Impacts of heat stress on blood metabolic in different periods of lactation and pregnancy in Holstein cows

O A Saeed¹, B T Jaber¹, MTA Mohammed², U M Sani³, K S Ziara⁴ and H M Saad⁵

¹ Department of Animal Production, Faculty of Agriculture, University of Anbar, Anbar, Ramadi, Iraq
² Environment Research Centre, University of Technology, Baghdad, Iraq
³ Department of Animal Science, Faculty of Agriculture, Taraba State University, P.M.P. 1167, Jalingo, Nigeria
⁴ Quality Assurance &. Academic Accreditation Directorate. Ministry of Higher Education and Scientific Research, Baghdad, Iraq
⁵ Al Qaim Hospital, Anbar Health Director, Anbar, Iraq

E-mail: osama_anwr85@uoanbar.edu.iq

Abstract. Heat stress (HS) greatly affects sustainable livestock production, thereby affecting the productive performance and welfare of animals. The effect of summer heat on lactating Holstein cows at different lactation, pregnancy phases, and blood metabolism were investigated in this study. Eighteen Holstein cows were selected randomly and housed in the cow farm. Climatic reads and blood parameters were measured. The temperature-humidity index has been designed as a measure of animal comfort and is a simple combination of temperature and humidity. The study showed that heat stress has a significant effect (P ≤ 0.01) on hematological and biochemical parameters. Mean platelet volume (MPV) and red blood cell distribution width (RDW) were reduced significantly (P ≤ 0.01) during lactation. There was a significant increase in creatinine level at 45 days in milk. A significant inverse correlation between HS with milk production and feed intake. So far, it was concluded that certain hematological and biochemical parameters were substantially altered by HS. A negative effect of HS on milk production and feed intake was observed.

Keywords: Heat stress, blood, Holstein cows, milk production, feed intake

1. Introduction

Iraq has a semi-arid climate that is characterized by high summer temperatures. In the middle and southern regions, the average summer temperature ranges from 40 to 50 °C with an average of 45 °C within a shade [1]. With the intensification of the greenhouse effect and global warming, one of the most important factors affecting profitability in the production of the dairy industry has been heated stress (HS). In addition, demand for animal products is expected to increase due to the growth of the human population, higher incomes, and increased urbanization. The sustainability of the livestock production system is largely affected by climate change. An imbalance between the production of metabolic heat within the animal body and its dissipation into the environment results in HS under high air temperatures and humid climates [2]. In summer, the hyper-thermal environment causes HS to dairy cows and causes enormous losses to the economy of dairy production. During the summer, the reduced milk production and conception rate of dairy cows reflects the HS on milking cows [3]. Over the years, several studies were carried out on the metabolism of HS in dairy cows [4, 5]. Studies suggest a suitable ambient temperature range from 5 °C to 25 °C for dairy cows. When the ambient temperature exceeds 25 °C, the temperature-humidity index (THI) will be
higher than 72 which may cause decreased milk yield [4]. In order to control biological reactions and physiological processes associated with normal metabolism, homeotherms' effort to stabilize body temperature within fairly narrow limits is essential. An animal must be within thermal equilibrium in order to maintain homeothermic, which is further affected by factors such as radiation, air temperature, air movement, and humidity [6]. In blood flow, acid-base chemistry, and hormones, substantial physiological changes occur, such as the responses of cows to HS. Neurons are temperature sensitive and transmit information to the hypothalamus, which induces various physiological and behavioral responses in the bodies of cows [7]. Blood metabolic processes are critical for disease diagnosis, treatment, and prognosis. In order to determine the normal physiological status of animals and to assist in the assessment of management activities, diet, and health status, the determination of metabolic blood profiles, including biochemical parameters, is very important. Information on blood metabolic processes and their association with THI in the environment of Iraq dairy cattle is lacking. Therefore, this research was conducted to examine the effects of HS on the hematological, biochemical characteristics, milk production, and feed intake in Holstein cows.

2. Materials and Methods

2.1 Location of study
The experiment was conducted in Ramadi, Al-Anbar, Iraq (33° 25’ 14” N latitude, 43° 18’ 28” E longitude, and 35 - 450 m altitude). The climate is classified according to Köppen as a hot desert climate, alternating between hot (May-September) and cool (November-March), with annual temperatures of 22.4 °C. The study was carried out during the summer months of 2011 - 2013 (June - September) at the cow farm of the Faculty of Agriculture at the University of Anbar.

2.2 Experiment design
Eighteen healthy Holstein cows at different lactation phases and parity were used for the study. The study was carried out during the summer (June – September). The dairy cows were reared under common management and provided with shade. Cows were healthy with no signs of disease and free from internal and external parasites. The dairy cattle were in various stages of lactation and the study targeted three periods which are dry period (DP), a day after calving (DFC), and 45 days in milk (DIM). During this period, the lactating cows were milked twice a day at (06:00 h and 17:00 h). The cows were fed on a basal diet to meet the requirements according to [8] with a roughage to concentrate ratio of 40:60 (DM basis). Drink water and hay were ad libitum during the study period.

2.3 Data collection
About 10 ml of blood samples were harvested randomly from a jugular vein by a sterile disposable syringe of dairy cows between 9:00 – 11:00 hr. The collected blood was transferred into an EDTA tube (2 ml) and plain tube (8 ml) which’s kept at room temperature for three hours. Then the blood samples were centrifuged at 3000 rpm for 15 minutes and serum was collected in the Eppendorf tube by using a pasture pipette and stored at -20 °C until analysis. Platelet, red blood cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leucocyte values of whole blood were measured in a semi-automated blood analyzer (Shenzhen Mind Ray Biomedical Electronics Ltd). For the estimation of biochemical parameters, the serum was used to determine the concentration of each parameter using a UV Spectrophotometer (SEAC, Slim, Florence, Italy). Milk yield was recorded daily and averaged on a weekly basis, and then followed weekly until the end of lactation. The milk production was adjusted to energy-corrected milk (ECM) which was calculated using the formula of [9]:

$$\text{ECM (kg)} = (\text{Milk yield (L)} \times 1.0295 \times (376 \times \text{fat%} + 209 \times \text{protein%} + 948)) \div 3138$$
The amounts of offered feed and refused were recorded daily for cows for calculation of the daily feed intake.

The environmental data were measured, temperatures and relative humidity were recorded daily for the minimum temperature at (9:00 – 10:00 h) and maximum temperature at (13:00 – 15:00 h). The environmental data were collected from a meteorology station with a distance from the farm of about 100 m. The temperature-humidity index (THI) was calculated using the following formula:

\[ \text{THI} = (1.8 \times T + 32) - (0.55 - 0.0055 \times \text{RH}) \times (1.8 \times T - 26). \]

Where; RH was relative humidity expressed as a fraction of the unit and T was ambient temperature expressed as degrees Celsius [10].

2.4 Data analysis

Data were analyzed using one-way ANOVA with THI and blood parameters of the cow as the primary variables. Pearson correlation coefficients between THI with feed consumption and milk production have also been computed. Associations between the HS and the factors also were tested using a repeated-measures ANOVA model using PROC MIXED (SAS version 9.2., SAS Institute, Inc., Cary, NC) [11].

3. Results

3.1 Ambient temperature

The average ambient temperature for this study was ranged from 33.16 to 34.98 °C, while relative humidity was 19.30 to 20.76 % as shown in Table 1.

| Year  | Temperature (°C) | Relative Humidity (%) |
|-------|------------------|-----------------------|
|       | Mean  | Minimum  | Maximum  |                   |
| 2011  | 34.28 ± 1.71 | 26.97 ± 1.66 | 41.59 ± 1.97 | 20.79 ± 4.06 |
| 2012  | 34.98 ± 1.83 | 27.69 ± 1.90 | 42.37 ± 1.68 | 19.30 ± 3.51 |
| 2013  | 33.16 ± 2.80 | 25.81 ± 2.33 | 40.70 ± 3.33 | 20.76 ± 6.30 |

The THI averages were 78.08, 78.51, and 76.94 in summer (June 1 to September 30) for the years 2011, 2012, and 2013 respectively. Mean monthly maximum THI ranged from 69.73 to 83.78, with heat events reaching their peak from July 13 to August 03 with an average THI during the entire study period exceeding 75 (see Figure 1).
3.2 Blood metabolic

The hematological parameters of dairy cows were influenced by HS (Table 2). The data revealed a significant decrease (P ≤ 0.05) of MPV during DAC and DIM compared with the DP period. Furthermore, RDW was statistically influenced (P ≤ 0.001) by the summer season and showed an inverse pattern in a linear relationship with different THI. The results of MCHC and neutrophils showed a significant (P ≤ 0.05) increased in DIM for cows exposed to HS. Meanwhile, platelet, PDW, MCV, MCH, lymphocytes, and eosinophils did not show significant effects of the variations of THI in the summer season.

Table 2. Hematology characteristics of metabolic blood collected in the summer season.

| Variables                              | Period     | SEM  | P value |
|----------------------------------------|------------|------|---------|
| Platelet (10⁵/µL)                      | DP         | 292.01 | 146.63 | 216.83 | 26.90 | 0.1245 |
|                                       | DAC        | 15.05 | 12.12 | 12.70 | 0.54 | 0.0518 |
|                                       | DIM        | 11.35a | 8.51b | 8.958ab | 0.52 | 0.0467 |
| Platelet Distribution Width (%)       | DP         | 14.28b | 17.43a | 14.41b | 0.33 | 0.0001 |
|                                       | DAC        | 56.74 | 52.56 | 53.49 | 0.84 | 0.1052 |
|                                       | DIM        | 15.27 | 17.35 | 17.31 | 0.51 | 0.1468 |
| Mean Platelet Volume (fL)             | DP         | 26.75b | 32.95a | 32.34a | 0.86 | 0.0010 |
| Mean Platelet Volume (fL)             | DAC        | 6.75b | 16.24ab | 27.30a | 3.12 | 0.0069 |
|                                       | DIM        | 61.38 | 67.68 | 55.73 | 2.47 | 0.1895 |
| Mean Cell Hemoglobin (pg/dL)          | DP         | 28.75 | 24.56 | 25.15 | 1.169 | 0.3505 |

DP; dry period. DAC; day after calving. DIM; day in milk. Means with different superscripts within a row are significantly different at (P ≤ 0.05).

The mean values of enzyme activities in the blood serum of dairy cows during summer are shown in Table 3. The serum concentration of creatinine has significantly increased (P ≤ 0.0001) at 45 days in milk and declined at the other stage of lactation. The aspartate aminotransferase (AST) activities of dairy cows during the three lactation periods and in the dry period show significant differences. The results indicated an increase of AST (P ≤ 0.05) values in the blood serum of observed cows at the DAC and the declined after
45 days of calving under HS. No significant variation was found in the overall selected biochemical parameters in the study.

| Variables          | Period     | SEM  | P value |
|--------------------|------------|------|---------|
| Creatinine (mg/dL) | DP         | 0.51 | 0.55    | 0.82    | 0.03 | 0.0001 |
|                    | DAC        | 131.38 | 124.38 | 137.18 | 4.38 | 0.5212 |
|                    | DIM        | 5.62  | 6.76    | 4.82    | 0.39 | 0.1668 |
| Cholesterol (mg/dL)|            | 7.12  | 7.63    | 7.12    | 0.70 | 0.0428 |
| ALP (U/L)          |            | 8.25  | 8.19    | 6.92    | 0.71 | 0.6994 |
| AST (U/L)          |            | 8.11  | 7.90    | 7.71    | 0.21 | 0.7742 |
| ALT (U/L)          |            | 15.49 | 17.40   | 9.77    | 1.99 | 0.2976 |
| Total protein (g/dL)|          |       |         |         |      |        |
| Procalcitonin (ng/L)|          |       |         |         |      |        |

DP; dry period. DAC; day after calving. DIM; day in milk. ALP; Alkaline phosphatase. AST; Aspartate aminotransferase. ALT; alanine aminotransferase. *Means with different superscripts within a row are significantly different at (P ≤ 0.05).

3.3 Relation of THI with dairy cow’s performance
Pearson correlation was carried out to examine the relationships between THI, milk production, ECM, and feed consumption indexes in dairy cows (Table 4). The results revealed an inverse association between THI and milk production that was strongly important (P ≤ 0.01). However, milk production was positively correlated with feed consumption in the summer. Furthermore, the ECM showed the same finding with MP and appeared a significant correlation with THI.

| Variables | THI | MP | ECM | FC |
|-----------|-----|----|-----|----|
| THI       | 1.00| 0.22 | 0.22 | 0.09|
| MP        | 0.22 | 1.00 | 1.00 | 0.30 |
| ECM       | 0.22 | 1.00 | 1.00 | -0.30 |
| FC        | 0.09 | -0.30 | -0.30 | 1.00 |

THI, Temperature humidity index, MP; milk production, ECM; energy-corrected milk, FC; feed consumption. a P ≤ 0.05, b P ≤ 0.01.

4. Discussion
4.1 Ambient temperature
Several studies reported the detrimental effect of HS on dairy animal production and welfare [5, 12, 13]. In arid and semi-arid areas, the problem is even greater as the majority of those areas are faced in the long summer with higher intensity of radiant solar energy and low humidity which contribute to a limited thermal relief. Our meteorological results are consistent with data obtained from [14] who found that daily maximum temperatures can reach up to 45 °C in Iraq from May – September. In comparison, between years of study, the temperature, humidity, and THI of the environmental assessment were not different and the variations were below ± 7 °C for the years 2011 to 2013.

Meteorological data indicated that cows are exposed to heat stress that the cows as THI exceeded the critical limit of 72 THI during summer for dairy cows, the present study is in agreement with the previous study reported by [15]. The evaluating THI showed a consistent result in dairy cows which’s the optimal
ambient temperature range of 5 °C to 25 °C. It becomes more difficult for a cow to cool itself sufficiently as air temperature rises thereby exposing cows to HS. The THI values of 70 or less are considered normal. However, 75-78 exposed animals to stress and values greater than 78 causes extreme discomfort with reduced ability of lactating cows to retain thermoregulatory mechanisms or maintain body temperatures as reported by [16] and [17]. Interestingly, it is extremely important to recognize and diagnose the condition of HS correctly before the occurrence of thermal stress in an animal.

4.2 Blood metabolic
Pregnancy and lactation significantly affect the blood metabolic profile, hence, the variation in HS level reported during different physiological stages. This is because dairy cows consume less feed in order to maintain optimal body temperature during these stages. In the present study, the MPV levels significantly affected and decreased during lactation as compared to late gestation (DP). These results are inconsistent with the report of [18] who also found that by releasing larger platelets into the circulation, megakaryocytes try to respond to the low number of platelets, but a low MPV can mean that there is an insufficient number of megakaryocytes or that they do not respond. Regarding, changes in MPV size during HS can potentially act as an HS biomarker. This finding is in agreement with [19] findings, which showed heat-stressed cattle have decreased platelet count associated with increased body temperature. However, because of the effects of body temperature on leucocyte variables and erythrocyte size, RDW has shown potential for use as biomarkers of thermal stress. The RDW in the current study was significantly affected (P ≤ 0.001) by THI, in fact, the low RDW in heat-stressed cows may indicate a decreased erythrocyte population of varying sizes or greater similarity in erythrocyte size [20]. Increased concentrations of MCHC, as shown during the summer season is an indication of a cow's tolerance to the environment. The result of MCHC was supported by previous studies [19, 21]. The high neutrophils concentration in lactation and the low neutrophils level in the late gestation, in the present study during the summer, was due to the cell-mediated and humoral immunity changes that have a direct effect on immune competence, which can make an animal more vulnerable to infection [22]. This result is in agreement with [23] the value of neutrophils has increased in animal expose to HS.

Heat stress has important effects on the different biochemical features of blood in this study as confirmed by previous studies [7, 19, 23]. In the present study, creatinine levels in serum increased under high environmental temperatures at the peak of lactation (45th day in milk), it appears that this adaptation is connected to the lactating cow's metabolic response to the hot environment. These relationships may partly be explained by [16] who stated cow’s exposure to hot environments can stimulate thermoregulatory mechanisms and reduce metabolism, feed consumption, and production rates. This finding is in contrast to [24] who linked variations in creatinine level to the maternal mobilization of protein for fetal muscle development and the removal of organic fetal residues. However, a rise in enzyme activity is due primarily to the leaking of liver cytosol enzymes into the bloodstream, indicating liver damage and normal liver function intervention. The present study showed a higher concentration of AST during the summer season at DAC of cows which might be due to the oxidative stress of dairy cows. This result also agreed with [16] who reported an increased AST blood concentration is known to be one of the markers of postpartum hepatic steatosis. However, procalcitoninin is another essential aspect of systemic inflammation in Holstein cows during summer. It has been used to identify and track systemic inflammatory reactions associated with diseases. Our findings showed a non-significant effect of HS on the procalcitonin, this finding was also reported by [25]. However, other blood parameters are within the normal range reported for dairy cattle in different parts of the world.
4.3 Relation of THI with dairy cow’s performance

The present study found a significant inverse correlation between indicated THI with milk production and feed consumption. These results similar to [5, 26, 27] who reported that daily milk yield and feed intake are affected by THI. However, feed efficiency increases per kg of feed when the value of THI is increased. The present study found that ECM also correlates with THI as reported by [28].

5. Conclusion

During the hot summer months, the HS has a severe negative effect on lactating dairy cows and thus poses a threat to the sustainability of the dairy sector in arid and semi-arid regions with the rising frequency and intensity of high heat events in response to climate change. The present study investigates the cow’s response to various THI. Blood metabolic parameters of dairy cows revealed that cows undergo few metabolic changes at THI 72. There was no dramatic shift in these parameters, indicating temporary acclimatization in animals to preserve homeostasis in this range. Further research on understanding thermoregulation dynamics, especially tissue-specific changes under HS, is required.

References

[1] Jaradat A 2003 Agriculture in Iraq: Resources, potentials, constraints, research needs and priorities J. Food. Agric. Environ. 1(2):160-166.

[2] Das R, Sailo L, Verma N, Bharti P and Saikia J 2016 Impact of heat stress on health and performance of dairy animals: A review Vet. world. 9: p 260

[3] Guo J, Gao S, Quan S, Zhang Y, Bu D and Wang J 2018 Blood amino acids profile responding to heat stress in dairy cows Asian-Australas. J. Anim. Sci. 31: p 47

[4] Hao L, Wang J, Sun P and Bu D 2016 The effect of heat stress on the metabolism of dairy cows: Updates & review Austin J. Nut. Met. 3: pp 10363-1041

[5] Saeed O and Khalaf S 2014 Replacement of liquorice pulp in diets of Holstein-Friesian cows under heat stress conditions: 1. effect on body temperature, feed intake, milk production and milk components Al- Anbar J. Vet. Sci. 7: pp 33-42

[6] Paudel T P, Acharya B R, Karki D B and Shrestha B S 2018 Effect of heat stress on crossbred dairy cattle in tropical Nepal: impact on blood parameters J. Agric. Nat. Resour. 1: pp223-30

[7] Wang J, Li J, Wang F, Xiao J, Wang Y, Yang H, Li S and Cao Z 2020 Heat stress on calves and heifers: a review J. Anim. Sci. Biotechnol. 11: pp 1-8

[8] National Research Council 2001 Nutrient requirements of dairy cattle, seventh revised addition 2001 National Research Council

[9] Tyrrell H and Reid J 1965 Prediction of the energy value of cow's milk J. Dairy Sci. 48: pp 1215-23

[10] National Research Council 1971 A guide to environmental research on animals: Nat. Acad. Sci., Washington, DC.

[11] SAS 2000 SAS User’s Guide: Statistics Version 9 ed. SAST Institute Inc. North Carolina, USA

[12] Lees A M, Sejian V, Wallage A L, Steel C C, Mader T L, Lees J C and Gaughan J B 2019 The impact of heat load on cattle Animals 9: pp322

[13] Osei-Amponsah R, Dunshea F R, Leury B J, Cheng L, Cullen B, Joy A, Abhijith A, Zhang M H and Chauhan S S 2020 Heat stress impacts on lactating cows grazing Australian summer pastures on an automatic robotic dairy Animals 10: pp 869

[14] Ahmed E S and Hassan A S 2018 The impact of the extreme Air temperatures on the characteristics of Iraq weather Iraqi J. Sci. 59: pp1139-45

[15] Tao S, Dahl G E, Laporta J, Bernard J K, Orellana Rivas R M and Marins T N 2019 Physiology symposium: Effects of heat stress during late gestation on the dam and its calf J. Anim. Sci. 97: pp 2245-57
Mazzullo G, Rifici C, Lombardo S, Agricola S, Rizzo M and Piccione G 2014 Seasonal variations of some blood parameters in cow Large Anim. Rev. 20 : pp81-4

Liu J, Li L, Chen X, Lu Y and 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: pp1332

Kocatürk M, Yeşilbağ K and Yılmaz Z 2010 Evaluation of red blood cell and platelet indices in cattle naturally infected with bovine viral diarrhea virus (BVDV) Uludag Univ. J. Fac. Vet. Med. 29 :pp17-21

Habibu B, Dzenda T, Ayo J, Yaqub L and Kawu M 2018 Haematological changes and plasma fluid dynamics in livestock during thermal stress, and response to mitigative measures Livest. Sci. 214 : pp189-201

de Souza A M, Camargo M B, Bacellar D T L, Campos S D E, de Almeida Torres Filho R, de Alencar N X, de Souza Xavier M, de Barros Macieira D and Almosny N R P 2012 Age and sex influence in canine Red Cell Distribution Width (RDW-CV and RDW-SD) values Rev. Bras. Cienc. Vet. 19 : pp 90-93

Regmi B and Pande K 2018 Hemato-biochemical analyses of lactating cross-bred Jersey cattle at kaski district, Nepal J. Inst. Agric. Anim. Sci. 35 : pp243-7

O’Driscoll D N, Greene C M and Molloy E J 2017 Immune function? A missing link in the gender disparity in preterm neonatal outcomes Expert Rev. Clin. Immunol. 13: pp 1061-71

Morar D, Ciulan V, Simiz F, Moț T, Hutu I and Văduva C 2018 Effect of heat stress on haematological parameters in dairy cows Lucr. Știint., Ser. Med. Vet. 51: pp 65-70

Soares G, Souto R, Cajueiro J, Afonso J, Rego R, Macêdo A, Soares P and Mendonça C 2018 Adaptive changes in blood biochemical profile of dairy goats during the period of transition Revue. Méd. Vét. 169: pp 65-75

Neethirajan S 2020 Transforming the adaptation physiology of farm animals through sensors Animals 10: p 1512

Bouraoui R, Lahmar M, Majdoub A and Belyea R 2002 The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate Animal Res. 51: pp 479-91

Könyves T, Zlatkovic N, Memisi N, Lukac D, Puvaca N, Stojsin M, Halász A and Miscevic B 2017 Relationship of temperature-humidity index with milk production and feed intake of holstein-frisian cows in different year seasons Thai. J. Vet. Med. 47: p 15

Ouellet V, Cabrera V, Fadul-Pacheco L and Charbonneau É 2019 The relationship between the number of consecutive days with heat stress and milk production of Holstein dairy cows raised in a humid continental climate J. Dairy Sci. 102: pp 8537-45

Acknowledgment
The authors are grateful to the Agricultural College Dairy farm employees at the University of Anbar for their assistance with collecting samples. Also, we thank the diagnostic laboratory staff of the Faculty of Veterinary Medicine at the University of Baghdad for their technical assistance.