Original Article

Impact of Management and Nutrition on *HSP70* Gene Expression in Heat-stressed Dairy Cows

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ABSTRACT

Background: This research investigated the effects of management and nutrition strategies on dairy cows under heat stress.

Objectives: We aimed to evaluate the effects of zinc mineral supplementation and mist spray, alone and in combination, on yield, milk production and composition, blood parameters and *HSP70* gene expression in dairy cows.

Methods: Sixteen Holstein lactating cows were used for our experiment in four treatment groups: Basal diet without heat stress alleviation methods (control), zinc supplementation in diet, basal diet and application of mist spray method and supplementation of zinc in basal diet with mist spray. We measured milk production and composition, blood parameters and *HSP70* gene expression in all cows.

Results: The results showed that the cows in the mist and zinc+mist treatments had significantly better performance and temperature-humidity index than the control group. The milk yield and its compounds were significantly affected by experimental treatments, with the best results seen in the treatment with both spray and zinc. The cows exposed to dry and lactation periods showed a significant increase in the concentration of blood biochemical factors and antioxidant indices in response to heat stress. *HSP70* gene expression was significantly decreased in all treatments compared to the control.

Conclusion: This experiment suggests that applying nutritional and management strategies can effectively mitigate the effects of heat stress on dairy cows. The study recommends using zinc supplementation and mist spray to alleviate heat stress effectively. Overall, this study highlights the importance of implementing management and nutrition strategies to improve the welfare and productivity of dairy cows under heat stress.

Keywords: Heat stress, Milk composition, Mist spray, Performance, Zinc

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Introduction

Breeding dairy cows has changed from a traditional activity to an integrated industry. This industrial system has advantages that allow it to operate more efficiently through improved nutrition, genetics, environment, and management (Ingvartsen & Moyes, 2013). Livestock experiences different stressors, such as nutritional, chemical, psychological, and thermal stress. Among them, thermal stress is the most intriguing factor influencing production, reproductive efficiency, health and the well-being of the high-producing animals. In tropical and subtropical regions, increased temperature and humidity are the major constraints in livestock production (Jyotiranjan, 2017). Heat stress is a biophysical condition that directly impacts the biological system of dairy cows (Less et al., 2019). Thermal stress can cause altered feed intake, digestion, discomfort, uneven growth and body weight, and altered metabolic function, leading to distress and increased mortality (Brito et al., 2020).

Over the past hundred years, the average temperature near the Earth's surface has increased by 0.18-0.74 ˚C. Climate change models show that the average surface air temperature has increased between 1.1 ˚C and 4.6 ˚C. This trend will also have adverse effects on animal husbandry, which is one of the key parts of activity on the Earth (Rhoads et al., 2009).

In lactating cows, milk production and secretion are special processes that differ from other animal metabolic pathways in terms of different biomolecular aspects. From the viewpoint of heat transmission, this process is the biggest challenge animals face after delivery. Therefore, any stress in terms of environmental conditions can affect the challenge and thus alter the production level and composition of this vital nutrient (Melo et al., 2016).

On the other hand, it is the biggest economic challenge in terms of cost, profit, volume, composition, and yield of milk produced in each dairy unit, and it can directly affect the sustainability and development of each dairy farm. For example, in milk pricing conditions, milk fat concentration is an important factor in the profitability of a cow in addition to production volume (Melo et al., 2016).

Genetic selection and the use of superior genes have been justified to ensure increased milk production over the past years. However, the available data indicate that this increase is one of the most important stressors affecting animal health and lifetime due to the lack of optimization in the environmental and biomolecular conditions of animals because an increase in milk production increases the interval of supplying livestock's feeding and the nutrients secreted by milk (Santana et al., 2016). Under such circumstances, the incidence of heat-transfer abnormalities from animal to milk increases.

Heat stress is one of the main problems in Iranian dairies, especially in the tropical and subtropical regions. Dairy cows are highly susceptible to heat stress (Dash et al., 2016). Heat stress causes adrenocorticotropic hormone release, delays ovulation, and inhibits luteinizing hormone secretion, cortisol, and other glucocorticoids (Khorsandi et al., 2016). It can also affect the adrenal glands, which can delay puberty, cause possibly silent and short estrous, and delay fetal development (Khorsandi et al., 2016). Heat stress leads to physiological and behavioral changes in dairy cows and affects reproductive success. Heat stress not only has a negative effect on yields, quality of milk, and reproduction rate but also has a detrimental effect on the health of dairy cows (Das et al., 2016). When the temperature rises abruptly or above the tolerance level of the animal, the body loses its balance, reducing appetite and production, lowering reproduction, and even leading to death (Smith & Harner, 2012). Behavioral, physiological, and endocrine mechanisms are used to mitigate the effects of heat stress (Mota-Rojas, 2021).

Temperature changes also increase the excretion of minerals and decrease the animal's longevity. The enzyme glutamate peroxidase, meanwhile, plays an essential regulatory role in the response of animals to stressful conditions, and this response will be effective when cofactors such as selenium peroxidase, copper, zinc, and manganese are available. Therefore, the role of proper feeding of animals and the inclusion of mineral supplements in their diet can be pointed out as the means to cope with heat stress (York et al., 2017). Malondialdehyde (MDA) indicates oxidative stress in cells and tissues because lipid peroxidation is a well-established mechanism of cellular harm. MDA testing is often used as a lipid peroxidation biomarker (Koohkan et al., 2023). SOD is a key anti-oxidative enzyme that acts as the first line of defense against reactive oxygen species (ROS) to reduce lipid peroxidation and oxidative stress by catalyzing the conversion of superoxide radicals into H_2O_2 that is detoxified by the activities of glutathione peroxidase (Gpx) and catalase (Shahsavari et al., 2023).

Although advances in nutrition and management strategies have mitigated some of the adverse effects of heat stress on dairy cows, the amount of production in the hot air of summer is significantly reduced (Shiao et al., 2011).

However, researchers believe that optimizing animal holding places, proper nutrition, and efficient management by utilizing modern scientific findings in relation to modern lactating cows is important and justified (Charpentier & Delagarde, 2018).

This study aimed to investigate the effects of nutritional and management strategies on production and physiological traits, heat shock protein gene expression, heat shock protein concentration, vaginal temperature before delivery, and the immune system of dairy cows under heat stress conditions.

Materials and Methods

The experiment was conducted during the summer of 2018 at a dairy farm in Sari City, Iran. Sixteen Holstein cows in the transition period were selected to perform this experiment. They were randomly divided into four treatments (each consisting of 4 cows). All cows had the same calving time, pre-production record, live weight, and gestation at similar conditions and were dried by stopping milking and antibiotic injection into each part of the breast within at least 45 days before the expected time for delivery. The animals used in the experiment underwent testing for brucellosis (Alamian et al., 2023). To confirm the absence of metabolic disorders and oxidative stress and ensure the test subjects' health, rumen-protected choline, and α-tocopherol supplementation were incorporated into the livestock diet (Salam Karim et al., 2022).

The cows were fed a mixed diet based on recommendations from the US National Research Council (2001) (Tables 1 and 2). After formulating the ration, samples were collected from various points to analyze and verify the uniform and homogeneous distribution of zinc and other essential nutrients within the feed (Khorrami et al., 2022). Experimental treatments included treatment with basal diet without heat stress alleviation methods (control), treatment fed by mineral zinc supplementation in basal diet (75 mg/kg feed), treatment with the base diet using mist spray method, and treatment fed by the addition of mineral zinc supplement to the base diet (75 mg/kg feed) and mist spray system (Marins et al., 2020).

Commercially available zinc supplement ($ZnSO_4$) was added to the diet 60 days before delivery, and the heat stress was assessed 45 days before delivery (at 39 ˚C) (El-Gindy et al., 2023; Zaghari et al., 2022; Falah et al., 2023). While mineral zinc sources are accessible in various supplement forms for animal feed, zinc sulfate is preferred over zinc phosphide. This substitution is attributed to the potential risk of phosphorus poisoning, which can originate from phosphorus fertilizers utilized in the cultivation and or mineral supplement (Sadeghinasab et al., 2021).

Performance

Feed intake was calculated daily by deducting the amount of waste and residue and multiplying the amount of dry matter in the feed. Weighting was performed weekly before meals in the morning, and the mean weight gain was obtained by dividing the total weight gain by the number of days spent in the respective period.

Vaginal temperature and heat-humidity index

The vaginal temperature was measured while the cows were in the special pan in the far-off phase using a calibrated temperature logger (DS1922L, Embedded Data Systems, Lawrenceburg, KY). The vaginal temperature was measured every 5 minutes for 4 consecutive days. At the same time as measuring the vaginal temperature, the thermo-humidity index was measured every 5 minutes at a height of approximately 3 m above the ground using the Equation 1:

1. *Temperature-humidity index (THI)=Dry bulb temperature (Tdb)–[0.55–(0.55×relative humidity/100)]×(Tdb–58)*

and, the temperature is measured in degrees Fahrenheit.

Production and composition of milk

Cows were milked twice a day at 8 AM and 4 PM for 30 days postpartum, and their milk yields were recorded daily. Milk samples were taken on the last day to determine the milk composition so that after milking, the milk tank was wholly shaken to mix the whole milk properly. A sample of milk produced from each cow is taken at a relatively constant level based on the amount of secreted milk, and the milk samples are poured into containers and immediately transferred to the laboratory for chemical analysis. Then, milk compounds, including lactose, fat, protein, non-fat solids, and urea nitrogen, were determined using standard methods and the MilkoScan apparatus.

Biochemical factors and antioxidant indices of blood

Blood samples were collected from the caudal vein 21 days before and 21 days after calving, two hours after the morning meal, and the experimental tubes of blood samples were centrifuged for 20 minutes at 3000 rpm for serum preparation. Then, the concentration of glucose, urea, creatinine, and total serum protein was determined using a spectrophotometer. NEFA and beta-hydroxybutyrate concentrations were obtained using a BioRex kit and alpha autoanalyzer separator (assay equipment). The activity of antioxidant indices was measured using Pars Test kits by ELISA reader (ELX800, Bio-Tek) at 412 nm for Gpx at the wavelength of 534 nm for MDA. Also, the concentration of superoxide dismutase (SOD) was calculated using the nitro blue tetrazolium method at a wavelength of 560 nm.

Heat shock protein 70 *HSP70* gene expression

Blood samples were collected from cows for *HSP70* gene expression at the end of the experiment and transferred by liquid nitrogen tanks to a molecular genetics and Biotechnology Laboratory of Sari University of Agricultural Sciences and Natural Resources and stored at -80 °C until RNA extraction. The steps were to evaluate the relative gene expression, RNA fragmentation, cDNA synthesis, and *HSP70* gene expression analysis using the real-time PCR (qPCR) method.

Blood samples were collected using the RNA extraction kit of Yekta Tajhiz Company according to the manufacturer's instructions. The cDNA was prepared by the QuienFast Reverse Transcriptase Kit of QIAGEN company (QIAGEN, 205311), and the cDNA of each sample was prepared from extracted RNA according to the manufacturer's instructions.

Real-time PCR (qPCR) reaction was performed using specific primers and Quant fast SYBR Green PCR kit from QIAGEN Company (QIAGEN, 204052) on Corbett's PCR machine (Corbett, Rotor gene 3000), and 18s rRNA was used as an internal control gene (Table 3). We used GAPDH as a reference gene to normalize mRNA expression levels of the *HSP70* gene (Karis et al., 2020). Finally, the cycle threshold obtained from real-time PCR was inserted for the genes in the computational method presented by Livak and Shmitgen (2001), which are in the form $2^{-\Delta\Delta CT}$, and the relative gene expression was accordingly calculated.

Statistical analysis

The data were analyzed in a completely randomized design using the general linear model and statistical software SAS software, version 2009. Mean comparisons were performed using Duncan's multiple range test at the 5% probability level. The statistical model of the design was in the form of $Y(i)=\mu+Ti+\varepsilon ij$, where Y (i) is the value of each observation, μ is the mean trait in the target population, Ti is the effect of experimental diets, and εij is the error of the experiment.

Results

The performance results of dairy cows are outlined in Table 4. In the current study, significant dry matter intake and body weight increases were observed in the cows subjected to the third and fourth treatments compared to the control group (P≤0.05). However, there were no notable differences in body scores between the two treatment groups and the control (P≤0.05).

Table 5 shows the effect of experimental groups on vaginal temperature and the heat-humidity index of dairy cows. The thermal-humidity index of dairy cows in the third and fourth treatments significantly decreased compared with the second treatment and the control (P≤0.05).

Table 6 shows the effect of experimental groups on milk production and composition. Daily milk yield was significantly affected by the third and fourth experimental treatments such that the highest production was related to the cows in the treatments with mist spray and zinc+mist spray (P≤0.05). Differences in milk composition in kilogram for fat, protein, lactose, and non-fat solids, and 4% fat-refined milk for the third and fourth treatments were significantly increased compared with the third and fourth treatments (P≤0.05). Fat percentage value significantly increased, and milk urea nitrogen significantly decreased for all the treatments compared with the control (P≤0.05).

Table 7 shows the experimental groups' effect on lactating cows' biochemical blood factors. This experiment showed that the cows in the dry and lactation periods significantly increased the concentration of glucose, NEFA, beta-hydroxybutyrate, and blood urea in response to heat stress (P≤0.05). There was also an increase in the concentration of blood urea to creatinine ratio during the dry season, and this increase was higher for the control treatment compared to the other treatments (P≤0.05). There was no significant difference between the treatments regarding blood protein concentration during the dry and lactation periods (P>0.05).

Table 8 illustrates the impact of experimental groups on the activity of blood antioxidant indices in lactating cows. The activity levels of MDA and SOD during both dry and lactation periods exhibited a significant decrease in the second, third, and fourth treatments when compared to the control treatment (P≤0.05). While the quantity of Gpx did not show a significant difference in any

Table 1. Ingredients (% dry matter) of the experimental diet

Ingredient	% Dry Matter
Corn silage	40.41
Wheat straw	3.29
Barley grain	11.10
Corn grain, ground	18.60
Molasses	4.94
Soybean shells	1.65
Soybean meal	9.85
Cottonseed meal	2.06
Balanced amino acid supplement	3.55
Fat powder	1.65
Salt	0.04
Calcium carbonate	0.49
Sodium bicarbonate	0.82
Magnesium oxide	0.28
Methionine	0.07
Lysine	0.44
Vitamin and mineral premix1	1.04

Table 2. Chemical composition of the experimental diet

Chemical Composition	% Dry Matter
Crude protein (%)	17.90
Acid detergent fiber (%)	30.00
Starch (%)	24.30
Ether extract (%)	4.70
Non-fiber carbohydrates (%)	41.60
Ash (%)	8.50
Ca (%)	1.20
P (%)	0.50
Mg (%)	0.40
K (%)	1.70
Na (%)	0.40
Zn (mg/kg DM)	35.00

Table 3. The details of primer sequences used for quantitative real-time PCR

Gene Symbol	Direction	Sequence of the Primers
HSP70	Forward	5′-GACGACGGCATCTTCGAG-3′
	Reverse	5′-GTTCTGGCTGATGTCCTTC-3′
18s rRNA	Forward	5′-GGTTGATCCTGCCAGTAGCATAT-3′
	Reverse	5′-TGAGCCATTCGCAGTTTCACT-3′
GAPDH	Forward	5′- CGACTTCAACAGCGACACTCAC-3′
	Reverse	5′-CCCTGTTGCTGTAGCCAAATTC-3′

Table 4. The effect of experimental treatments on dry matter intake, body weight, and body condition score of lactating cows

Item	T1	T2	T3	T4	SEM	P
Dry matter intake (kg/cow per d)	21.00^{b}	22.10^{b}	26.10^{a}	27.30^{a}	0.6	0.0143
Body weight (kg)	662^{b}	666^{b}	711^{a}	712^{a}	4.7	0.0064
Body condition score	2.73	2.76	2.88	2.91	0.027	0.1152

T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet + zinc mineral supplement (75 mg/kg feed); T3: Control diet + fog system and T4: control diet + (zinc mineral supplement (75 mg/kg feed) + fog system); SEM: Standard error of the means.

Notes: The means within the same row with at least one common letter do not differ significantly (P>0.05).

of the treatment groups compared to the control, it was lower than that in the control (P>0.05).

Figure 1 shows the effect of experimental groups on the relative expression of the *HSP70* gene in the blood of dairy cows. The graph shows that the difference in *HSP70* gene expression was significantly decreased in all treatments compared to the control (P <0.004).

Discussion

As observed from the results, cows in the control treatment exhibited lower dry matter intake and body weight than those in the mist spray and mist spray + zinc treatments. This finding aligns with the results reported by Savsani et al. (2015).

Savsani et al. (2015) stated in their results that the bodyweight loss in the cows exposed to heat stress reflects a high energy demand for maintaining body temperature, and they also concluded that the amount of dry matter consumed decreased under heat stress conditions. This condition can also affect the weight of cows during the dry season. On the other hand, some researchers have reported that the embryos of dry cows exposed to heat stress strategies have grown to some extent, possibly due to the difference in body weight of those cows to the cows in heat stress conditions (Rhoads et al., 2009).

Table 5. The effect of experimental treatments on vaginal temperature and temperature humidity index of lactating cows

Item	T1	T2	T3	T4	SEM	P
Vaginal temperature (̊C)	40.00	39.88	39.06	39.00	0.26	0.4298
Temperature humidity index	77.70^{a}	77.31^{a}	60.00^{b}	59.70^{b}	0.52	0.0001

T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet+ zinc mineral supplement (75 mg/kg feed); T3: Control diet+ fog system, T4: control diet+ (zinc mineral supplement (75 mg/kg feed) +fog system); SEM: Standard error of the means.

Notes: The means within the same row with at least one common letter do not differ significantly (P>0.05).

Table 6. The effect of experimental treatments on milk yield and milk compositions of lactating cows

Item	T1	T2	T3	T4	SEM	P
Milk yield (kg/cow per d)	25.8[b]	25.9[b]	25.3[b]	35.5[a]	0.55	0.0002
Milk urea nitrogen (mg/dL)	11.26[a]	10.78[b]	9.15[c]	8.99[c]	0.064	0.0001
Milk fat (%)	3.14[b]	3.34[a]	3.38[a]	3.49[a]	0.029	0.0158
Milk fat (kg/cow per d)	0.84[c]	0.86[c]	1.11[b]	1.24[a]	0.013	0.0001
Milk protein (%)	2.81	2.85	2.86	2.86	0.027	0.9
Milk protein (kg/cow per d)	0.71[b]	0.73[b]	0.99[a]	1.02[a]	0.014	0.0001
Lactose (%)	4.55	4.57	4.67	4.70	0.029	0.2626
Lactose (kg/d)	1.15[b]	1.17[b]	1.66[a]	1.68[a]	0.021	0.0001
Total solids nonfat (%)	8.31	8.33	8.42	8.50	0.058	0.6442
Total solids nonfat (kg/cow per d)	2.09[b]	2.13[b]	2.98[a]	3.03[a]	0.032	0.0001
Fat corrected milk (3.5%)	24.6[b]	25.00[b]	23.3[b]	35.5[a]	0.61	0.0004

T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet + zinc mineral supplement (75 mg/kg feed); T3:Control diet + fog system, T4: control diet + (zinc mineral supplement (75 mg/kg feed) + fog system); SEM: Standard error of the means.

Notes: The means within the same row with at least one common letter do not differ significantly (P>0.05).

In the present study, the heat-humidity index during the heat challenge was 77.7, indicating that all the cows were exposed to heat stress throughout the experiment. The difference in temperature-humidity index of other treatments compared to the control treatment showed that mist spray + zinc treatment was more effective in reducing heat stress on cows. The mechanisms involved in the process of the possible regulatory effects of zinc on heat regulation are not fully understood. Still, zinc is reported to affect the thermal conditions of cattle due to its antioxidant role and positive effect on cows' immune function (Kellogg et al., 2004).

As evident from the results, the control treatment yielded lower milk production than the other treatments, consistent with findings from prior studies (Garcia et al.,

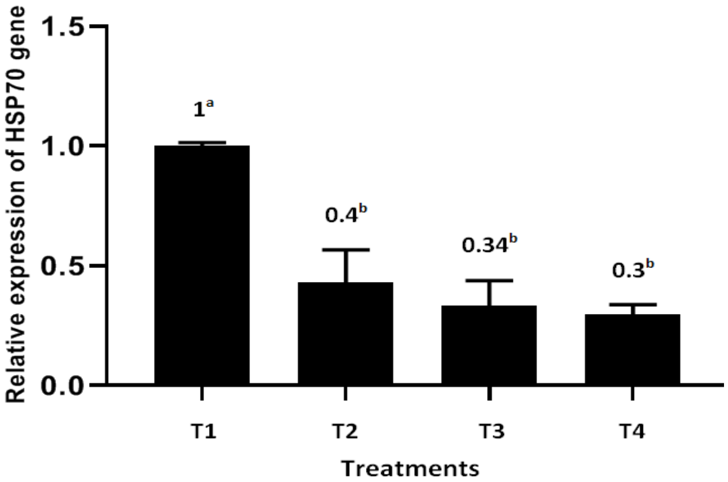

Figure 1. The effect of experimental treatments on *HSP70* gene expression of lactating cows

Notes: T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet + zinc mineral supplement (75 mg/kg feed); T3: Control diet + fog system and T4: Control diet + (zinc mineral supplement (75 mg/kg feed) + fog system).

Table 7. The effect of experimental treatments on blood biochemical factors of lactating cows

Item	Calving	T1	T2	T3	T4	SEM	P
Glucose (mmol/L)	-21	3.94[a]	3.75a[b]	3.56[b]	3.28[c]	0.041	0.0025
	+21	3.31[a]	3.22[b]	3.09[b]	2.83[c]	0.041	0.0010
Free fatty acid (mmol/L)	-21	189.76[a]	177.01[b]	164.19[c]	156.67[d]	2.90	0.0001
	+21	748.34[a]	642.80[b]	506.73[c]	425.01[d]	3.31	0.0001
Beta-hydroxy butyric (mmol/L)	-21	0.48	0.47	0.45	0.41	0.17	0.5285
	+21	3.31[a]	3.22[b]	3.09[b]	2.83[c]	0.041	0.0010
Total protein (g/dL)	-21	7.40	7.35	7.29	7.22	0.026	0.1690
	+21	6.89	6.85	6.78	6.71	0.030	0.2473
BUN (mmol/L)	-21	3.88[a]	3.34[b]	2.86[c]	2.48[d]	0.029	0.0001
	+21	3.59[a]	3.31[b]	3.15[b]	2.92[c]	0.033	0.0006
Creatinine (mmol/L)	-21	93.78	93.10	91.34	88.55	1.22	0.4678
	+21	80.3	79.15	77.27	73.61	1.24	0.3019
BUN/Creatinine	-21	0.040[a]	0.035[b]	0.031[c]	0.027[d]	0.0002	0.0001
	+21	0.044	0.041	0.040	0.039	0.0005	0.1254

T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet + zinc mineral supplement (75 mg/kg feed); T3: Control diet + fog system and T4: Control Diet + (zinc mineral supplement (75 mg/kg feed) + fog system); SEM: Standard error of the means.

Notes: The means within the same row with at least one common letter do not differ significantly (P>0.05).

2015). Heat stress, leading to reduced feed intake, can diminish nutrient intake and elevate body maintenance requirements, a significant factor in decreased milk production (Garcia et al., 2015). Researchers have also noted that glucose is vital for mammary gland lactose synthesis. Lactose is a crucial regulator of milk osmolarity, so its levels determine milk production. Under stressful conditions, skeletal muscles in cows tend to consume more glucose to lower metabolic heat production, resulting in inadequate glucose supply to mammary glands, leading to decreased lactose production and, ultimately, reduced milk production (Hill & Wall, 2015).

Table 8. The effect of experimental treatments on blood oxidation factors of lactating cows

Item	Calving	T1	T2	T3	T4	SEM	P
Malondialdehyde (nmol/mL)	-21	8.3[a]	7.9[b]	7.5[c]	7.00[d]	0.044	0.0001
	+21	8.8[a]	8.1[b]	7.6[c]	7.1[d]	0.046	0.0001
Glutathione peroxidase (UI/mL)	-21	65.70[a]	51.23[b]	46.40[bc]	41.50[c]	1.16	0.0004
	+21	56.60	54.43	52.20	49.70	1.13	0.2322
Superoxide dismutase (UI/mL)	-21	176.5[a]	155.8[b]	141.4[c]	135.7[c]	2.24	0.0008
	+21	172.8[a]	154.02[bc]	153.03[bc]	143.3[c]	2.27	0.0096

T1: Control diet (without zinc mineral supplement and fog system); T2: Control diet + zinc mineral supplement (75 mg/kg feed); T3: Control diet + fog system, T4: Control diet + (zinc mineral supplement (75 mg/kg feed) + fog system); SEM: Standard error of the means. Notes: The means within the same row with at least one common letter do not differ significantly (P>0.05).

This study observed treatment effects on milk fat percentage, kilogram of milk fat, protein, and lactose, all of which increased compared to the control, consistent with earlier research (Griffiths et al., 2007; Hackbart et al., 2010). Researchers have reported that management and nutrition strategies to mitigate heat stress positively impact milk composition, potentially due to their role in optimizing resource utilization and facilitating milk protein production in mammary tissue. Stelwagen and Singh (2014) emphasized the unique nature of mammary epithelial tissue responsible for milk synthesis and secretion, highlighting the necessity for the existence and maintenance of epithelial transduction pathways. Adequate maintenance of mammary attachments indicates optimal mammary function, as the loss of mammary epithelial tissue during lactation can diminish milk synthesis and secretion. Given how heat stress affects mammary epithelial tissues, preventive measures can influence milk production and composition (Weng et al., 2018). Numerous feed additives, including live yeast cultures, buffers, fat-soluble vitamins (such as A, D, and E), niacin, selenium, and zinc, can be considered for their potential to enhance rumen function and immunological response, optimize energy utilization, and improve feed conversion efficiency (Nzeyimana et al., 2023).

Thermal stress prevention strategies could reduce milk urea nitrogen levels compared to the control group. Previous research has shown that urea nitrogen is a byproduct of protein metabolism in the body, and its excessive concentration in blood and or milk indicates some problems with adequately utilizing the nitrogen-rich feeds for tissue and milk protein synthesis. Since feed intake reduces under heat stress, it can be effective in feed efficiency (Cowley et al., 2015).

The percentage of non-fat solids in milk was lower in the control group than in the other experimental group. This result is consistent with previous findings and may be related to higher milk protein levels and, to some extent, milk fat levels in the groups exposed to mist spray + zinc supplement (Ballantine et al., 2002).

The results of the present experiment showed that the concentration of glucose, non-esterified free fatty acids, and beta-hydroxybutyrate in the control had a significant increase in response to heat stress, which is similar to the results of Wheelock et al. (2010) and Duffield (2006).

Wheelock et al. (2010) found that hormones such as adrenaline and noradrenaline, glucagon, growth hormone, and cortisol are also released during heat stress, releasing glucose from the body's reserves to feed the skeletal muscle to reduce metabolic heat. This process also raises glucose in the blood of the cows exposed to heat stress in the dry season. They also stated that the accumulation of fetal carbohydrates and the priority of native carbohydrate oxidation under heat stress cause increased blood glucose concentrations in cows after calving (Dehghan-Banadaky et al., 2013).

According to the results of the Duffield study, NEFA and beta-hydroxybutyrate in the blood indicate the status of body fat mobilization in response to negative energy balance or exposure of cows to various stresses. During diminishing dry matter intake and energy shortage, the cows break down their body's triacylglycerol (fat) stores during delivery and afterward. This breakdown of fatty tissues results in the production of NEFA and column bodies and their entry into the bloodstream (Duffield, 2006).

In the present study, an increase in blood urea concentration was observed, and this increase was greater in the control treatments that were exposed to heat stress than in the other treatments. Several other studies have reported higher blood urea nitrogen concentrations in the case of lactating cows under heat-stress conditions. The reasons for increased blood urea nitrogen concentrations due to heat stress are unknown but may reflect changes in ruminal nitrogen metabolism and or systemic amino acid metabolism (Rhoads et al., 2009).

Blood creatinine was measured as an indicator constantly produced for renal clearance to investigate the causes of elevated blood urea (uremia) concentrations in the experimental treatments.

For cows, like other animal species, creatinine synthesis is dependent on muscle mass and is not diet-dependent (Schneider, 1988). Thus, although the blood creatinine concentration was not significant in either treatment, the increase in blood creatinine concentration observed in the control may be due to a decrease in renal clearance and partly due to the rise in muscle catabolism (Schneider, 1988). To eliminate the effect of renal inefficiency on changes in urea and creatinine concentrations in the blood, the ratio of urea to creatinine was calculated to evaluate the balance between protein oxidation and proteolysis.

According to the present results, the ratio of urea to creatinine in the cows exposed to heat stress before calving was significantly increased compared to other treatments. It seems that in cows with heat stress during the pre-calving period, the mobilization of amino acids in muscle protein gets higher than the amino acid oxidation in the liver to be used for metabolism or fetal growth, which may be the reason for the increased urea to creatinine ratio in the cows exposed to heat stress (Schneider, 1988).

Temperature and external dietary factors are the most important factors affecting the efficiency of the living antioxidant system. Natural and neutral antioxidants, along with the levels of selenium, manganese, zinc, and copper in the diet, help maintain efficient levels of external antioxidants in the tissue. Proper diet composition and ambient temperature allow the dietary antioxidants to be efficiently absorbed and metabolized (Schwartz et al., 2009).

The concentration of blood factors of the cows exposed to zinc, mist spray, and mist spray + zinc significantly decreased compared with the control. According to West (2003), temperature changes affect feed intake, body weight gain, and the immune system of animals, and they decrease the concentration of minerals such as iron, zinc, selenium, and chromium. The cow's overall ability decreases as the ambient temperature and relative humidity exceed normal, leading to physiological changes in the animal's body. Some enzymes play an important regulatory role in the response of animals to stressful conditions, and this response will be effective when cofactors such as selenium, copper, zinc, and manganese are available (Genther et al., 2015). Therefore, managing the ambient temperature of the animal and proper nutrition can prevent the physiological changes of the animal's body.

As was shown in the results, the activity of MDA, Gpx, and SOD was higher in control, which was under heat stress than the other treatments. The higher level of oxidative indices in the cows exposed to heat stress indicates that heat has caused oxidative stress in these cattle. Our results are consistent with the findings of Ghasemian Karyak et al. (2011) that heat stress increases lipid oxidation and production of free radicals in dairy cows in the dry and lactation periods through increased metabolic rate. Since the superoxide dismutase and glutathione peroxidase enzymes play important roles in the cleansing of free radicals such as superoxide and hydroxyl, it can be concluded that the increased activity of these enzymes in the control treatment is due to higher production of free radicals (Bernabucci et al., 2002).

The decrease in oxidative indices in the cows exposed to mist spray and zinc diet can be attributed to decreased temperature and antioxidant properties of zinc. Zinc deficiency in the diet increases oxidative damage to the cell membrane due to increased free radicals in the cell (Sahin et al., 2005). The zinc element stimulates the production of metallothionein, which is an effective factor in the cleansing of hydroxyl radicals and thus plays a key role in reducing free radical production (Sahin et al., 2005). Heat stress causes oxidative damage, which could be minimized by supplementing vitamins C, E, and A and minerals such as zinc. Vitamin E is an inhibitor chain blocker of lipid peroxidation, and ascorbic acid prevents lipid peroxidation due to peroxyl radicals. It also recycles vitamin E; vitamin C and zinc are known to scavenge ROS during oxidative stress (Somvanshi et al., 2018)

According to the results of this study, high levels of heat shock protein 70 gene expression were observed in the control group compared with the other treatments.

Heat shock protein gene expression in dairy cows has been previously reported by Collier et al. (2008). Heat stress is one factor that increases the relative expression of the *HSP70* gene. The essential role of heat shock proteins in cell protection during heat stress is illustrated by the overexpression of heat shock protein, which protects the living organism from heat shock of blood circulation and cerebral ischemia during heat stress (Febbraio & Koukoulas, 2000). Therefore, management strategies to keep the livestock site cool during breeding can prevent the increased relative expression of the *HSP70* gene.

Padmani et al. (2008) found that the increased internal capacity of antioxidants was a factor in the relative decrease of *HSP70* gene expression. Dufresne and Farnworth (2001) also described the antioxidant activity mechanism of antioxidants as directly inhibiting or removing oxygen-free radicals and/or reactive oxygen species and inhibiting oxidative enzymes in reducing *HSP70* gene expression. This outcome is consistent with our research findings.

Therefore, the decrease in the relative expression of the *HSP70* gene in the treatments containing zinc supplementation indicates the antioxidant property of this element, which could play a role in essential enzymes that have a significant effect on maintaining the balance between free radicals and the antioxidant system (Febbraio & Koukoulas, 2000).

Conclusion

This experiment showed that management and nutrition strategies can effectively manage thermal stress. As can be seen, applying mist and zinc improved the amount of dry matter intake, milk yield, and its compounds. Significant differences existed between heat stress treatment and blood factors and the antioxidant indices of cows exposed to heat stress alleviation strategies. Also, the high level of heat shock protein 70 gene expression in the control compared to other treatments showed the effect of management and nutrition strategies on heat stress reduction. Although using zinc and mist spray separately could reduce the thermal stress in cows, their combination had a more favorable effect.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Ali Mahdavi and Yadollah Chashnidel; Methodology: Ali Mahdavi, Yadollah Chashnidel, Mohammad Hasan Yousefi and Reza Narenji Sani; Data collection: Ayoub Farhadi, and Pourya Mollaie Berneti, Data analysis: Ali Mahdavi and Ayoub Farhadi Investigation, writing, funding acquisition and resource: All authors; Review and editing: Ali Mahdavi.

Conflict of interest

The authors declared no conflict of interest.

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مطالعه پژوهشی

تأثیر مدیریت و تغذیه بر بیان ژن HSP70 گاوهای شیری تحت استرس گرمایی

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چکیده

زمینه مطالعه: در این آزمایش، تأثیر روش‌های مدیریت و تغذیه بر گاوهای شیری در شرایط استرس حرارتی بررسی شد.

هدف: این تحقیق با هدف ارزیابی تأثیر مکمل‌کردن جیره با روی و روش‌های اسپری آب، به‌طور جداگانه و ترکیبی بر عملکرد، میزان تولید و ترکیبات شیر، پارامترهای خونی و بیان ژن *HSP70* در گاوهای شیری انجام شد.

روش کار: در این مطالعه از ۱۶ گاو شیری نژاد هلشتاین استفاده شد و آزمایش شامل ۴ تیمار بود: جیره پایه بدون روش‌های کاهش استرس حرارتی (کنترل)، جیره پایه همراه با مکمل معدنی روی، جیره پایه همراه با استفاده از روش اسپری آب و جیره پایه همراه با مکمل معدنی روی همراه با اسپری آب تولید و ترکیبات شیر، پارامترهای خونی و بیان ژن *HSP70* اندازه گیری شدند.

نتایج: مطابق یافته‌ها گاوهای مورداستفاده در تیمار اسپری آب و تیمار مکمل روی+اسپری آب نتایج بهتری در مقایسه با گروه کنترل داشتند. تولید و ترکیبات شیر تحت تأثیر تیمارهای آزمایشی قرار گرفته بودند و بهترین نتایج در گروه‌هایی بود که در تیمار ترکیبی بودند. گاوهایی که در دوره‌های خشکی و شیردهی قرار گرفته بودند، در پاسخ به استرس حرارتی، افزایش قابل‌توجهی در غلظت فاکتورهای بیوشیمیایی خون و شاخص‌های آنتی‌اکسیدانی نشان دادند. بیان ژن *HSP70* در تمام درمان‌ها نسبت به کنترل به‌طور قابل‌توجهی کاهش یافت.

نتیجه‌گیری نهایی: استفاده از روش‌های مدیریتی و تغذیه‌ای می‌تواند در کاهش اثرات استرس گرمایی بر روی گاوهای شیری مؤثر باشد. بنابراین پیشنهاد می‌شود استفاده از مکمل روی و اسپری آب به‌عنوان روش‌های مؤثر در کاهش استرس گرمایی استفاده شوند. به‌طورکلی، این مطالعه اهمیت پیاده‌سازی روش‌های تغذیه‌ای و مدیریتی را برای بهبود بهره‌وری و سلامت گاوهای شیری در شرایط استرس گرمایی بیان می‌کند.

کلیدواژه‌ها: استرس گرمایی، *HSP70*، ترکیبات شیر، اسپری آب، عملکرد تولید شیر، روی

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