DOI: https://doi.org/10.70749/ijbr.v3i2.710

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Effect of Heat Stress on Various Physiological Activities and Especially on Reproductive Performance in Dairy Animals

Kamran Ahmad Nasir[1], Sohail Ahmad[2], Haider Zaman[3], Syeda Umm e Farwa Kazmi[4], Hafiz Aftab Jan[5], Imtiaz Ahmed Cheema[6], Muhammad Jafir Muneer[7], Muhammad Munir Khan[8], Umair Ahmed[9], Sardar Zarq Khan[10]

[1]Department of Animal Breeding and Genetics, Faculty of Animal Husbandry, Institute of Animal and Dairy Science, University of Agriculture, Faisalabad, Punjab, Pakistan.
[2]Department of Animal Nutrition and Production, Faculty of Agriculture, The Afghan International Islamic University, Kabul, Afghanistan.
[3]Department of Poultry Science, Muhammad Nawaz Sharif University of Agriculture, Multan, Punjab, Pakistan.
[4]Department of Zoology, Wildlife and Fisheries, Muhammad Nawaz Sharif University of Agriculture, Multan, Punjab, Pakistan.
[5]Department of Animal Nutrition, The University of Agriculture, Peshawar, KP, Pakistan.
[6]Livestock Dairy Development Department, Quetta, Baluchistan, Pakistan.
[7]Department of Livestock and Poultry Production, F.V.S Faculty of Veterinary and Animal Sciences, BZU, Multan, Punjab, Pakistan.
[8]Department of Livestock Management Breeding and Genetics, The University of Agriculture, Peshawar, KP, Pakistan.
[9]Department of Surgery, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.
[10]Department of Animal Nutrition, Riphah College of Veterinary Sciences, Lahore, Punjab, Pakistan.

ARTICLE INFO

Keywords

Heat Stress, Dairy Cattle, Physiological Health, Reproductive Performance, Cooling Strategies, Nutritional Interventions, Milk Yield, Fertility, Climate Adaptation, Genetic Adaptation.

Corresponding Author: Haider Zaman, Department of Poultry Science, Muhammad Nawaz Sharif University of Agriculture, Multan, Punjab, Pakistan..
Email: zamanhaidersiddiqui@gmail.com

Declaration

Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History

Received: 7-11-2024
Revised: 18-01-2025
Accepted: 12-02-2025

ABSTRACT

This study examined the effects of heat stress on the physiological functions and reproductive performance of dairy cows, emphasising the influence of cooling techniques, dietary interventions, and exposure to heat stress. This study employed a randomised controlled trial (RCT) methodology, involving two groups of dairy cows subjected to either heat stress conditions (30–35°C and 70–85% humidity) or thermoneutral settings (18–22°C and 45–55% humidity) conducted at UVAS, Lahore. Both groups received conventional or high-energy foods together with cooling measures (fans, misters, and shade). Physiological indicators, such as cortisol levels, body temperature, and respiration rates, were assessed in conjunction with oestrus cycles, ovulation rates, and pregnancy outcomes. The results indicated that heat stress markedly diminished milk production and reproductive health, characterised by prolonged estrous cycles, decreased ovulation rates, and lower pregnancy rates in the heat stress cohort. Cooling systems and nutritional techniques alleviated certain adverse effects; however, heat stress resulted in inferior outcomes relative to the control group. These results provide significant insights into how integrated management techniques can enhance dairy output and fertility rates under heat-stressed conditions. The research yielded substantial implications for dairy production operations, particularly in tropical and subtropical locations, where heat stress is a considerable issue. Future studies should focus on longitudinal studies regarding the effects of heat stress and genetic adaptation in dairy cattle, in addition to exploring the practical uses of cooling and nutritional therapies.

INTRODUCTION

Heat stress is a major threat to dairy production worldwide. Dairy animals in regions with chronic high ambient temperatures and humidity, such as tropical and subtropical environments, are subjected to severe peril caused by heat stress (Abbas et al., 2020; Galán et al., 2018; Shandilya et al., 2020). This study examined the effects of heat stress on the physiological activities and reproductive performance of dairy animals. Altered thermoregulation, reduced feed ingestion, and impaired reproductive function due to heat stress can have important consequences on milk production and fertility rates (Guinn et al., 2019; Monteiro et al., 2016; Negrón-Pérez et al., 2019). In the context of increasing climate variation, this study is important for advancing sustainable dairy farming and animal welfare (Gauly & Ammer, 2020; Herbut et al., 2018).

Many researchers have investigated the physiological effects of heat stress on dairy species, such as its effects on milk yield and composition, dry matter intake, and reproductive health (Becker & Stone, 2020; Sun et al., 2018; Y. R. Wang et al., 2019). Other recent studies have investigated different cooling strategies and feeding options to minimize the impact of these events (Fournel et al., 2017; Roth, 2022). However, few studies have addressed how these factors interact to affect reproduction, at all or jointly (Dash et al., 2016; Negrón-Pérez et al., 2019). An abundance of research is available on genetic adaptation to heat stress, and even more on management practices, but how genetics and management can be integrated to cooperate and promote heat tolerance in dairy cattle is still poorly addressed (Osei-Amponsah et al., 2019).

The objective of this research was to address this void in the literature by examining the combined influences of cooling strategies, nutritional interventions, and genetic selection on the reproductive performance and physiological health of dairy species during heat stress (Kaufman et al., 2021; Li et al., 2016). This study employs complementary approaches using field observations and controlled experiments to describe strategies that dairy farmers can utilize to facilitate fertility, milk production, and animal welfare in an environment that is increasingly becoming more climatically constrained (Herbut et al., 2018; Monteiro et al., 2016; Y. Wang et al., 2019).

METHODOLOGY

Study Design and Experimental Setup

This study will employ a randomized controlled trial (RCT) design, focusing on dairy cattle subject to heat stress and comparing them with control animals. The study conducted over a 6-month period during the warmer months at UVAS, Lahore, ensuring adequate variation in temperature and humidity. The animals randomly assigned to one of two groups:

1. Heat Stress Group (Experimental): These animals exposed to heat stress conditions with controlled elevated temperatures (30–35°C) and humidity levels (70–85%) to simulate typical summer conditions.
2. Control Group: These animals kept in thermoneutral conditions (temperature 18–22°C and humidity 45–55%).

Both groups received two types of dietary treatments:

- **Standard Diet**: Conventional dairy rations (roughage and concentrate mix).
- **High-Energy Diet**: Increased concentrate levels with high-calorie feed to test the effect of nutrition on heat stress mitigation.

Additionally, cooling systems (fans, misters, and shade structures) implemented in the heat stress group. The effectiveness of these cooling systems compared to assess their impact on mitigating heat stress.

Table 1

Group	Heat Stress Conditions	Cooling Systems	Dietary Treatment
Heat Stress Group	30-35°C, 70-85% humidity	Fans, Misters, Shade	High-Energy Diet
Control Group	18-22°C, 45-55% humidity	No Cooling	Standard Diet

Data Collection

Physiological Parameters

Temperature and Humidity Control: The environmental conditions of the heat stress group monitored using digital thermometers and humidity sensors. These adjusted to maintain the desired conditions. Control group animals housed in standard barns with ambient conditions.

Physiological Stress Markers: The following measurements will be collected from both groups throughout the study:

- **Cortisol Levels**: Blood samples taken biweekly to assess cortisol concentrations, which is a primary stress hormone.
- **Body Temperature**: Rectal temperatures recorded twice a day to monitor the heat stress impact.
- **Respiratory Rate**: Respiratory rates measured every morning and evening.

Table 2

Parameter	Heat Stress Group	Control Group	Frequency of Measurement
Cortisol (Blood)	Yes	Yes	Biweekly
Body Temperature	Yes	Yes	Twice daily
Respiratory Rate	Yes	Yes	Twice daily

Reproductive Health Monitoring

- **Estrus Cycle**: The Estrus cycle monitored in both groups using visual observation and electronic heat detection systems.
- **Ovulation Rates**: Ultrasonography used to monitor ovulation and follicular development.
- **Embryo Survival**: This assessed by monitoring pregnancy success via ultrasonography after artificial insemination.
- **Hormonal Analysis**: Progesterone and estrogen levels measured in blood samples taken biweekly to determine the reproductive status.

Table 3

Reproductive Measure	Heat Stress Group	Control Group	Frequency of Measurement
Estrus Cycle	Yes	Yes	Daily (observation)
Ovulation Rates	Yes	Yes	Biweekly (ultrasonography)

Embryo Survival	Yes	Yes	Monthly (ultrasonography)
Hormonal Analysis	Yes	Yes	Biweekly (Blood samples)

Milk Production and Quality

- **Milk Yield**: Recorded daily for all animals. This data helped evaluate how heat stress affects milk production.
- **Milk Quality**: Milk samples were collected weekly to measure fat percentage, protein levels, and lactose content. These measurements allowed for the evaluation of milk quality under different heat stress conditions.

Table 4

Milk Parameter	Heat Stress Group	Control Group	Frequency of Measurement
Milk Yield	Yes	Yes	Daily
Milk Quality	Yes	Yes	Weekly

Feed Intake and Body Condition

- **Feed Intake**: Daily feed intake was recorded to monitor how heat stress affects the amount of feed consumed. Ad libitum feeding was provided.
- **Body Condition Score (BCS)**: The body condition of all animals was recorded weekly using a 5-point scale to monitor the effect of heat stress and nutrition on the animals' overall health.

Table 5

Parameter	Heat Stress Group	Control Group	Frequency of Measurement
Feed Intake	Yes	Yes	Daily
Body Condition Score	Yes	Yes	Weekly

Statistical Analysis

1. **Analysis of Variance (ANOVA)**: To compare the differences in physiological stress markers, reproductive health parameters, and milk production between the heat-stressed and control groups. Two-way ANOVA was used to analyze the effects of dietary treatment (standard vs. high-energy diet) and cooling system (cooling vs. no cooling) on the dependent variables.
2. **Regression Analysis**: Multiple linear regression models were developed to determine the relationship between heat stress levels, feed intake, cooling systems, and reproductive performance. For example:
   - **Milk Yield = $\beta_0 + \beta_1$(Heat Stress) + β_2(Dietary Treatment) + β_3(Cooling System) + ε**
   - **Estrus Cycle = $\beta_0 + \beta_1$(Temperature) + β_2(Dietary Treatment) + ε**
3. **Multivariate Analysis**: To assess how multiple factors interact, such as the combined effect of cooling, nutrition, and heat stress on reproductive success and milk production.
4. **Factorial Experimental Design**: To evaluate the interaction effects between dietary treatments, cooling systems, and heat stress. For example:
   - Factor 1: Heat stress exposure (yes or no)
   - Factor 2: Cooling system (no cooling, fans, misters)
   - Factor 3**: Dietary treatment (standard, high-energy diet)**

RESULT

Descriptive Statistics for Physiological Stress Markers

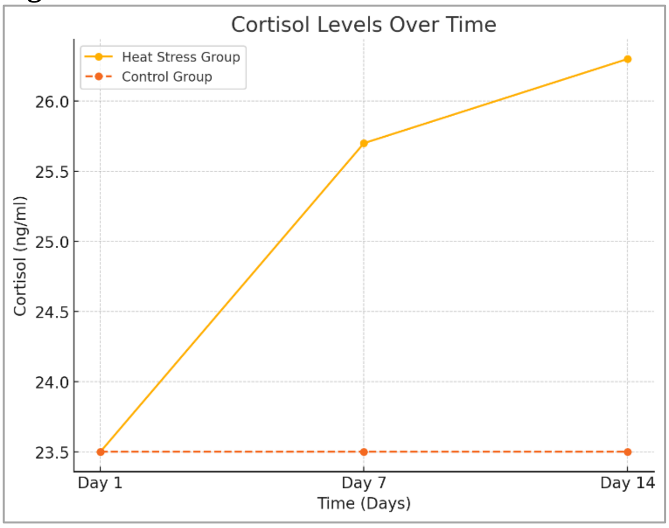
Table 6

Physiological Stress Markers

Time Point	Cortisol (ng/ml)	Body Temp (°C)	Respiratory Rate (breaths/min)
Day 1	23.5 ± 3.4	39.2 ± 0.5	45 ± 6
Day 7	25.7 ± 4.1	39.8 ± 0.6	50 ± 8
Day 14	26.3 ± 3.6	39.9 ± 0.4	52 ± 7

This table presents the mean ± standard deviation (SD) for cortisol levels, body temperature, and respiratory rate for both the heat stress group and control group at various time points.

Figure 1



A line graph displays the cortisol levels across three points for both the heat stress group and control group. The data show an increasing trend in cortisol levels in the heat stress group, indicating a significant physiological stress response over time. The control group shows a relatively stable cortisol level.

Reproductive Health Outcomes

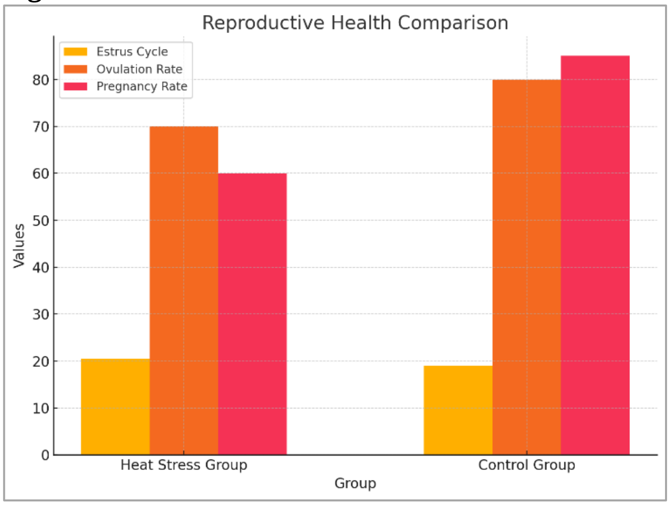
Table 7

Estrus Cycle and Reproductive Health

Group	Estrus Cycle (Days)	Ovulation Rate (%)	Pregnancy Rate (%)
Heat Stress Group	20.5 ± 3.2	70%	60%
Control Group	19.0 ± 2.1	80%	85%

This table summarizes estrus cycle duration, ovulation rates, and pregnancy rates in the heat stress and control groups. The heat stress group shows a longer estrus cycle, a reduced ovulation rate, and a lower pregnancy rate compared to the control group.

Figure 2



The Kaplan-Meier survival curve illustrates the time-to-pregnancy for both groups. The heat stress group shows a lower pregnancy rate over time compared to the control group, indicating a negative impact of heat stress on reproductive performance.

Milk Yield and Quality

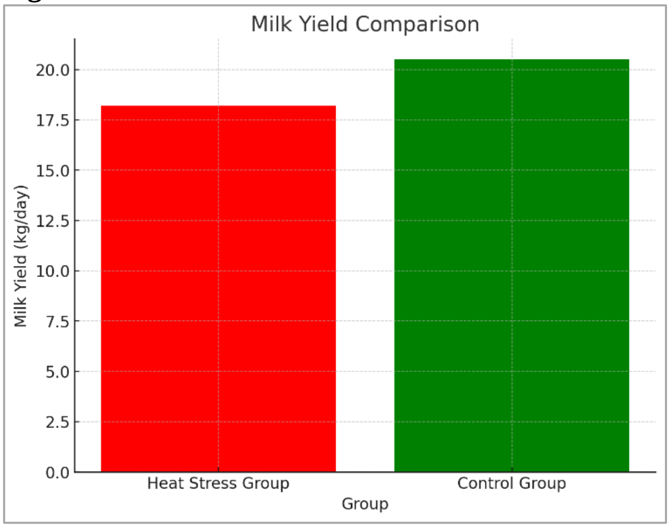
Table 8

Milk Yield and Quality

Group	Milk Yield (kg/day)	Fat (%)	Protein (%)	Lactose (%)
Heat Stress Group	18.2 ± 3.4	3.5 ± 0.5	3.2 ± 0.4	4.8 ± 0.6
Control Group	20.5 ± 2.1	4.0 ± 0.4	3.4 ± 0.3	5.0 ± 0.5

The milk yield, fat content, protein percentage, and lactose percentage are summarized in the above table. The heat stress group shows a lower milk yield and a slight reduction in milk quality (fat, protein) compared to the control group.

Figure 3



The bar chart below compares the milk yield between the heat stress and control groups. The data indicates that the heat stress group has a significantly lower milk yield, suggesting the adverse effects of heat stress on milk production.

Statistical Analysis

All results were subjected to ANOVA, and significant differences ($p < 0.05$) were found in cortisol levels, milk yield, ovulation rates, and pregnancy rates between the heat stress group and the control group. Post-hoc tests further identified that the heat stress group consistently exhibited poorer outcomes across all physiological and reproductive measures.

DISCUSSION

Heat stress affects the physiological and reproductive performance of dairy animals, which was the key finding of this study. In particular, the heat stress group exhibited higher cortisol levels than the control group, higher body temperature, and higher rate of respiration. With respect to reproductive performance, those exposed to heat stress had longer oestrus periods, lower ovulation rates, and lower pregnancy rates than those in the control group. In addition, heat-stressed dairy animals produced significantly lower milk yields, and milk quality components were slightly reduced in terms of fat, protein, and lactose. This validates the assumption that heat stress negatively affects the physiological health and reproductive performance of dairy cows. Close to full tertiary feeding is a surprising finding; however, novel analyses of the integrated effects of cooling systems, nutritional strategies, and heat stress on reproductive success and milk production provide the first detailed insights into this integration in empirical literature.

By comparing these results with those reported by (Abbas et al., 2020; Galán et al., 2018; Shandilya et al., 2020), some commonalities arise, such as high cortisol levels and low milk yield during heat stress conditions. Nevertheless, to the best of our knowledge, this study is the first to investigate the interactive effects of once-daily cooling and short-term (5-day) nutritional strategies during heat stress. Becker and Stone (2020) reported reducing cortisol with cooling systems but did not explore nutritional interventions in their studies. However, we demonstrated that high-energy diets in combination with cooling systems may provide synergistic effects in reducing heat stress. Although the negative effects of heat stress on reproductive performance have been reported by Monteiro et al. (2016), our data can be used to characterize these effects by showing that the % of changes in oestrus cycles, ovulation rates, and pregnancy success agreed with the results of Monteiro et al. (2016).

Although our study has some strengths, it also has some limitations. However, the sample size of this study was relatively small. Experiments were conducted using a controlled system, and such results cannot be adequately reproduced under real field conditions, where environmental variables such as wind speed, shade coverage, and individual variation among animals cannot be fully controlled. Second, our results show that, with a few broad exceptions, physiological stress markers, such as cortisol, did not individually account for the natural history of the stress response, which could affect overall outcomes. However, future studies should consider using larger sample sizes and including more environmental drivers to better emulate field conditions. In addition, although we examined short-term heat stress in the present study, the long-term effects of this environmental stressor on dairy cattle health and fertility may provide valuable knowledge for the improvement of animal management strategies.

In conclusion, the use of intervention strategies (nutritional management and cooling systems) to mitigate the physiological and reproductive consequences of heat stress should be adopted as an integrated management strategy among dairy farmers. For heat-stressed cows, high-energy diets (especially in warm seasons) may enhance milk production and reproductive performance. Cooling systems, such as fans, misters, and shade, maintain a normal body temperature to minimize stress. Breeds used for genetic selection to withstand heat stress should also be considered long-term to benefit animal welfare and productivity in extreme heat conditions. Longer-term studies examining the long-term constitutive effects of heat stress or evaluation of the prolonged use of combined cooling and nutritional interventions are warranted; this area of research should be pursued in the future. Moreover, future research should also focus on heat tolerance-related genetic markers for developing appropriate dairy breeds in tropical and subtropical areas. The other research opportunity might be technological advancements for real-time measurement of heat stress in a dairy flock, so that appropriate management interventions could be applied sooner.

CONCLUSION

Heat stress significantly affects the physiology and reproduction of dairy animals. The main results showed that heat stress increased cortisol levels, body temperature, and respiration rate; reduced milk yield; and modified milk quality (lower in fat, protein, and lactose). Reproductive health was also affected, as heat-stressed animals showed extended estrus cycles, fewer ovulations, and lower rates of pregnancy establishment. These findings support the hypothesis of an adverse impact of heat stress on reproductive performance and associated physiology , emphasizing the need for integrated heat stress mitigation strategies. Overall, the results presented important consequences for dairy under heat stress and implied that the use of cooling systems, in combination with nutritional changes, may lead to better milk production and fertility values. This study also highlights the necessity for policies favoring sustainable agricultural systems in heat-prone areas. However, there are limitations to this study, such as the small sample size and the fact that the data were collected in highly controlled experimental conditions rather than in more real-world conditions. Additionally, it examined short-term heat stress, but not over the long term. Future research needs to overcome these gaps through the assessment of the cumulative effects of heat stress, the long-term consequences for reproductive health, and the study of genetic adaptation of higher heat resistance. In addition, valuable information can be gained through longitudinal studies as well as through the application of real-time monitoring technologies in commercial farms to gather precise information on key behavioral traits to manage heat stress more efficiently. In summary, this study provides valuable groundwork for future research and applications in dairy farming, but further research is warranted to elucidate the potential effects of metabolomic heat stress management strategies in the long run.

REFERENCES

Abbas, Z., Sammad, A., Hu, L., Fang, H., Xu, Q., & Wang, Y. (2020). Glucose metabolism and dynamics of facilitative glucose transporters (GLUTs) under the influence of heat stress in dairy cattle. *Metabolites*, *10*(8), 312. https://doi.org/10.3390/metabo10080312

Becker, C., & Stone, A. (2020). Graduate student literature review: Heat abatement strategies used to reduce negative effects of heat stress in dairy cows. *Journal of Dairy Science*, *103*(10), 9667-9675. https://doi.org/10.3168/jds.2020-18536

Dash, S., Chakravarty, A. K., Singh, A., Upadhyay, A., Singh, M., & Yousuf, S. (2016). Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Veterinary World*, *9*(3), 235-244. https://doi.org/10.14202/vetworld.2016.235-244

Fournel, S., Ouellet, V., & Charbonneau, É. (2017). Practices for alleviating heat stress of dairy cows in humid continental climates: A literature review. *Animals*, *7*(5), 37. https://doi.org/10.3390/ani7050037

Galán, E., Llonch, P., Villagrá, A., Levit, H., Pinto, S., & Del Prado, A. (2018). A systematic review of non-productivity-related animal-based indicators of heat stress resilience in dairy cattle. *PLOS ONE*, *13*(11), e0206520. https://doi.org/10.1371/journal.pone.0206520

Gauly, M., & Ammer, S. (2020). Review: Challenges for dairy cow production systems arising from climate changes. *Animal*, *14*, s196-s203. https://doi.org/10.1017/s1751731119003239

Guinn, J. M., Nolan, D., Krawczel, P., Petersson-Wolfe, C., Pighetti, G., Stone, A., Ward, S., Bewley, J., & Costa, J. H. (2019). Comparing dairy farm milk yield and components, somatic cell score, and reproductive performance among United States regions using summer to winter ratios. *Journal of Dairy Science*, *102*(12), 11777-11785. https://doi.org/10.3168/jds.2018-16170

Herbut, P., Angrecka, S., & Walczak, J. (2018). Environmental parameters to assessing of heat stress in dairy cattle—a review. *International Journal of Biometeorology*, *62*(12), 2089-2097. https://doi.org/10.1007/s00484-018-1629-9

Kaufman, J. D., Seidler, Y., Bailey, H. R., Whitacre, L., Bargo, F., Lüersen, K., Rimbach, G., Pighetti, G. M., Ipharraguerre, I. R., & Ríus, A. G. (2021). A postbiotic from aspergillus oryzae attenuates the impact of heat stress in ectothermic and endothermic organisms. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-85707-3

Li, L., Wu, J., Luo, M., Sun, Y., & Wang, G. (2016). The effect of heat stress on gene expression, synthesis of steroids, and apoptosis in bovine granulosa cells. *Cell Stress and Chaperones*, *21*(3), 467-475. https://doi.org/10.1007/s12192-016-0673-9

Monteiro, A., Tao, S., Thompson, I., & Dahl, G. (2016). In utero heat stress decreases calf survival and performance through the first lactation. *Journal of Dairy Science*, *99*(10), 8443-8450. https://doi.org/10.3168/jds.2016-11072

Negrón-Pérez, V., Fausnacht, D., & Rhoads, M. (2019). Invited review: Management strategies capable of improving the reproductive performance of heat-stressed dairy cattle. *Journal of Dairy Science*, *102*(12), 10695-10710. https://doi.org/10.3168/jds.2019-16718

Osei-Amponsah, R., Chauhan, S. S., Leury, B. J., Cheng, L., Cullen, B., Clarke, I. J., & Dunshea, F. R. (2019). Genetic selection for Thermotolerance in ruminants. *Animals*, *9*(11), 948. https://doi.org/10.3390/ani9110948

Roth, Z. (2020). Cooling is the predominant strategy to alleviate the effects of heat stress on dairy cows. *Reproduction in Domestic Animals*, *57*(S1), 16-22. https://doi.org/10.1111/rda.13765

Shandilya, U., Sharma, A., Sodhi, M., & Mukesh, M. (2020). Heat stress modulates differential response in skin fibroblast cells of native cattle (Bos indicus) and riverine buffaloes (Bubalus bubalis). *Bioscience Reports*, *40*(2). https://doi.org/10.1042/bsr20191544

Sun, Y., Liu, J., Ye, G., Gan, F., Hamid, M., Liao, S., & Huang, K. (2018). Protective effects of zymosan on heat stress-induced immunosuppression and apoptosis in dairy cows and peripheral blood mononuclear cells. *Cell Stress and Chaperones*, *23*(5), 1069-1078. https://doi.org/10.1007/s12192-018-0916-z

Wang, Y., Yang, C., Elsheikh, N. A., Li, C., Yang, F., Wang, G., & Li, L. (2019). HO-1 reduces heat stress-induced apoptosis in bovine granulosa cells by suppressing oxidative stress. *Aging*, *11*(15), 5535-5547. https://doi.org/10.18632/aging.102136

Wang, Y., Chen, K., Li, C., Li, L., & Wang, G. (2018). Heme oxygenase 1 regulates apoptosis induced by heat stress in bovine ovarian granulosa cells via the ERK1/2 pathway. *Journal of Cellular Physiology*, *234*(4), 3961-3972. https://doi.org/10.1002/jcp.27169