South African Journal of Animal Science 2024, VOL 54

Effect of seasonal thermal stress on serum hormones, oxidative status, and immune response of periparturient dairy cows

Yang Geng[1], Tianzun Li[1], Hongyang Xu[1], Yulai Lu[1], Yinga Wu[1], Rouqi Wang[1], DeYuan Luo[1], Zhili Qi[1#]

[1]Department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, 430070 Wuhan, China

(Submitted 18 March 2024; Accepted 13 May 2024; Published 30 May 2024)

Abstract

The objective of this study was to investigate the effect of seasonal thermal stress on serum hormones, oxidative status, and immune response of periparturient dairy cows. A total of 32 healthy Holstein dairy cows in the perinatal period with the same date of delivery were selected (eight cows per season) and housed in a free-stall barn. Different seasons corresponded to different temperature–humidity indices (THI). Regression analysis revealed a strong positive correlation between THI and rectal temperature and respiratory rate. On the day of calving, the level of progesterone and oestradiol increased and prolactin decreased substantially at a high THI. For oxidative stress biomarkers, the contents of malondialdehyde, superoxide dismutase, and catalase were substantially higher at a low THI. Glutathione peroxidase was increased at a high THI. For immune responses, the data indicated that low and high THI conditions led to an increase in interleukin-2 and interleukin-10. Low THI cows exhibited a substantially higher level of tumour necrosis factor-α before calving. Oxidative stress, inflammatory response, and endocrine imbalance therefore occur in lactating dairy cows in hot summers and cold–wet winters in comparison with comfortable seasons.

Keywords: cold stress, heat stress, periparturient dairy cow, serum biochemical indicators, temperature–humidity index

[#]Corresponding author: zhiliqi@mail.hzau.edu.cn

Introduction

The perinatal period in cows typically spans three weeks before and after calving (Li *et al*., 2015). During this time, there are important shifts in both endocrine and metabolic functions (Yasmin *et al*., 2020). Cows in the perinatal phase are particularly sensitive to fluctuations in environmental

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v54i2.07

temperature and humidity. Perinatal dairy cows are more prone to the effects of seasonal variations in temperature and humidity, which can consequently impact their production performance (Zhang, 2012).

Although efforts have been made to mitigate summer production losses, metabolic disorders, and health issues in dairy cows, heat stress remains a persistent challenge for the global dairy industry (Qu *et al*., 2015). For Holstein cows, maintaining body temperature balance becomes critical at 25–26 °C. Beyond this threshold, typically above 25 °C, cows enter a state of heat stress (Berman, 2005). The Temperature–Humidity index (THI) provides a comprehensive assessment by integrating the effects of both humidity and temperature, serving as the most authoritative tool for evaluating heat stress (Bohmanova, 2007).

Thermal stress can induce various physiological changes in dairy cows, including alterations in rectal temperature, respiratory rate, hormone secretion, oxidative stress, and other biological functions (Grewal *et al*., 2019; Jeelani *et al*., 2019). Nevertheless, our understanding of how seasonal heat and cold stress specifically affect the endocrine status and immunity of periparturient dairy cows is still limited and warrants further investigation. Studies have shown that as the THI rises, dairy cows experience substantial increases in rectal temperature and respiratory rate (Liu *et al*., 2019). Research by Sandhya *et al*. (2015) demonstrated elevated levels of serum cortisol and progesterone, with decreased levels of luteinizing hormone, oestradiol, and prolactin, in response to heat stress.

During heat stress, the body generates numerous highly-reactive molecules. The body's antioxidant system works to counteract these molecules, inhibiting and eliminating free radicals to prevent oxidative damage (Yang *et al*., 2010; Zeng *et al*., 2014). Research by Yang *et al*. (2006) revealed that during heat stress, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) decreased, whereas the concentration of malondialdehyde (MDA) and reactive oxygen species (ROS) increased substantially. Stress impacts the immune performance of animals, leading to changes in immunoglobulin G and related immune factors, such as interleukin (IL)-2, IL-10, and tumour necrosis factor (TNF)-α (Chen *et al*., 2018). Understanding the effects of seasonal cold and heat stress on the physiological state of perinatal cows is crucial for enhancing management practices and farming efficiency. Hence, the objective of this study was to investigate the impact of seasonal thermal stress on the serum hormones, oxidative status, and immune response of periparturient dairy cows. This research aims to provide valuable insights for improving the management of perinatal cows and optimizing farming practices.

Material and Methods

The protocol of this experiment was approved by the Institutional Animal Care and Use Committee at HuaZhong Agricultural University (Wuhan, China), and the animal experiment was conducted in accordance with the National Institute of Health Guidelines for the Care and Use of Experimental Animals (Beijing, China).

A total number of 32 healthy, Holstein dairy cows in the perinatal period (the same date of delivery; body weight: 737.35 ± 30.20 kg; age: 3.27 ± 0.96 years; parity: 2.33 ± 0.90;) were selected (eight cows per season) and housed in free-stall barn. The cows were fed with total mixed ration at 06:00 and at 18: 00 before milking. During the experimental period, the animals had a free access to feed and water. Feed formula and nutrients are reported in Table 1.

Table 1 Ingredients and nutrient composition (% of dry matter) of diets fed to Holstein dairy cows in the perinatal period

Ingredients[1]	Composition	Nutrient composition	Content
Corn silage	47.06	NE_L (MJ/kg)[2]	5.68
Peanut vine	5.88	Crude protein (CP)	13.76
Leymus chinensis	11.76	Neutral detergent fibre (NDF)	41.02
Brewer's grains	11.47	Acid detergent fibre (ADF)	23.05
Concentrate supplement[1]	23.54	Calcium	0.59
$NaHCO_3$	0.29	Phosphorous	0.39
Total	100		

[1]raw material composition of concentrate supplement: maize, soybean meal, maize husk, dried distiller's grains and solubles, sugarcane molasses, sodium chloride, complex minerals (including copper sulphate, ferrous sulphate, manganese sulphate), compound vitamins (including vitamin A, vitamin E)

[2] Net energy (lactation) calculated according to NRC (1989)

This study was conducted at the Academy of Agricultural Sciences commercial dairy farm (latitude and longitude 30°28′ N, 114°16′ E, respectively) located in Wuhan, China. The area is characterized by a subtropical monsoon climate. Four experiments were respectively carried out in different seasons with 30 days and eight cows in each experiment: the first experiment was conducted in spring (4th April–3rd May), the second experiment in summer (12th July–10th August), the third experiment in autumn (19th October–17th November), and the fourth experiment in winter (28th December–26th January). To detect the effect of THI on thermal tolerance, antioxidant status, immune response, and serum hormones of Holstein dairy cows, the physiological parameters were categorized into low THI (LTHI) in winter with mean daily THI of 42.97 ± 0.95, moderate THI (MTHI) in spring and autumn with mean daily THI of 61.84 ± 0.42, and high THI period (HTHI) in summer with mean daily THI of 86.09 ± 0.23.

Ambient temperature and relative humidity were recorded four times daily (06:00, 08:00, 12:00, and 18:00) at the barn area using an electronic thermometer and hygrometer (Zhengzhou Boyang Instrument Factory, China). THI was calculated using following equation (Mader *et al*., 2006):

$$THI = [0.8 \times \text{ambient temperature (°C)}] + [(\text{relative humidity (\%)} / 100) \times (\text{ambient temperature} - 14.4)] + 46.4 \quad (1)$$

Thermal tolerance data were measured three times daily (06:00, 12:00, and 18:00) for 6 d. Rectal temperature was measured with a clinical thermometer inserted 3 cm into the rectum and held in place for 5 min minutes. Respiratory rate was determined using a stopwatch to count the flank movements of the individual cows for one minute and was expressed as breaths per minute (bpm).

Representative samples of the total mixed ration (150 g) and refusals were taken daily during days 22–27 of each period. Samples of feeds and refusals were oven-dried at 60 °C and ground with a Wiley mill grinder through a 1-mm screen. Standard methods (AOAC, 2012) were applied to analyse

dry matter (DM; method 934.01), crude protein (CP) using an automatic Kjeldahl system (Kjeltec 8400, Foss, Sweden; method 981.10), calcium (method 968.08), and phosphorus (method 965.1). Determinations of neutral (NDF) and acid detergent fibre (ADF) were carried out according to the method of Van Soest *et al*. (1991). Predicted net energy of lactation (NE_L) was calculated according to the method of the NRC (2001).

Blood samples were collected through the jugular vein before morning feeding on day 14 before calving and day 14 after calving of each experimental season, and samples were placed into separation gel, coagulation-promoting tubes, followed by centrifugation within 2 h at 3000 × *g* for 15 min. The serum thus obtained was immediately stored at -20 °C for further analysis. The levels of prolactin (PRL, H095), progesterone (PROG, H089), luteinizing hormone (LH, H206), and oestradiol (E2, H102) in the serum were assessed using commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The levels of glutathione peroxidase (GSH-Px, A005-1-2), superoxide dismutase (SOD, A001-1-2), catalase (CAT, A007-1-1), and malondialdehyde (MDA, A003-1-2) in the serum were assessed using commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The optical densities were measured at 412 nm, 550 nm, 405 nm, and 532 nm using a microplate reader (model 320, Labsystems Multiskan MS, Finland) for GSH-Px, SOD, CAT, and MDA levels, respectively. The levels of IL-10 (H009), IL-2 (H003), IgG (H106), and TNF-α (H052) in the serum were determined using commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The ELISA results were obtained by using the microplate reader (DNM-9602, Pulang, New technology, Beijing, China) at a wavelength of 450 nm.

Statistical analysis was performed using SPSS software (SPSS v. 21, SPSS Inc.; Chicago, IL, USA). Significance analysis of the environmental parameters, thermal tolerance, the biomarkers of oxidative stress, immune response, and serum hormones in different seasons was conducted using one-way ANOVA in SPSS. The relationship between THI value and respiratory rate, as well as rectal temperature, were assessed by using Pearson's correlation coefficients. The significant differences were presented at $P < 0.05$.

Results

Environmental conditions in southern China typically range from hot–humid to cold–wet weather during the time when this study was carried out. The values of THI varied from 50 to 68 in spring and summer was characterised as MTHI. The entire summer experimental period was characterised as HTHI and presented high daily temperature and THI, which exceeded the critical values of 25 °C and 72, respectively. The cold winter with a low temperature and high relative humidity was characterized as LTHI (Figure 1). Rectal temperature and respiratory rate also varied substantially according to the THI. Regression analysis revealed a strongly positive correlation between THI and rectal temperature (r = 0.6272) and respiratory rate (r = 0.8226) (Figure 2). Dairy cows exhibited higher rectal temperatures under HTHI than under MTHI and LTHI. A similar result was observed in the alteration of respiratory rate with the increasing THI.

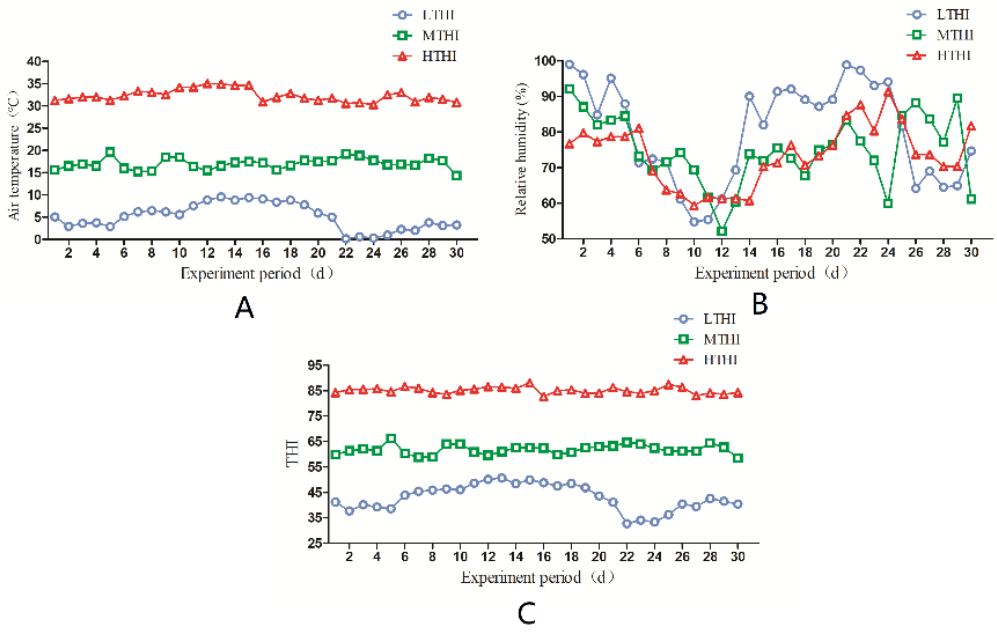

Figure 1 Environmental conditions during the experimental period of different THI: (A) daily temperature, (B) daily relative humidity, (C) daily temperature–humidity index (THI)

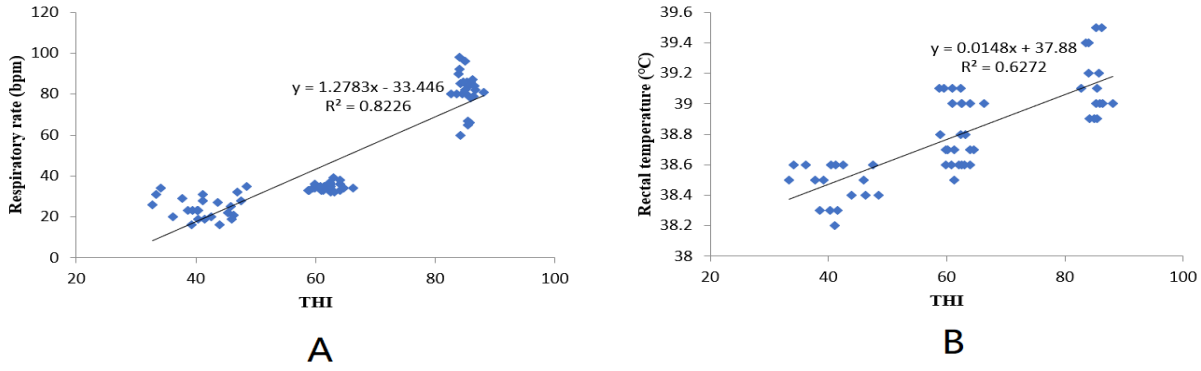

Figure 2 Relationship between physiological parameters and temperature–humidity index (THI): (A) rectal temperature and (B) respiratory rate. Points indicate individual observations; solid lines represent simple linear regression equations; R^2 represents the regression coefficient

The level of serum PRL was lower in HTHI than in MTHI and LTHI at the day of calving (P <0.01, Figure 3A). However, HTHI cows exhibited a lower level in PROG and LH than MTHI and LTHI cows post-calving (P <0.01, Figure 3B and 3C). On the day of calving, the level of PROG and E2 was higher in HTHI than in MTHI (P <0.01, Figure 3B and 3D). However, the E2 level was lower with a HTHI than a LTHI post-calving (P <0.01, Figure 3D).

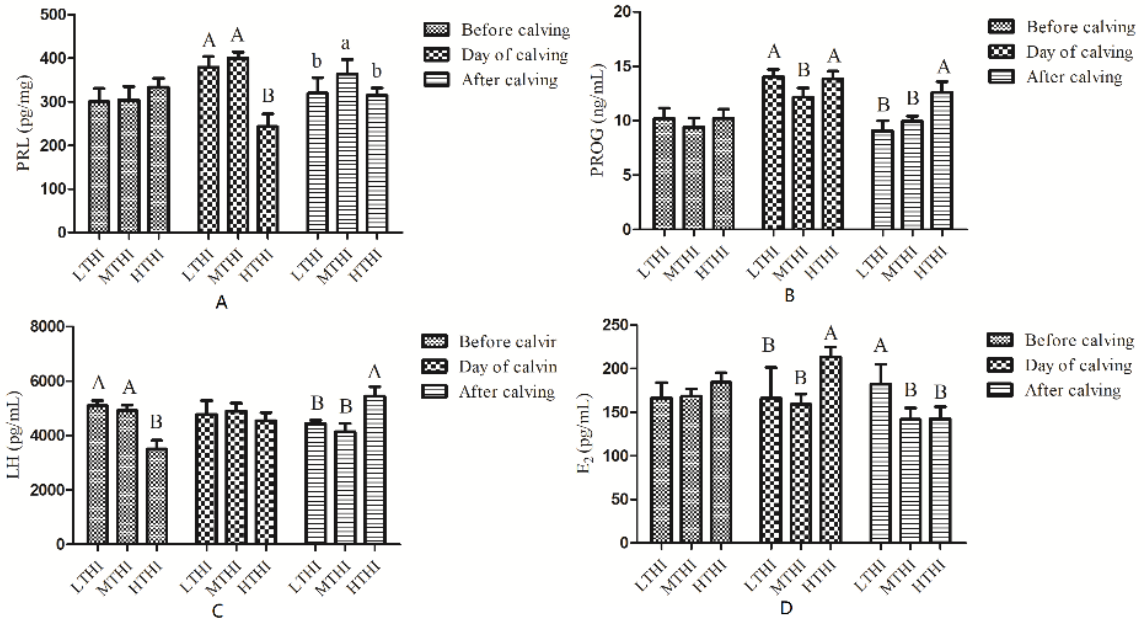

Figure 3 Effect of temperature–humidity index (THI) on reproductive hormones of lactating Holstein cows: (A) prolactin, (B) progesterone, (C) luteinizing hormone, (D) oestradiol. Values with superscript letters (A, B, C) and (a, b, c) differ (P <0.01) and (P <0.05)

The levels of MDA, SOD, and CAT were higher in LTHI than in MTHI and HTHI in the perinatal period (P <0.01, Figure 4A, 4C, and 4D). However, the glutathione peroxidase level was higher in HTHI than MTHI on the day of calving (P <0.01, Figure 4B).

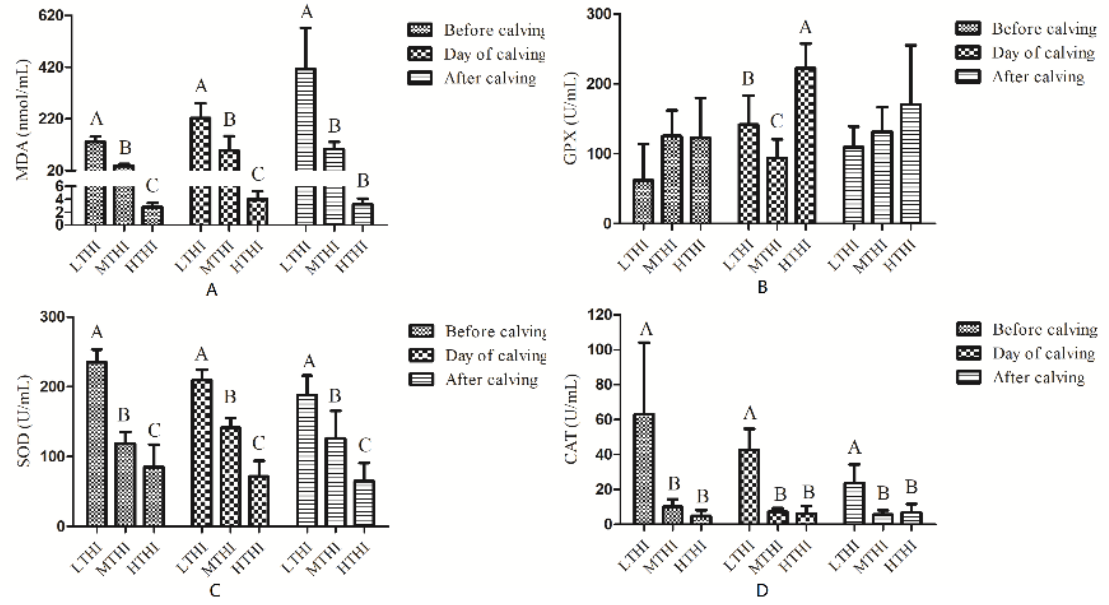

Figure 4 Effect of temperature–humidity index (THI) on oxidative status of periparturient Holstein dairy cows: (A) malondialdehyde, (B) glutathione peroxidase, (C) superoxide dismutase, (D) catalase. Values with superscript letters (A, B, C) and (a, b, c) differ (P <0.01) and (P <0.05)

Biomarkers of immunity function such as cytokines, endotoxin, and IgG were strongly influenced by THI (Figure 5). The levels of IL-2 and IgG in serum were substantially higher in LTHI than in MTHI and HTHI before calving; opposite results were observed on the day of calving. After calving, the IL-2 and IgG levels in serum were lower in LTHI and HTHI cows than in MTHI cows. The trend in IL-10 in serum was contrary to IL-2 on the day of calving and after calving. LTHI cows exhibited a substantially higher level of TNF-α before calving and a lower level on the day of calving.

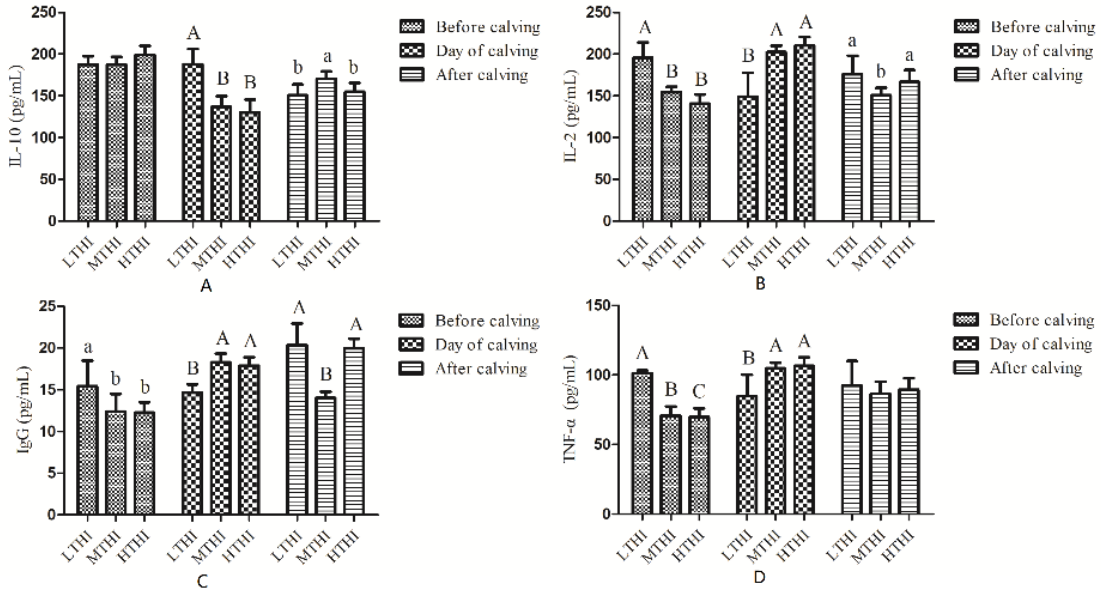

Figure 5 Effect of temperature–humidity index (THI) on immune response of periparturient Holstein dairy cows (A) Interleukin-10, (B) Interleukin-2, (C) Immunoglobulin G, (D) Tumour necrosis factor-α Values with superscript letters (A, B, C) and (a, b, c) differ (P <0.01) and (P <0.05)

Discussion

THI has been well known as an effective indicator of the degree of thermal stress in dairy cows (Yan *et al*., 2020). Rectal temperature (RT) is a sensitive index of dairy cows to external stress, which can reflect the response of cows to the external environment more than other physiological indices and is an indicator of heat balance in animal body (Ammer *et al*., 2016; Chen *et al*., 2018). The rectal temperature of dairy cows was ~38.5 °C when dairy cows were exposed to a THI of 72–80 (Hajer *et al*., 2019). Rectal temperature (Chaudhary *et al*., 2015) and respiratory rate in the present study increased with an increase in THI. In order to maintain the balance of cow body temperature when cows are in HTHI and LTHI, the hypothalamic thermoregulation centre is activated. Under the regulation of nerves and body fluid, physiological methods such as increasing or decreasing RR are used to accelerate or weaken body heat dissipation. This is one of the ways to regulate the heat dissipation of dairy cows, and RT is the result of this heat balance (Dangi *et al*., 2014).

Different THIs will have an impact on the changes in serum hormone concentration in dairy cows. Lu (2006) found that no matter the LTHI or HTHI, oestradiol in the serum of dairy cows increased, while PRL content decreased substantially. Studies have found that serum cortisol and E_2 of cows with HTHI increased, while PRL and LH were substantially decreased (Li, 2018). Progesterone content increases

in HTHI, while cortisol, LH, and E_2 decrease substantially (Li *et al*., 1998). In the current study, LTHI and HTHI conditions led to a decrease in serum PRL content in dairy cows after calving, which is not conducive to the development of the mammary acinar system, the production of milk, or the maintenance of pregnancy. HTHI leads to an increase in LH and a decrease in E_2, which indicates a disorder of the endocrine system (Lopez, 2018).

Heat stress leads to oxidative stress, which leads to an increase in ROS in different cells and tissues of heat-stressed animals. The animal body has a defence mechanism against oxidative stress in the form of enzymes, SOD, GSH, and CAT antioxidants, which are increased by heat stress (Rakhshan *et al*., 2019). At present, MDA is widely-used to indicate the degree of oxidative stress (Castillo *et al*., 2006). The main source of MDA is the peroxides of polyunsaturated fatty acids in biological systems; increased lipid mobilisation to gain energy is an adaption to cold stress (Turk *et al*., 2015). In the current study, the sharp increase in MDA under LTHI indicates a negative energy balance under cold stress (Nonnecke *et al*., 2009), as well as a decrease in antioxidant capacity and an increase in free radicals (Zhang *et al*., 2011). SOD, as an essential antioxidant enzyme, in the blood can eliminate superoxide radicals in the body (Lei *et al*., 2015). The content of SOD in LTHI was substantially higher than that in MTHI and HTHI, which is consistent with other studies (Yatoo *et al.,* 2014). CAT and GPX are important cell protective enzymes in animals. They can effectively remove hydrogen peroxide in plants, catalyse the decomposition of H_20_2, and prevent excessive levels of reactive oxygen species in cells (Colakoglu *et al.,* 2017). In the current study, LTHI induced stress in dairy cows, resulting in a sharp increase in CAT content.

Damage to the immune system weakens the disease resistance of dairy cows, increases the risk of susceptibility, and affects the health of dairy cows (Liu, 2018). IL-2 and IL-10 are cytokines with immunomodulatory activity, which act on lymphocytes and macrophages; regulate the activation, proliferation, and differentiation of T cells and B cells; and play an important role in the inflammatory response (Ge, 2018). Consistent with previous studies, LTHI and HTHI led to the increase in IL-2 and IL-10, which may control the risk of immune dysfunction through a stress protection mechanism. TNF-α is a cytokine that can kill or necrotise tumour cells. An increase in TNF-α indicates that cows are suffering from stress and inflammation has been caused (Min, 2016). The data indicate that LTHI led to an increase in TNF-α with calving; specific reasons need to be investigated further. In the current study, LTHI and HTHI promoted the body to resist stress by increasing the secretion of serum IgG after calving (Xu *et al*., 2017). The transfer of IgG from blood to the udder is related to the synthesis of milk. If the degree of endogenous inflammation exceeds the regulatory capacity of the immune system, lactation performance will deteriorate (McCarthy *et al*., 2016).

In summary, the results clearly show that oxidative stress, an inflammatory response, and endocrine imbalance of lactating dairy cows occur in hot summers and cold–wet winters in comparison with more comfortable seasons, i.e., spring and autumn. It is recommended that environmental management of keeping cows cool or warm should be taken in adaption to extreme weather during this sensitive periparturient period.

Acknowledgements

This research was supported by grants from the National Key Research and Development Project (2023YFD1301004), Natural Science Foundation of Hubei Province (2022CFB327), National Key Research and

Development Program Intergovernmental Cooperation on International Science and Technology innovation (2021YFE0115500), National Dairy Technology Innovation Center project (No.2022-Scientific Research-3), and Hubei International Cooperation Project (2022EHB043).

Authors' contributions

Conceptualization, Yang Geng; Data curation, Yang Geng and Tianzun Li; Formal analysis, Yang Geng and Hongyang Xu; Funding acquisition, ZhiLi Qi; Investigation, Yang Geng, Tianzun Li, Hongyang Xu, Yulai Lu, Yinga Wu, Rouqi Wang and Deyuan Luo; Methodology, Hongyang Xu; Project administration, ZhiLi Qi; Resources, ZhiLi Qi; Supervision, ZhiLi Qi; Validation, Yang Geng, Tianzun Li, Hongyang Xu, Yulai Lu, Yinga Wu, Rouqi Wang, and Deyuan Luo; Writing-original draft, Hongyang Xu; Writing-review & editing, Yang Geng

Conflict of interest declaration

The authors declare that there is no conflict of interest related to this article.

References

Amamou, H., Beckers, Y., Mahouachi, M., Hammami, H., 2019. Thermotolerance indicators related to production and physiological responses to heat stress of Holstein cows. J Therm Biol. 82, 90–98.

Ammer, S., Lambertz, C. & Gauly, M., 2016. Comparison of different measuring methods for body temperature in lactating cows under different climatic conditions. J. Dairy Res. 83, 165–172.

Berman, A., 2005. Estimates of heat stress relief needs for Holstein dairy cows. J Anim Sci. 83(6), 1377–1384.

Bohmanova, J., Misztal, I., Cole, J. B., 2007. Temperature–humidity Indices as indicators of milk production losses due to heat stress. J Dairy Sci. 90, 1947–1956

Castillo, C., Hernández, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M.L., Benedito, J.L., 2006. Plasma malondialdehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Res Vet Sci. 80, 133–139.

Chaudhary, S.S., Singh, V.K., Upadhyay, R.C., Puri, G., Odedara, A.B., Patel, P.A., 2015. Evaluation of physiological and biochemical responses in different seasons in Surti buffaloes. Vet World. 8(6), 727–731

Chen, S.Y., Wang, J., Peng, D.D., Li, G., Chen, J., Gu, X.H., 2018. Exposure to heat-stress environment affects the physiology, circulation levels of cytokines, and microbiome in dairy cows. Sci Rep. 8(1).

Colakoglu, H.E., Yazlik, M.O., Kaya, U., Colakoglu, E.C., Kurt, S., Oz, B., Bayramoglu, R., Vural, M.R., Kuplulu, S., 2017. MDA and GSH-Px activity in transition dairy cows under seasonal variations and their relationship with reproductive performance. J Vet Res. 61(4), 497–502.

Dangi, S. S., Gupta, M., Nagar, V., Yadav, V. P., Dangi, S. K., Shankar, O., Chouhan, V. S., Kumar, P., Singh, G., & Sarkar, M., 2014. Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats. Int J Biomet. 58(10), 2085–2093.

Ge Ying, Y., Wang, G.L., Zhang, Z., Xue, Y.K., 2018. Changes of serum related indexes of cow mastitis. Heilongjiang Animal Husbandry and Veterinary, (03), 39–43

Grewal, S., Aggarwal, A., Alhussien, M.N., 2019. Integrated effects of season and parturition-associated stress on the inflammatory response and metabolic status in Sahiwal (*Bos indicus*) cows. Biol. Rhythm Res. *52*(8), 1179–1190. doi:10.1080/09291016.2019.1627657

Lei, X.G., Zhu, J.H., Cheng, W.H., Bao, Y., Ho, Y.S., Reddi, A.R., *et al*., 2015. Paradoxical roles of antioxidant enzymes: Basic mechanisms and health implications. Physiol Rev. 96, 307–314.

Liu. J., Jinhuan, D., Lirong, H., Qing, X., Yachun, W., 2018. Effects of heat stress on physiological and immune functions of dairy cows and its mechanism. Chinese Animal Husbandry and Veterinary. 45(01), 263–270.

Li, j., Runzi, S., Zhengshan, Z., Tiezheng, W., Huizhong, W., Yongfu, J., Yufeng, C., Yingcai, T., Zengfu, X., 1998. Effects of heat stress on physiological constant, blood biochemical indexes, reproduction, and lactation performance of dairy cows. Journal of Hebei Agricultural University. 04, 69–75.

Li, j., Zhang Z., Yin, H., Yong, L., Lina, Y., Huanrong, W., Wei, G., 2015. Research progress on occurrence and preventive measures of hypocalcemia in perinatal dairy cows. Dairy Sci Tech. 38(05), 29–32.

Liu, J.J., Li, L.Q., Chen, X.L., Lu, Y.Q., Wang, D., 2019. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: A novel idea for monitoring and evaluation of heat stress — A review. Asian-Australas J Anim Sci. 32, 1332–1339.

Lopez, E., Mellado, M., Martínez, A.M., Veliz, F.G., Garcia, J.E., de Santiago, A., Carrillo, E., 2018. Stress-related hormonal alterations, growth and pelleted starter intake in pre-weaning Holstein calves in response to thermal stress. Int J Biomet. 62(4), 493–500.

Lu, X., 2006. Effects of chronic cold and heat stress on serum enzyme activity, endocrine hormone level and maintenance behavior of Holstein cows. Dissertation. Inner Mongolia Agricultural University.

Mader, T.L., Davis, M.S., Brown-Brandl, T., 2006. Environmental factors influencing heat stress in feedlot cattle. J Anim Sci. 84, 712–719.

McCarthy, M., Yasui, T., Felippe, M., Overton, T., 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J Dairy Sci. 99, 680–700.

Min, L., Zheng, N., Zhao, S., Cheng, J., Yang, Y., Zhang, Y., Yang, H., Wang, J., 2016. Long-term heat stress induces the inflammatory response in dairy cows revealed by plasma proteome analysis. Biochem Bioph Res. 471, 296–302.

Nonnecke, B.J., Foote, M.R., Miller, B.L., Fowler, M., Johnson, T.E., Horst, R.L., 2009. Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of pre-ruminant calves. J Dairy Sci. 92, 6134–6143.

Qu, M., Wei, S., Chen, Z., Wang, G., Zheng, Y., Yan, P., 2015. Differences of hormones involved in adipose metabolism and lactation between high and low producing Holstein cows during heat stress. Anim Nutr. 1, 339–343.

Rakhshan, J., Konwar, D., Khan, A., Kumar, D, Chakraborty, D., Brahma, B., 2019. Reassessment of temperature–humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region. J Therm Biol. 82, 99–106.

Turk, R., Podpečan, O., Mrkun, J., Flegar-Meštrić, Z., Perkov, S., Zrimšek, P., 2015. The effect of seasonal thermal stress on lipid mobilisation, antioxidant status, and reproductive performance in dairy cows. Reprod Domest Anim. 50, 595–603.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74, 3583–3589.

Xu, H, Huang, W., Hou, Q., Kwok, L.Y., Sun, Z., Ma, H., *et al*., 2017. The effects of probiotics administration on the milk production, milk components, and fecal bacteria microbiota of dairy cows. Sci Bull. 62, 667–674.

Yan, G., Li, H., Zhao, W. *et al.,* 2020. Evaluation of thermal indices based on their relationships with some physiological responses of housed lactating cows under heat stress. Int J Biometeorol. 64, 2077–2091. doi.org/10.1007/s00484-020-01999-6

Yang, Y.L., Ye, B.K., Liu, H.Y., 2010. Occurrence, danger, prevention and treatment of heat stress in dairy cattle.

China Cattle Sci. 36, 63–66.

Yatoo, M.I., Dimri, U., Sharma, M.C., 2014. Seasonal changes in certain blood antioxidants in cattle and buffaloes. Indian J Anim Sci. 84, 173–176.

Zeng, T., Li, J.J., Wang, D.Q., Li, G.Q., Wang, G.L., Lu, L.Z., 2014. Effects of heat stress on antioxidant defense system, inflammatory injury, and heat shock proteins of Muscovy and Pekin ducks: Evidence for differential thermal sensitivities. Cell Stress Chaperon 19, 895–901.

Zhang, X., 2012. Physiological characteristics and nutritional requirements of peripartum dairy cows. Chinese Dairy Cows. 4, 62–65.

Zhang, Z., Lv, Z., Li, J., Li, S., Xu, S., Wang, X., 2011. Effects of cold stress on nitric oxide in duodenum of chicks. Poult Sci. 90, 1555–1561.