Journal of Advances in Biology & Biotechnology

Volume 27, Issue 7, Page 1515-1522, 2024; Article no.JABB.119281
ISSN: 2394-1081

Impacts of Chronic Heat Stress on Lymphocytic Proliferation and Phagocytic Assay in Sahiwal and Tharparkar Cattle

Lata Kant [a], Priyanka M. Kittur [a], Susmita Majumder [a], Neha Rajawat [a], Hari Abdul Samad [a], Vikrant Singh Chouhan [a], Gyanendra Singh [a] and V.P. Maurya [a*]

[a] *Division of Physiology & Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh - 243122, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors GS, VPM and HAS conceived the study. Authors GS, VPM, VSC and HAS provided the resources. Author LK collected the samples and performed the experiments. Author HAS analyzed the data. Author LK written the original draft. Authors VSC and PMK edited the original draft. All authors discussed the results, edited the manuscript and approved the final version of the manuscript for publication. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i71114

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers,peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119281

Original Research Article

Received: 01/05/2024
Accepted: 04/07/2024
Published: 10/07/2024

**Corresponding author: E-mail: vpmaurya@rediffmail.com;*

Cite as: *Kant, Lata, Priyanka M. Kittur, Susmita Majumder, Neha Rajawat, Hari Abdul Samad, Vikrant Singh Chouhan, Gyanendra Singh, and V.P. Maurya. 2024. "Impacts of Chronic Heat Stress on Lymphocytic Proliferation and Phagocytic Assay in Sahiwal and Tharparkar Cattle". Journal of Advances in Biology & Biotechnology 27 (7):1515-22. https://doi.org/10.9734/jabb/2024/v27i71114.*

ABSTRACT

High temperatures cause many immune and physiological alterations in livestock, making them more vulnerable to a wide range of diseases. Lymphocyte proliferation assay and neutrophil phagocytic assay are frequently employed to evaluate cell-mediated immunity. The present study used in vitro culture of blood polymorphonuclear cells after animals were exposed to in vivo heat stress to examine the differing effects of heat stress on defensive responses in Sahiwal and Tharparkar calves. The objectives of this research were to contrast the thermo-adaptability of Sahiwal and Tharparkar breeds based on their immune responses. In the present study, ten male cattle aged between 1 to 1.5 years were selected and divided into two groups with five animals, each of Sahiwal and Tharparkar cattle. Animals were maintained inside a psychrometric chamber under the following conditions: 7 days acclimatization period at a thermo-neutral zone, 38°C temperature exposure for 6 hours up to 49 days, followed by seven days recovery period. Blood was collected once a week on the following days: -7, 0, 7, 14, 21, 28, 35, 42, 49 and 56. Physiological responses such as rectal temperature and respiration rate were measured daily. THI was calculated by temperature and relative humidity (RH). Following their isolation from blood, the polymorphonuclear cells were cultured at 38°C. Then, using NBT and MTT tests, respectively, phagocytosis and lymphocyte proliferation were assessed. The entire mean THI was significantly ($p< 0.5$) higher during heat exposure period (88.41± 1.54) when compared to the control period (64.75 ± 0.97). There is a significant high lymphocytic proliferation in Tharparkar Group when compared to Sahiwal Group cattle. Additionally, the level of PA significantly decreases during the heat exposure period when compared to control period in both the breeds and in Sahiwal group I there is a significant reduction in LPA during heat stress. The current study's findings imply that during the heat stress period, Tharparkar showed greater tolerance to heat stress than Sahiwal group.

Keywords: Cattle; heat stress; immune response; PMN; lymphocyte proliferation assay; phagocytic assay; Sahiwal; Tharparkar.

ABBREVIATIONS

LPA : Lymphocyte Proliferation Assay
PA : Phagocytic assay
PMN : Poly Morphonuclear Cells

1. INTRODUCTION

"Global warming directly impacts livestock welfare by increasing the incidence of heat stress. Dairy cows have been identified as the livestock species most sensitive to elevated temperatures and humidity due to their high metabolic heat load and therefore are quite susceptible to heat stress" [1]. "Acute heat stress may have a stimulatory effect on the immune system, however chronic heat stress may have an inhibitory role on the capacity of the immune system to maintain homeostasis" [2]. "Heat stress inhibits the expression of genes involved in T-cell activation and cytokine production, which compromises cellular immunological capabilities through the release of cortisol" [3]. "Heat stress is well documented to reduce dry matter intake (DMI) in cattle, which consequently has a negative influence on nutrient absorption overall impacting the immune system and inflammatory response of cattle" [4]. According to Dahl et al. [5], "heat stress has a detrimental impact on the health and productivity of dairy cattle. It also raises the risk of disease and renders the immune system". "Heat stress impacts the cellular immune response by increasing the cortisol concentrations, as it binds to DNA and suppresses the expression of genes related to cytokine production and T-cell activation" [6]. "The anti-inflammatory properties of corticosteroids result in a decrease in phagocytic cell activity and alter lymphocyte function" [7]. "Heat stress increases the secretion of glucocorticoids which acts as inhibitor of the pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 initiating the innate immune responses by the inhibition of the p38 MAPK pathway which maintains the stability of the immune system in animals" (Abraham et al. 2006). "Stimulation of the HPA axis results in the production of cortisol, which is associated with a suppression of the immune system in cattle" [8]. "Glucocorticoids influence the balance of T-helper 1 (Th1) and T-helper 2 (Th2) through the inhibition of IL-12, whereas catecholamines inhibit IL-12 and enhance IL-10 production" [9]. "Therefore, glucocorticoids and catecholamines may suppress cellular immunity and result in a preferential shift toward Th2-mediated HI" [10]. "These health-related issues could be due to the impaired immune function observed in heat-stressed dairy cattle. Heat stress can impact both the innate and adaptive arms of the immune system. The disruption of the balance between T-helper 1 (TH1) and T-helper 2 (TH2) responses is one of the impacts of heat stress on the adaptive immune response, causing a shift towards a TH2 response" [11]. "This bias can lead to impaired cell-mediated immune response (CMIR). In similar circumstances, it has been demonstrated that heat stress in dairy cattle decreases lymphocyte proliferation" (Cartwright et al., 2023). "Lymphocytes, which include B and T-cells, rapidly proliferate in response to invading pathogens in order to facilitate clearance. Accordingly, if lymphocyte proliferation is reduced it is more difficult for cattle to defend against invading pathogens. Several studies have assessed immunological changes in Holstein dairy cows under heat stress conditions. For example, the conditions associated with high temperature and dry environmental seasons have been found to affect immune responses, including reduction in lymphocyte proliferation, neutrophil, phagocytosis, and cytokine expression in Holstein dairy cows" [12,13]. "Moreover, heat-stressed Holstein cattle reportedly show reduced cellular immunity and enhanced humoral immune responses under prolonged heat stress" [14]. Prompt and appropriate action is required to protect native bovine genetic resources; failure to do so could cause irreversible harm to India's subsistence farming system. Nevertheless, there is a dearth of scientific data on immune system modification in heat-stressed environments, especially in the case of cattle. There hasn't been nearly enough fieldwork done on the subject of native cattle's capacity for adaptation, particularly to the harsh weather typical of a tropical country like India.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Site

The experiment involved 10 healthy Sahiwal and Tharparkar cattle of the same age group between 1 and 1.5 years maintained at, Indian Veterinary Research Institute, Izatnagar, UP, India. The institute is situated in a subtropical climate at a height of 564 feet above mean sea level, with latitudes of 28°21′N and longitudes of 79°24′E.

2.2 Experimental Design

Temperature and Relative humidity of the animal sheds were recorded daily.There were two different types of immunity tests used to assess the immunological status during the study period by using PBMCs and Neutrophils isolated from the blood within two hours of sample collection.

2.3 Blood Sampling

Sterilized vacutainers were used to collect blood samples (spray-coated lithium heparin and clot activators containing tubes) at the seven day interval and during the acclimatization period. During the fifty six days experiment period at 38°C, blood collection was done on day 0, 7, 14, 21, 28, 35, 42, 49, 56 days. On 56th day blood sample was collected as a recovery sample. The blood sample was collected immediately after the heat exposure period in each day.

2.4 Measurement of Meteorological Variable

The minimum temperature, maximum temperature, and relative humidity (RH) were recorded at the experimental shed daily during the experiment period using dry and wet bulb thermometers, and then the THI, a measure of thermal load on animals, was calculated from a formula by McDowell et al.,[15].

2.5 Assessment of Lymphocyte Proliferation Assay (LPA) and Phagocytic Assay (PA)

Peripheral blood mononuclear cells were isolated using Histopaque according to the method described by English and Andersen [16], and The lymphocyte proliferation and Phagocytic assays were done as per the method described by Khatti et al. [17]. The phagocytic assay (PA) and lymphocyte proliferation assay (LPA) were used to estimate the phagocytic activity of neutrophils and proliferation of lymphocytes in vitro respectively. Peripheral blood samples were subjected to hypotonic lysis of erythrocytes followed by isolation of neutrophils. The neutrophils were pipetted in RPMI 1640 (Sigma - Aldrich, USA) culture media containing 10 % FB S (Gibco). Cell viability was determined using trypan blue and coun ting was done thereafter cell concentration was adjusted to 10^5 -10^6 live cells/ml.

2.6 Lymphocyte Proliferation Assay (LPA)

After centrifugation of blood at 1000g for time period of 30 min; the buffy coat was collected and resuspended in phosphate buffer saline (PBS). The entire content was precisely layered on a lymphocyte separation medium in sterile 15ml polypropylene centrifuge tube and centrifuged at 700g for 30 min at room temperature Khatti et al., [17]. The layer rich in lymphocytes was removed and washed twice with PBS. The washed cells were then resuspended in RPMI 1640 culture media with supplementation of 10% FBS and antibiotics. The lymphocyte suspension was adjusted to 5×106 live lymphocytes per ml. A 96-well tissue culture plate with a flat bottom was filled with 200µl of diluted cell suspension per well, made in triplicate. The mitogen used for the study was concanavalin A (5µg/ml), which stimulated T lymphocytes. "During LPA, concanavalin A (5 µ g /ml), a mitogen was used to stimulate T lymphocytes, and B lymphocytes were stimulated by lipopolysaccharide (5 0 µ g /ml)" [18]."The cells were allowed to incubate with and without mitogen, to determine the difference in cell proliferation at 37°C in a humidified CO2 incubator (5% CO2 and 95% air) for 24 to 72 hours. The proliferative response of lymphocytes was estimated using the colourimetric MTT" [19]. Approximately 50µl/ml of MTT solution to each well was added after culturing lymphocytes for the standard incubation period. The further incubation of plates at 38.5°C for 4 hours in a humidified CO_2 incubator was done. This results in the formation of formazan crystals at bottom of each well during this period. The residual supernatant along with the suspended cells was pipette out without disturbing the forazman crystal layer. Then 100µl DMSO in each well was added and thoroughly mixed to dissolve the dark blue crystal. The optical density was read using ELISA reader in the dual-wavelength measuring system, at a test wavelength of 503nm and a reference wavelength of 630nm after incubating the plate at room temperature for 15 min. Plates were read within 1 hour of adding DMSO. Stimulation index (SI) or proliferation index is calculated by using the formula:

SI= Average OD with mitogen / Average OD without mitogen

2.7 Phagocytic Activity or Assay (PA)

Isolation of neutrophills from peripheral blood using Granulosep and Histopaque according to the methods described by English and Anderson [16] and modified by Khatti et al. [17]. The neutrophils cell suspension was adjusted to 1 to 5×10 live cells per ml by culture media containing 10% FCS and antibiotics. 200 µl of the diluted cell suspension per well, in triplicate, was added to a 96-well flat-bottomed tissue culture plate. Seeding was done at the rate of 20 0 µ L /well in triplicate. During PA , incubation neutrophils with Zymosan (650 µ g /ml) and Nitro blue Tetrazolium (NBT , 25 0 µ g /ml) at 37 °C [18] for 2 hours. The quantity of phagocytosed zymosan was utilized as a PA indicator. The OD values were taken at 540 nm in Absorbance reader (Bio - rad). In a humidified CO2 incubator (5% CO2 and 95% air) all cultures are allowed to incubate at a temperature of 37°C. The amount of zymosan which was phagocytozed was used as Phagocytic activity indicator. The yellow color of Nitro-blue tetrazolium changes to blue formazan after phagocytosis, which is measured spectrophotometrically [20]. OD was taken using ELISA reader at 540nm.

3. RESULTS AND DISCUSSION

Fig. 1 shows the changes in the temperature-humidity index (THI) over a period of different weeks. During the period of experiment, it was observed that THI was lowest during control period (25ºC) and was more during heat stress period (38ºC). During the period of heat stress, the overall mean THI was observed to be significantly ($p<0.05$) higher as compared to control period. The differences in lymphocyte proliferation and phagocytic activity index among the groups during summer were presented in Figs. 2 and 3, respectively. Both breeds when compared on heat exposure period showed a significant ($p<0.05$) decrease in value of LPA during heat stress (38 ºC) as compared to control period (25ºC). In both the breeds LPA values reduced significantly ($p<0.05$) up to 28 days of heat exposure and then started to increase. In case of Tharparkar cattle significant ($p<0.05$) decrease was observed at 21st day of heat stress whereas in Sahiwal significant ($p<0.05$) reduction was observed on 14th and 21st day of heat exposure. Both breeds when compared during heat exposure period (38ºC), a significant difference was observed on 21st, 35th and 42nd day of heat exposure.

While comparing both the breeds, a significant ($p<0.05$) decline in values of PA during heat stress (38^{o}C) as compared to control period (25^{o}C) was observed.Significant difference was observed between both on day 7 and 28 day. On comparing both the breeds, a significant ($p<0.05$) difference in phagocytic activity was observed on all the days of heat exposure period, whereas no significant ($p>0.05$) difference was observed in control period between both the breeds.

“In some cases, in vivo heat stress has been found to be associated with a reduction in the levels of neutrophil phagocytosis” [21]. LPA is used for assessment of the proliferation ability of peripheral blood mononuclear cells (PBMCs) in response to antigen stimuli like concanavalin A. Al-Busaidi et al., [22] reported “a significant increase in lymphocytes during summers as compared to winters in goats”. The decline in lymphocyte proliferation in reported by Elvinger et al., [23] in cows exposed to high temperatures. In the study conducted, it was observed that LPA value of both Tharparkar and Sahiwal breeds significantly ($p<0.05$) decreased during heat exposure as compared to control period.The reduction in LPA values was more evident in Sahiwal than Tharparkar breed during heat exposure period as compared to the control period. The observations were found to be similar with the observations of Mukherjee et al., [24] who reported, “during heat exposure in buffaloes there was a decrease in mitogen-induced lymphocyte proliferation response”.

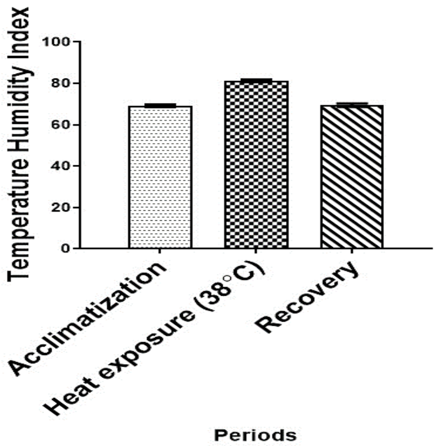

Fig. 1. Temperature Humidity Index (THI) before, during and after heat exposure (38°C). All the values are shown as Mean±SE

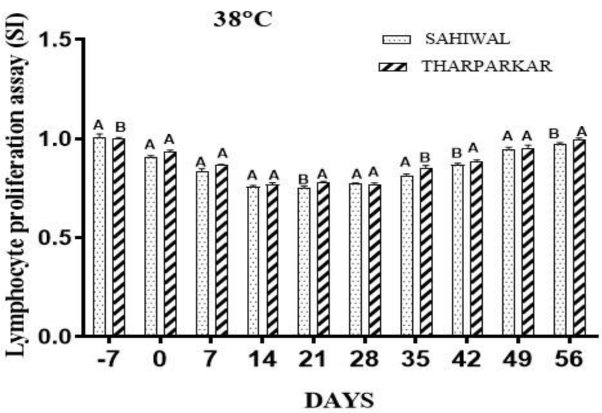

Fig. 2. Lymphocyte Proliferation Assay (SI) in serum of Sahiwal and Tharparkar breeds compared before, during and after heat exposure (38°C). Samples collected at -7 day, before heat exposure was taken as control. All the values are shown as Mean±SEM.Different superscripts denotes statistically different values (P<0.05)

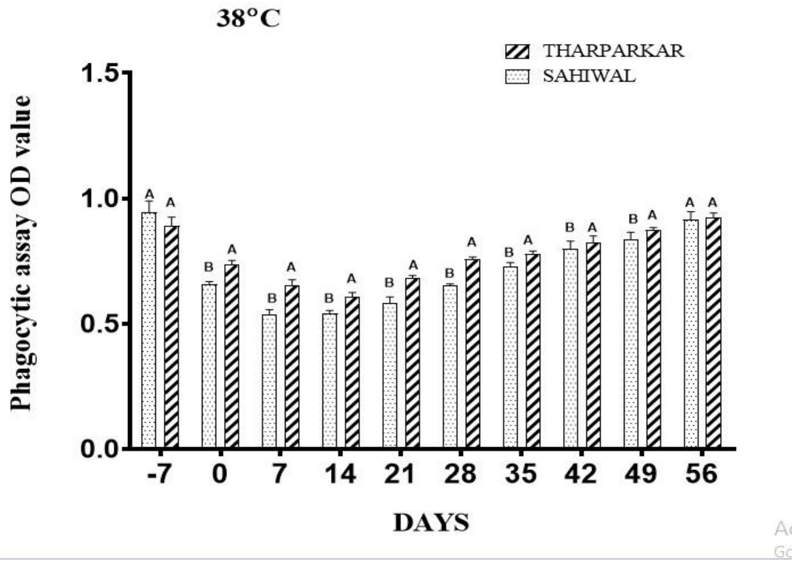

Fig. 3. Phagocytic Assay (OD value) in serum of Sahiwal and Tharparkar breeds compared before, during and after heat exposure (38°C). Samples collected at -7 day, before heat exposure was taken as control. All the values are shown as Mean±SEM.Different superscripts denotes statistically different values (P<0.05)

The initial line of defence against pathogen invasion by phagocytic cells is crucial. In-vitro assessment of phagocytosis by engulfment of substrate by phagocytic cells. According to Callahan et al. [25] "heat shock response is enhanced during heat stress and exerts anti-inflammatory action. In the experiment conducted, It was noted that phagocytosis rate was significantly ($p<0.05$) reduced in both Tharparkar and Sahiwal breed cattle during heat exposure (38ºC) as compared to control period (25ºC)". The results were found to be similar with Lecchi et al.,[26],Sgorlon [27].

4. CONCLUSION

In contrast to Tharparkar cattle, Sahiwal cattle exhibit decreased lymphocyte proliferation and phagocytic activity (LPA and PA), which explain the decline in immune cell reactivity and increased susceptibility to infections during heat stress. Furthermore, it can be inferred from the study that Tharparkar has higher heat stress resistance than Sahiwal.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICS APPROVAL

All the experiments, procedures, and protocols on animals were conducted following the approval of the Ethics Committee, Indian Veterinary Research Institute, No.F.1-53/2012-13/J.D(R).

DATA AVAILABILITY

The data will be made available and will be shared on reasonable request.

FUNDING

The funds were provided under NICRA project; award number: F.No.- BUD/REL/NICRA/2013-141 Recipient:Gyanendra Singh, Ph.D.

ACKNOWLEDGEMENTS

We thank and gratefully acknowledged the support and funding received from the National Initiative on Climate Resilient Agriculture (NICRA) and Indian Council of Agricultural Research (ICAR).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Das R, Sailo L, Verma N, Bharti P, Saikia J, Kumar R. Impact of heat stress on health and performance of dairy animals: A review.Veterinary World.2016;9(3):260.
2. Cantet JM, Yu Z, Ríus AG. Heat stress-mediated activation of immune–inflammatory pathways.Antibiotics.2021;10(11):1285.
3. Bagath M, Krishnan G, Devaraj C, Rashamol VP, Pragna P, Lees AM, Sejian V. The impact of heat stress on the immune system in dairy cattle: A review.Research in Veterinary Science.2019;126:94-102.
4. Yadav B, Singh G, Wankar A, Dutta N, Chaturvedi VB, Verma M.R. Effect of simulated heat stress on digestibility, methane emission and metabolic adaptability in crossbred cattle. Asian-australas. Journal of Animal Science. 2016;29(11):1585.
5. Dahl GE, Tao S, Laporta J. Heat stress impacts immune status in cows across the life cycle. Frontiers in Veterinary Science. 2020;7:116.
6. Caroprese M, Ciliberti MG, Annicchiarico G, Albenzio M, Muscio A, Sevi A. Hypothalamic-pituitary-adrenal axis activation and immune regulation in heat-stressed sheep after supplementation with polyunsaturated fatty acids. Journal of Dairy Science. 2014;97(7):4247-4258.
7. Caroprese M, Albenzio M, Bruno A, Annicchiarico G, Marino Rosaria, Sevi A. Effects of shade and flaxseed supplementation on the welfare of lactating ewes under high ambient temperatures. Small Ruminant Research. 2012;102(2-3):177-185.
8. Grandin T. Welfare problems in cattle, pigs, and sheep that persist even though scientific research clearly shows how to prevent them. Animals.2018;8(7):124.
9. Inbaraj S, Sejian V, Bagath M, Bhatta R. Impact of heat stress on immune responses of livestock: A review. Pertanika Journal of Tropical Agricultural Science. 2016;39(4).
10. Elenkov IJ, Chrousos GP, Wilder R.L. Neuroendocrine regulation of IL-12 and TNF-α/IL-10 balance: clinical implications. Annals of the New York Academy of Sciences.2000;917(1):94-105.
11. Cartwright SL, McKechnie M, Schmied J, Livernois AM, Mallard B.A. Effect of *In-vitro* heat stress challenge on the function of blood mononuclear cells from dairy cattle ranked as high, average and low immune responders. BMC Veterinary Research. 2021;17:1-11.
12. Do Amaral BC, Connor EE, Tao S, Hayen J, Bubolz J, Dahl GE. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. Domestic Animal Endocrinology. 2010; 38(1):38-45.
13. Do Amaral BC, Connor EE, Tao S, Hayen MJ, Bubolz JW and Dahl GE. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. Journal of Dairy Science. 2011;94(1): 86-96.
14. Lacetera N, Bernabucci U, Scalia D, Basiricò L, Morera P, Nardone A. Heat stress elicits different responses in peripheral blood mononuclear cells from Brown Swiss and Holstein cows. Journal of Dairy Science. 2006;89(12):4606-4612.
15. McDowell RE, Hooven NW, Camoens JK. Effect of climate on performance of Holsteins in first lactation. Journal of Dairy Science. 1976;59(5):965-971.
16. English D, Andersen BR. Single-step separation of red blood cells, granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. Journal of Immunological Methods.1974;5(3):249-252.
17. Khatti A, Mehrotra S, Patel PK, Singh G, Maurya VP, Mahla AS, Chaudhari RK, Das GK, Singh M, Sarkar M, Kumar H. Supplementation of vitamin E, selenium and increased energy allowance mitigates the transition stress and improves postpartum reproductive performance in the crossbred cow. Theriogenology. 2017;104:142-148.
18. Dang AK, Prasad S, De K, Pal S, Mukherjee J, Sandeep IVR, Mutoni G, Pathan MM, Jamwal M, Kapila S, Kapila R. Effect of supplementation of vitamin E, copper and zinc on the in vitro phagocytic activity and lymphocyte proliferation index of peripartum Sahiwal (Bos indicus) cows. The Journal ofAnimal Physiology and Animal Nutrition. 2013;97(2):315-321.
19. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55-63.

20. Sim Choi H, Woo Kim J, Cha YN, Kim C. A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. Journal o fImmunoassay and Immunochemistry. 2006;27(1):31-44.
21. Tejaswi V, Balachander B, Samad HA, Sarkar M, Maurya VP and Singh G. Assessment of heat stress induced alterations in polymorphonuclear (PMN) cell activity in native and crossbred cows. Journal of Applied Animal Research. 2020;48(1):549-552.
22. Al-Busaidi R, Johnson EH, Mahgoub O. Seasonal variations of phagocytic response, immunoglobulin G (IgG) and plasma cortisol levels in Dhofari goats. Small Ruminant Research. 2008;79(2-3):118-123.
23. Elvinger F, Hansen PJ, Head HH, Natzke RP. Actions of bovine somatotropin on polymorphonuclear leukocytes and lymphocytes in cattle. Journal of Dairy Science. 1991;74(7):2145-2152.
24. Mukherjee J, Pandita S, Huozha R, Ashutosh M. *In vitro* immune competence of buffaloes (*Bubalus bubalis*) of different production potential: Effect of heat stress and cortisol. Veterinary Medicine International. 2011;(1).860252.
25. Callahan TE, Marins J, Welch WJ and Horn JK. Heat shock attenuates oxidation and accelerates apoptosis in human neutrophils. Journal of Surgical Research. 1999;85(2):317-322.
26. Lecchi C, Rota N, Vitali A, Ceciliani F, Lacetera N. *In vitro* assessment of the effects of temperature on phagocytosis, reactive oxygen species production and apoptosis in bovine polymorphonuclear cells.Veterinary Immunologyand Immunopathology. 2016;182:89-94.
27. Sgorlon S, Colitti M, Asquini ELISA, Ferrarini A, Pallavicini A, Stefanon B. Administration of botanicals with the diet regulates gene expression in peripheral blood cells of Sarda sheep during ACTH challenge. Domestic Animal Endocrinology. 2012;43(3):213-226.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/119281