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The Effect of Management and Nutrition Strategies on the Function and Expression of HSP70 Gene in Dairy Cows under Heat Stress

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Abstract

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

Objectives: The aim was to evaluate the effects of zinc mineral supplementation and mist spray, both individually 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, and experiment was conducted in four treatments: 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. Milk production and composition, blood parameters and HSP70 gene expression were measured

Results: The results showed that the cows in the mist and zinc+mist treatments had significantly better performance and temperature-humidity index compared to 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.

Conclusions: This experiment suggests that applying nutritional and management strategies can be effective in mitigating the effects of heat stress on dairy cows. The study recommends using zinc supplementation and mist spray method as effective strategies to alleviate heat stress.. 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.

Introduction

Breeding dairy cows has changed from a traditional activity to an integrated industry over the years. This industrial system has advantages that allow it to operate more efficiently through improved nutrition, genetics, environment and management (Ingvartsen and Moyes, 2013). Livestock experiences different stressors throughout their lives 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 sub-tropical 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, 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 and 4.6 degrees Celsius. 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 is a special process that is different from other animal metabolic pathways in terms of different biomolecular aspects. From the viewpoint of heat transmission, this process is the biggest challenge facing the animal after delivery. Therefore, the presence of 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 that 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 as a means of ensuring 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 ACTH release, delays ovulation, and inhibits LH 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 oestrus 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 rises above the tolerance level of the animal, the body loses its balance, reducing appetite and production, lowering reproduction, and even leads to death (Smith *et al*., 2007). Behavioral, physiological, and endocrine mechanisms are used to mitigate the effects of heat stress (mota 2021).

Temperature changes also increase the excretion of minerals and decrease the longevity of the animal. The enzyme glutamate peroxidase, meanwhile, plays an important 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 ROS to reduce lipid peroxidation and oxidative stress by catalyzing the conversion of superoxide radicals into H2 O2 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 negative 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 place, proper nutrition, and efficient management by utilizing modern scientific findings in relation to modern lactating cows is important and justified (Charpentier and Delagarde, 2018).

The aim of this study was 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 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. 16 Holstein cows in the transition period were selected to perform this experiment and they were selected and randomly divided into four treatments (each treatment consisting of 4 cows). All cows had the same time of calving, 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 prior to 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 health of the test subjects, rumenprotected choline and α-tocopherol supplementation were incorporated into the livestock diet (Salam Karim *et al.,* 2022).

The cows were fed by a mixed diet based on NRC (2001) recommendations (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: 1- treatment with basal diet without heat stress alleviation methods (control), 2- treatment fed by mineral zinc supplementation in basal diet (75 mg/kg feed), 3-

treatment with the base diet using mist spray method; and 4- 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 (ZnSO4), was added to the diet 60 days prior to delivery and the heat stress was assessed 45 days before delivery (at 39˚of Celsius) (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, the use of 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 (Sadeghi-nasab *et al.*, 2021).

Performance

Feed intake was calculated daily by deducting the amount of wasted 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 amount of 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 3m above the ground using the following formula.

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

Production and composition of milk

Cows were milked twice in 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 completely 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 were poured into containers and immediately transferred to the laboratory in order to take chemical analysis. Then, various milk compounds including lactose, fat, protein, non-fat solids and urea nitrogen were determined by standard methods and using 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 preparation of serum. Then, the concentration of glucose, urea, creatinine and total protein of serum were determined by spectrophotometer. NEFA and beta-hydroxybutyrate concentrations were obtained using 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 glutathione peroxidase and at the wavelength of 534 nm for malondialdehyde. Also, the concentration of superoxide dismutase was calculated by Nitrobutetrazolium (NBT) method at the 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 Real-Time PCR (qPCR) method.

Blood samples were collected using the RNA Extraction Kit of YektaTajhiz Company according to the manufacturer's instructions. The cDNA was prepared by 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 for normalization of mRNA expression levels of the geneHSP70 (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 general linear model (GLM) and statistical software SAS (2009). Mean comparisons were performed using Duncan's multiple range test at the 5% level of probability. The statistical model of the design was in the form $Y(i)=\mu+Ti+\varepsilon ij$ where Y (i)

is the value of each observation, μ is 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, there were significant increases in dry matter intake and body weight observed in the cows subjected to the third and fourth treatments compared to the control group ($P \leq 0.05$). However, there were no notable differences in body scores between each of the two treatment groups and the control ($P \leq 0.05$).

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 in comparison with the second treatment and the control ($P \leq 0.05$).

Table 6 shows the effect of experimental groups on milk production and milk 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 ($P \leq 0.05$). Differences in milk composition in terms of kilogram for fat, protein, lactose and non-fat solids, as well as 4% fat refined milk for the third and fourth treatments, were significantly increased in comparison with the third and fourth treatments ($P \leq 0.05$). The percentage value of fat significantly increased and

urea nitrogen of milk significantly decreased for all the treatments in comparison with the control ($P \leq 0.05$).

Table 7 shows the effect of experimental groups on the biochemical blood factors of lactating cows. The results of this experiment showed that the cows in the dry and lactation period had a significant increase in the concentration of glucose, NEFA, beta-hydroxybutyrate and blood urea in response to heat stress ($P \leq 0.05$). 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 ($P \leq 0.05$). There was no significant difference between the treatments in terms of blood protein concentration during the dry and lactation period ($P > 0.05$).

Table 8 illustrates the impact of experimental groups on the activity of blood antioxidant indices in lactating cows. The activity levels of Malondialdehyde and superoxide dismutase (SOD) during both dry and lactation periods exhibited a significant decrease in the second, third, and fourth treatments when compared to the control treatment ($P \leq 0.05$). While the quantity of glutathione peroxidase did not show a significant difference in any 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. As it is shown in the graph, the difference in HSP70 gene expression was significantly decreased in all treatments in comparison to the control (P <0.004).

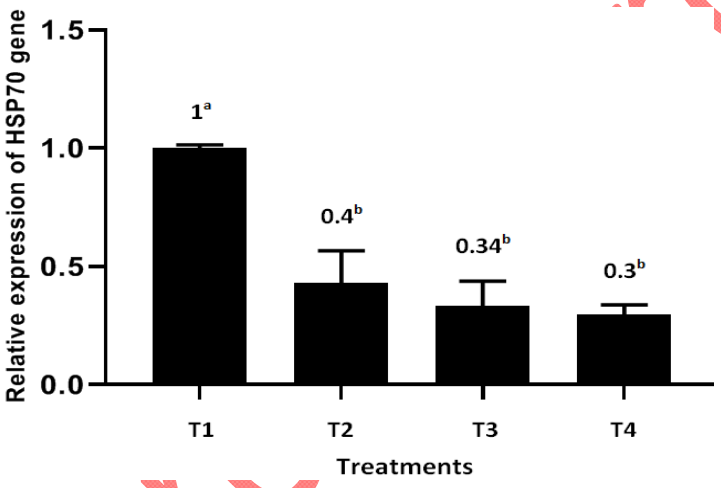

Figure 1: The effect of experimental treatments on HSP70 gene expressionof lactating cows.

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).

Discussion

As observed from the results, cows in the control treatment exhibited lower dry matter intake and body weight in comparison to the cows 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 consumed dry matter consumed decreased under heat stress conditions. This 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, which may be due to the difference in body weight of those cows than the cows in heat stress conditions (Rhoads *et al*., 2009).

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 of 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, but zinc is reported to have an effect on the thermal conditions of cattle due to its antioxidant role and its positive effect on immune function in cows (Kellogg *et al*., 2004).

As evident from the results, the control treatment yielded lower milk production compared to the other treatments, consistent with findings from prior studies (Garcia *et al*., 2015). Heat stress, leading to reduced feed intake, can diminish nutrient intake and elevate body maintenance requirements, thereby acting as a significant factor in decreased milk production (Garcia et al., 2015). Researchers have also noted that glucose is vital for mammary gland lactose synthesis. Given lactose's role as a crucial regulator of milk osmolarity, 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 and Wall, 2015). In this study, treatment effects were observed 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 serves as an indicator of optimal mammary function, as the loss of mammary epithelial tissue during

lactation can diminish milk synthesis and secretion. Given the various mechanisms through which 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 were able to 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 the proper utilization from the nitrogen-rich feeds for tissue and milk protein synthesis. Since feed intake reduces under heat stress, it can, therefore, be effective in feed efficiency (Cowley *et al*., 2015).

The percentage of non-fat solids in milk was lower in the control than the other experimental groups. 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, which releases glucose from the body's reserves to feed the skeletal muscle in order 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 are the cause of increased blood glucose concentrations in cows after calving (Dehghan-Banadaky *et al*., 2013).

According to the results of Duffield's, (2006), non-esterified free fatty acids 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. The result of this breakdown of fatty tissues is 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 more in the control treatments that were exposed to heat stress compared to 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 the increase in 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).

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

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 an increase 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 the cows with heat stress during 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 for 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 (Shwartz *et al*., 2009).

The concentration of blood factors of the cows exposed to zinc, mist spray and mist spray + zinc significantly decreased in comparison with the control. According to West (2003), changes in temperature affect feed intake, body weight gain and the immune system of animals and decrease the concentration of minerals such as iron, zinc, selenium and chromium. As the ambient temperature and relative humidity exceed the normal, the cow's overall ability decreases, 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 malondialdehyde, glutathione peroxidase and superoxide dismutase (SOD) was higher in the 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 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 the decrease in temperature and antioxidant properties of zinc. Zinc deficiency in the diet increases oxidative damage to the cell membrane due to the increase in 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 through supplementation of vitamins C, E and A and minerals such as zinc. Vitamin E acts as 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 in comparison with the other treatments.

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

Padmani *et al*. (2008) found that increased internal capacity of antioxidants was a factor in the relative decrease of HSP70 gene expression. Farnworth and Dufresne, (2001) also described the antioxidant activity mechanism of antioxidants by direct inhibition and/or removing oxygen free radicals and/or

reactive oxygen species and inhibiting oxidative enzymes in reducing HSP70 gene expression. This 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 important enzymes that have a significant effect on maintaining the balance between free radicals and the antioxidant system (Febbraio and Koukoulas, 2000).

Conclusion

The results of this experiment showed that management and nutrition strategies can be generally effective in thermal stress. As can be seen, the application of mist and zinc improved the amount of dry matter intake, milk yield and its compounds. In terms of blood factors and antioxidant indices of cows exposed to heat stress alleviation strategies, there were significant differences with respect to heat stress treatment. 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 the use of zinc and mist spray separately could reduce the thermal stress in cows, their combination had a more favorable effect.

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در گاو های شیری تحت HSP70 تأثیر راه کارهای مدیریتی و تغذیه ای بر عملکرد و بیان ژن تنش گرمایی

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چکیده:

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

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

روش کار: شانزده گاو شیری نژاد هلشتاین مورد استفاده قرار گرفت و آزمایش شامل چهار تیمار بود: جیره پایه بدون روش‌های کاهش استرس حرارتی (کنترل)، جیره پایه به همراه مکمل معدنی روی، جیره پایه به همراه استفاده از روش اسپری آب و جیره پایه به همراه مکمل معدنی روی به همراه اسپری آب. تولید و ترکیبات شیر، پارامترهای خونی و بیان ژن HSP70 مورد اندازه گیری قرار گرفت.

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

نتیجه گیری نهایی: استفاده از روش های مدیریتی و تغذیه ای می تواند در کاهش اثرات استرس گرمایی بر روی گاوهای شیری مؤثر باشد. این مطالعه توصیه می‌کند که استفاده از مکمل روی و اسپری آب به عنوان روش‌های مؤثر در کاهش استرس گرمایی مورد استفاده قرار گیرند. به طور کلی، این مطالعه اهمیت پیاده‌سازی روش‌های تغذیه‌ای و مدیریتی برای بهبود بهره‌وری و سلامت گاوهای شیری در شرایط استرس گرمایی را بیان می‌کند.

کلمات کلیدی: استرس گرمایی، HSP70، ترکیبات شیر، اسپری آب، عملکرد تولید شیر، روی.

Table 1: Ingredients (% DM) 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	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	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	41.60
Ash (%)	8.50
Ca(%)	1.20
P(%)	0.50
Mg(%)	0.40
K(%)	1.70
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′

Table 4: The effect of experimental treatments ondry matter intake, body weight and body condition scoreof lactating cows

Item	T1	T2	T3	T4	SEM	P-value
Dry matter	21.00[b]	22.10[b]	26.10[a]	27.30[a]	0.6	0.0143
Body weight	662[b]	666[b]	711[a]	712[a]	4.7	0.0064
Body	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).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM:standard error of the means.

Table 5: The effect of experimental treatments onvaginal temperature and temperature humidity indexof lactating cows

Item	T1	T2	T3	T4	SEM	P-value
Vaginal	40.00	39.88	39.06	39.00	0.26	0.4298
Temperature	77.70[a]	77.31[a]	60.00[b]	59.70[b]	0.52	0.0001

T1:controldiet (withoutzincmineralsupplement and fog system); T2:controldiet+ 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).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 6: The effect of experimental treatments on milk yield and milk compositionsof lactating cows

Item	T1	T2	T3	T4	SEM	P-value
Milk yield	25.8^{b}	25.9^{b}	25.3^{b}	35.5^{a}	0.55	0.0002
Milk urea	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	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	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	8.31	8.33	8.42	8.50	0.058	0.6442
Total solids	2.09^{b}	2.13^{b}	2.98^{a}	3.03^{a}	0.032	0.0001
Fat corrected	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 and T4: control diet + (zinc mineral supplement (75 mg/kg feed) + fog system).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

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

Item	Calving	T1	T2	T3	T4	SEM	P-value
Glucose (mmol/L)	-21	3.94^{a}	3.75^{ab}	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
Freefattyacid (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	-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).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 8: The effect of experimental treatments on blood oxidation factorsof lactating cows

Item	Calving	T1	T2	T3	T4	SEM	P-value
Malondialdehyde	-21	8.3^{a}	7.9^{b}	7.5^{c}	7.00^{d}	0.044	0.0001
(nmol/mL)	+21	8.8^{a}	8.1^{b}	7.6^{c}	7.1^{d}	0.046	0.0001
Glutathione	-21	65.70^{a}	51.23^{b}	46.40^{bc}	41.50^{c}	1.16	0.0004
peroxidase	+21	56.60	54.43	52.20	49.70	1.13	0.2322
Superoxide	-21	176.5^{a}	155.8^{b}	141.4^{c}	135.7^{c}	2.24	0.0008
dismutase	+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 and T4: control diet + (zinc mineral supplement (75 mg/kg feed) + fog system).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.