increase of blood NEFA (Baumgard et al., 2017). Therefore, the lower GLU and higher NEFA content of the HMSeBA group may be related to the greater milk yields of the HMSeBA group. Meanwhile, our result showed that the milk protein in the HMSeBA group was lower, which may be due to the decreased concentration of branched-chain fatty acids (BCFA) in the rumen. Previous studies have shown that a large number of branched-chain amino acids (BCAA) are synthesized from BCFA in rumen (Allison and Bryant, 1963), and that BCAA are mainly used for synthesizing milk protein in dairy cows (Leal Yepes et al., 2019;

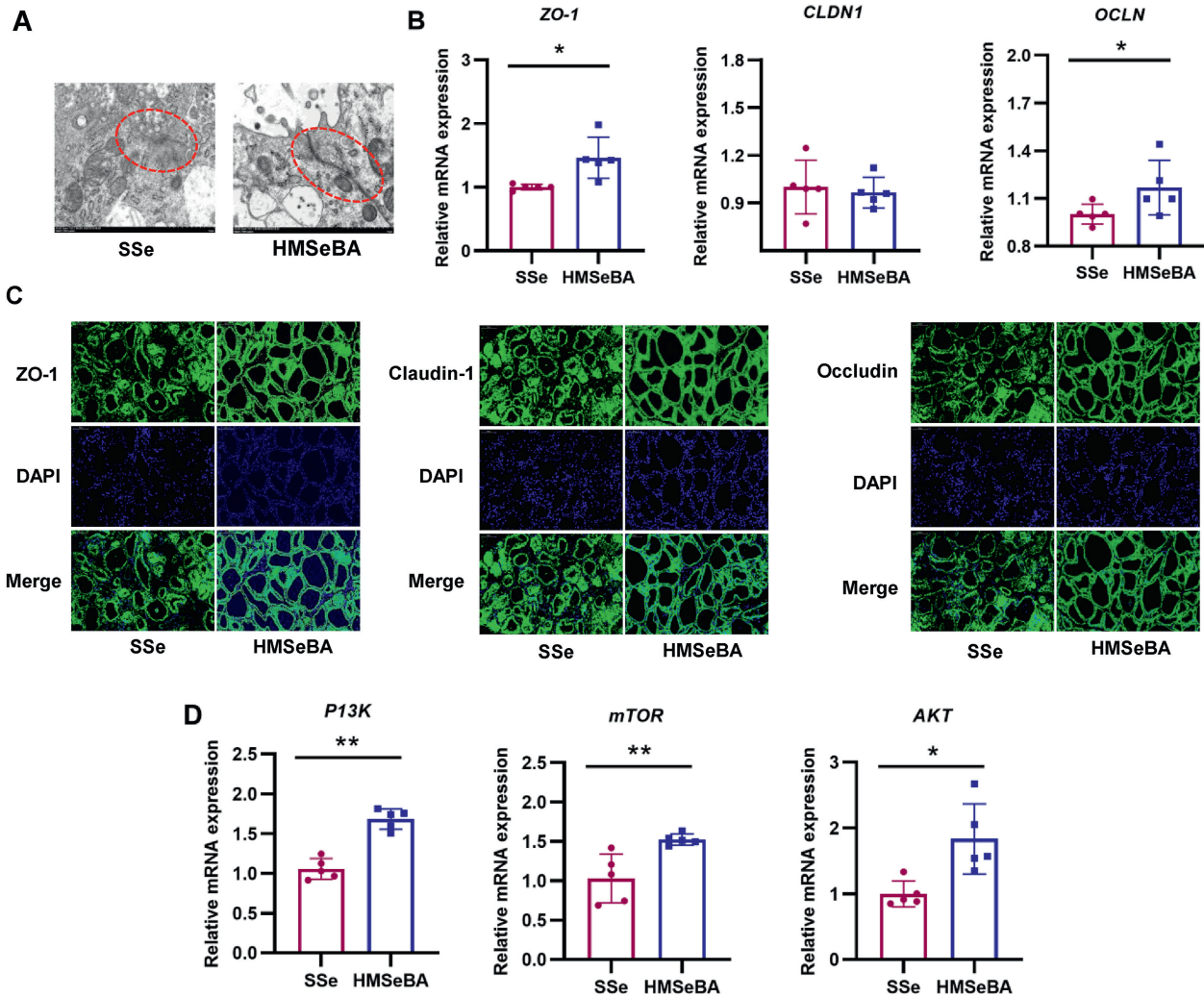


Fig. 4. The tight junction structure and expression of tight junction proteins of mammary gland tissue in SSe and HMSeBA cows. (A) Tight junction structure under transmission electron microscopy. (B) The mRNA expression of *ZO-1*, *CLDN1* and *OCLN*. (C) The immunofluorescence of *ZO-1*, claudin 1 and occluding, DAPI marks the nucleus of neutrophils, and the green label marks the tight junction proteins. (D) The expression of *P13K/AKT/mTOR* pathway of mammary gland tissue in SSe and HMSeBA cows. *ZO-1* = zonula occludens-1; *CLDN1* = claudin 1; *OCLN* = occluding; DAPI = 4',6-Diamidino-2'-phenylindole; *P13K* = phosphatidylinositol 3-kinase; *AKT* = protein kinase B; *mTOR* = mammalian target of rapamycin. SSe: 35 mg sodium selenite group per kilogram of premix; HMSeBA: 35 mg hydroxy-selenomethionine group per kilogram of premix. Significance was tested using *t*-test. Data with error bars are expressed as mean \pm standard deviation (SD). * $P < 0.05$, ** $P < 0.01$.

Xue et al., 2020). Additionally, our study found that the Se content in milk of the HMSeBA group was also higher. On the one hand, previous studies found that the Se content in milk was higher in HMSeBA cows compared to the yeast selenium and SSe (Hachemi et al., 2023; Sun et al., 2017), which in agreement with our findings in dairy cows. On the other hand, the higher Se content in milk was related to the higher Se content in plasma in the HMSeBA group in our experiment. The total plasma Se content of HMSeBA cows was higher than the SSe cows, which supports the common view that organic Se is more effective in raising blood selenium concentrations (Phipps et al., 2008). Furthermore, HMSeBA has been proved to be absorbed more easily from the intestine than SSe (Sun et al., 2017). These results indicate that HMSeBA can more effectively increase the concentration of Se in milk and plasma.

Previous studies have found that rumen TVFA and propionate concentrations in HMSeBA groups were higher than control groups without Se supplementation (Wei et al., 2019; Zheng et al., 2022a), but the rumen TVFA and acetate concentrations showed no difference between the HMSeBA and SSe groups under the same Se addition (Wei et al., 2019). In our study, HMSeBA was used to

replace SSe, ensuring the consistency of Se content among the treatment groups, and no differences in acetate, propionate, and TVFA concentrations were found between the two groups. *Ruminococcus* is a Gram-positive coccus involved in fermentation metabolism, which uses carbohydrates as substrates and ferments cellulose in the rumen (Jami and Mizrahi, 2012). Zheng et al., (2022a) found that the addition of HMSeBA reduced the relative abundance of *Ruminococcus* in the rumen, which is consistent with our results. *Prevotellaceae-UCG-003* belongs to the *Prevotellae* family, representing a class of bacteria that can improve nitrogen utilization, generate succinate and acetate from carbohydrates, and improve feed type utilization (Morotomi et al., 2009; Purushe et al., 2010). In the present study, *Prevotellaceae-UCG-003* was significantly decreased in the HMSeBA group, which may be one of the reasons for the observed decrease in ruminal acetate and butyrate. Additionally, the correlation results showed that both *Ruminococcus* and *Prevotellaceae-UCG-003* were positively correlated to milk protein. Therefore, the decrease in milk protein of HMSeBA group may also be related to the decrease in abundance of *Ruminococcus* and *Prevotellaceae-UCG-003*. *Syntrophococcus*, *Dialister*,

and *Erysipelotrichaceae_UCG_002* are members of *Firmicutes* (Ahmad et al., 2020; Rey et al., 2014), which can digest multiple carbohydrates to produce SCFA. Butyrate is the metabolite of *Shuttleworthia* in the rumen (Downes et al., 2002). These bacteria were significantly increased in the HMSeBA group, but we found that the concentrations of acetate and butyrate decreased in the HMSeBA group. This may be due to the fact that the metabolites of *Ruminococcus* and *Prevotellaceae_UCG-003* in the rumen are also VFA such as acetate and butyrate, while the relative abundance of *Ruminococcus* and *Prevotellaceae_UCG-003* in the rumen was higher and decreased in the HMSeBA group compared to the SSe group.

Inflammation is a subsequent event of heat stress-induced oxidative stress in the body and is the first line of defense to deal with all forms of cell damage and initiate cell repair (Chen et al., 2019; Zhang et al., 2020). Heat stress can cause the loss of intestinal barrier integrity, allowing for the paracellular transport of LPS into the bloodstream, leading to the activation of the innate immune system and systemic inflammation (Koch et al., 2019), thus disrupting the redox equilibrium and causing oxidative stress in cells (Belhadj Slimen et al., 2016). In addition, heat stress can activate inflammatory genes expression through the NF- κ B pathway and produce cytokines such as TNF- α , IL -1, IL-6 and IL-4 (Nawab et al., 2019; Vidya et al., 2018). Serum amyloid A can be used to evaluate the inflammatory state of the body, which can disrupt normal physiological functions and cause organ exhaustion (Sack, 2020). Our results indicated that the serum contents of IL-1 β , IL-6, LPS, and SAA in the HMSeBA group were lower, whereas the serum contents of IgG, IgM, and IgA were higher compared to the SSe group. This illustrates that compared with SSe, HMSeBA could more effectively improve the immunity level of dairy cows to deal with the inflammatory injury induced by heat stress. As an important indicator for evaluating liver injury, the ALP content in the HMSeBA group decreased compared to the SSe group, further reflecting the alleviating effect of HMSeBA on heat stress in cows. Selenium is a multifunctional nutrient closely related to the antioxidant capacity of animals (Mehdi and Dufresne, 2016; Qazi et al., 2018), which exerts its antioxidant function mainly through 25 selenoproteins that have important physiological functions, including GSH-Px, thioredoxin reductase (TrxR), and selenoprotein P (Kieliszek, 2019). Previous experiments have found that the addition of Se to diet can improve the blood Se and GSH-Px activity of calves, broilers and cows (Abdel-Moneim et al., 2022; Bordignon et al., 2019; Sun et al., 2019). Our result showed that the content of GSH-Px, T-AOC, and SOD in the HMSeBA cows were higher than the cows in SSe group, whereas the content of MDA was significantly reduced, indicating that HMSeBA was more effective in improving the antioxidant capacity under heat stress conditions.

The inflammatory response induced by heat stress can be observed in multiple organs of animals such as hypothalamus, liver, and intestinal tract (Liu et al., 2022; Nawab et al., 2019; Zhao et al., 2021). A study also found that heat stress induced inflammation in the MEC of mice and dairy cows (Zheng et al., 2022b). Studies have found that Se deficiency promotes inflammation by activating TLR2-related pathways in the mammary gland (Wang et al., 2018), whereas Se supplementation inhibits TLR2 signaling pathways (Melehani et al., 2015). In this study, we found that the mammary gland injury in the SSe group was more serious, and the mRNA expressions of *TNF- α* and *IL-6* were higher compared to the HMSeBA group; indicating that the HMSeBA group had better resistance to mammary gland injury and inflammation caused by heat stress. Heat shock protein 90 accounts for 1 to 2% of cellular proteins under normal circumstances, but this proportion increases to 4 to 6% in stressed cells (Picard, 2002; Taipale et al., 2010). Cellular levels of HSP90 depend on the main heat shock response regulator HSF1, which binds to HSP90 under normal conditions.

Heat shock protein will separate from HSF1 when the body or cells are stimulated by HS, inducing the expression of genes regulated by downstream heat shock elements (Koch et al., 2019). The HSF1 and HSP90 expressions in the mammary gland of the HMSeBA cows were lower compared to the cows in SSe group in this study, which implied that the mammary gland of cows in the SSe group suffered more serious heat stress. Moreover, in previous study we supplemented HMSeBA in the diet of mice under heat stress and found similar results in the mammary gland of mice (Zheng et al., 2022b).

The blood-milk barrier is semi-permeable and only allows the selective transfer of nutrients required for lactation. Tight junctions in the mammary gland are an important point in cutting off milk from the vascular system and ambient extracellular fluid (Stelwagen and Singh, 2014). Tight junctions are complex structures composed of over 50 proteins (Zheng et al., 2022b), among which claudin-1 (Furuse et al., 1998), *ZO-1* (Fanning and Anderson, 1999) and occludin (Balda et al., 1996) plays important roles. Studies have shown that Se addition in diet upregulates the mRNA expression of *OCN*, and *ZO-1* and *CLDN1* in the jejunum of broilers (Ali et al., 2022), and Tang et al., (2019) confirmed that dietary Se enhanced the mRNA and protein expression of tight junction proteins in the intestinal barrier of piglets under heat stress. In our research, we compared the expression of these three tight junction proteins in dairy cow mammary gland through real-time quantitative PCR and immunofluorescence. In the HMSeBA group, the mRNA expressions of *ZO-1* and *OCN* were higher. The results of immunofluorescence showed that the expressions of three tight junction proteins were more evident in the HMSeBA group, and the acinar structure was intact and defined compared to the SSe group. This was consistent with previous results (Ali et al., 2022; Tang et al., 2019), and may be relevant to the higher expression of mammary gland inflammatory cytokines in the SSe group. Previous studies have found that inflammatory cytokines (TNF- α) can increase the sensitivity of NF- κ B and induce the increase of myosin light-chain kinase, which leads to cytoskeleton contraction and tight junctions opening (Al-Sadi et al., 2009). This may also be the reason for the decreased expression of tight junction proteins in SSe group cows. The *PI3K/AKT/mTOR* pathway participates in multiple functions of cells, including cell proliferation, migration, apoptosis (Miricescu et al., 2020). Activated *AKT* can mediate the regulation of cell proliferation, growth, and energy metabolism (Risso et al., 2015), and *mTOR* can upregulate cell growth by stimulating nutrient absorption and inhibiting protein degradation (Dummler and Hemmings, 2007). Moreover, the expression of the *PI3K/AKT/mTOR* pathway is closely related to blood-brain barriers (BBB) and intestinal epithelial barriers (IEB). For example, the activation of the *PI3K/AKT/mTOR* pathway can strengthen IEB function by increasing the expression of intercellular tight junction protein *ZO-1* (Shao et al., 2017) and reduce harm to the BBB under hyperglycemic conditions by increasing the expression of tight junction protein, reducing BBB permeability and decreasing autophagy levels (Yang et al., 2021). Our results showed that the expression of *PI3K/AKT/mTOR* was higher in the HMSeBA group. This indicates that HMSeBA could promote the expression of tight junction proteins in the mammary gland by increasing the expression of the *PI3K/AKT/mTOR* signal molecule mRNA, effectively helping to maintain the integrity of the blood-milk barrier structure in dairy cows.

5. Conclusion

In conclusion, HMSeBA can improve the antioxidant capacity of cows under heat stress, alleviate the damage to the blood-milk barrier caused by heat stress, and prevent the harmful effects of heat stress on the milk yield of cows. Hydroxy-selenomethionine increases the antioxidant and immune capacities of cows under

heat stress, which could help reduce the inflammatory response caused by heat stress. Moreover, HMSeBA improves the expression of tight junction proteins by increasing the expression of the *PI3K/AKT/mTOR* signaling pathway in the mammary gland, which could help reduce the damage to the blood-milk barrier and mammary gland caused by heat stress, alleviating the decrease of milk production under heat stress.

CRedit authorship contribution statement

Zhantao Yang: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Yuhui Zheng:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Kai Ren:** Investigation. **Wei Wang:** Writing – review & editing. **Shengli Li:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization.

Availability of data and material

The rumen 16S rRNA sequences data were deposited into national center for biotechnology information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA1017408.

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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Appendix supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2024.10.003>.

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