

Influence of Season and Pregnancy on Thermoregulation and Haematological Profile in Crossbred Dairy Cows in Tropical Environment

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Abstract: The objective of this study was to evaluate the effects of season (summer vs winter) and stage of pregnancy on thermoregulation and haematological responses of crossbred dairy cows (Holstein-Friesian X Zebu) under tropical conditions. The rectal temperature (T_r) and respiration rate (RR) were significantly higher during summer at all stages of pregnancy. There was significant increase in T_r and RR with the advance of pregnancy in summer. Summer was associated with significantly lower packed cell volume (PCV) and haemoglobin (Hb) concentration in non pregnant cows. The PCV and Hb concentration tended to increase with the advance in pregnancy course in both seasons. The total leukocyte count (TLC) was significantly higher only in early pregnancy in winter. Environmental and nutritional measures should be adopted for alleviation of environmental stress in dairy farms.

Key words: Dairy cows • Season • Pregnancy • Thermoregulation • Blood parameters

INTRODUCTION

Dairy cattle are exposed to stressful climatic conditions in tropical regions, which influence their productivity and welfare. The indigenous Zebu (*Bos indicus*) breeds are adapted and tolerant to the hot environments because of low metabolic rate and great sweating capacity [1,2], but generally they exhibit low productive and reproductive performance [3]. Crossbreeding of cattle has been adopted for blending the adaptability of tropical cattle with the high milking potential of exotic breeds. However, the local environment can sustain only composite genotypes of a moderate level of *Bos taurus* blood [4]. Friesian crossbreds were noted to be the most suitable for their adaptability in addition to their high milking capacity. Adaptation to tropical environment has been reduced when the local Butana and Kenana cattle were crossbred with high producing, non-adapted Holstein-Friesian breed. Furthermore, selection for high milk yield was reported to reduce the heat tolerance of cows which magnifies the seasonal depression in productivity caused by climatic stress [5,6]; accordingly, high yielding cows are more sensitive to hot environment compared to low yielding cows [7,8].

Exposure of dairy cows to hot environment during summer could stimulates thermoregulatory mechanisms

and produces reduction in the rates of metabolism, feed intake and productivity [9]. Heat stress influences reproduction and represents a major factor for lower fertility during summer in tropical environments. It reduces the length and intensity of oestrus [10, 11]. The competence of oocyte for fertilization and subsequent development is reduced during times of the year associated with heat stress [12, 13] and depression in conception rate in hot environment has been documented [14-16].

The blood constituents may reflect the influences of thermal stress and the physiological state of dairy cows. Assessment of PCV and Hb concentration was included in metabolic profile test amongst other biochemical constituents of blood [17-19]. Haemoconcentration may occur during hot weather. Toharmat *et al.* [20] reported an increase in PCV value and haemoglobin concentrations during summer, that could be associated with dehydration. Stressors including high ambient temperature which increase the level of glucocorticoids are linked to lower cellular immunity in animals [21]. A higher susceptibility to infections has been observed in cows suffering from heat stress [22]. However, reports on the effects of heat stress on the cell-mediated immunity of bovines are conflicting [23]. Additionally, stress-induced immunosuppression may be inhibited by pregnancy [24].

Research is needed to evaluate the physiological performance of dairy cows under tropical conditions, so that the patterns of responses to climatic stress and physiological state can be established. Although the changes in indices as rectal temperature and respiration rate are ultimate indicators of heat stress, such data are not available for cattle in the tropics. The information obtained can be utilized in adopting environmental control and nutritional strategies that can alleviate stress and improve reproductive performance and milk yield. The findings could also be used to monitor the health status of cows. Therefore, the present study was undertaken to assess the effects of seasonal change in thermal environment and stage of pregnancy on thermoregulation and some haematological variables of crossbred dairy cows under local tropical conditions.

MATERIALS AND METHODS

Animals and Management: Clinically healthy, multiparous crossbred (Butana X Friesian) dairy cows, aged 5-8 years were used in the study. The cows were selected from the herd of the governmental dairy farm at Khartoum North (15° 36' N; 32° 35' E). The animals were generally maintained on the normal grazing programme under natural summer and winter conditions. Animals were kept in shaded pens located close to the milking parlor, with appropriate facilities for feeding and watering. The non-grazing nutritional regimen comprised two types of feeds: roughages and concentrates. There were two types of roughages: alfalfa (*Medicago sativa*) and Abu 70 (*Sorghum bicolor*). The lactating cows were fed supplemental concentrate mixture twice daily, before milking at 8.00 a.m. and at 11.00 p.m. The roughages were offered twice daily at 10.00 a.m. and 5.00 p.m. Table 1 shows the nutrient composition of the two types of roughage and the concentrate consumed by cows during the experimental period. All animals had free access to tap water during the course of the study.

Experimental Plan: The effect of season (summer vs winter) and physiological status of dairy cows on thermoregulation and blood constituents were assessed. In each season, 24 cows were assigned to 4 groups of 6 each: a group of non pregnant cows served as control and 3 groups represented, early, mid-and late pregnancy. The baseline data were obtained for all experimental groups of animals. Then the rectal temperature (Tr) and respiration rate (RR) were measured and blood samples were collected weekly at 9.00 to 11.00 a.m. for 9 weeks in each season. The haematological parameters were determined immediately after blood sampling.

Table 1: The nutrient composition of roughages and concentrate (g/kg)

Ingredients	Alfalfa	Abu 70	Concentrate
	(<i>Medicago sativa</i>)	(<i>Sorghum bicolor</i>)	
Dry matter	230	280	952
Crude protein	46.1	19.2	29.25
Crude fibre	70.0	108.0	35.3
Ether extract	3.3	8.4	3.99
Ash	28.2	23.9	7.88

Climatic Conditions: The climatic data were obtained from the Meteorological Department of the Ministry of Science and Technology; the mean values of ambient temperature (Ta) and relative humidity (RH) recorded during the experimental period were calculated. The temperature-humidity index (THI) was computed using the equation described by Ravagnolo *et al.* [25]:

$$THI = (1.8 \times T + 32) - [(0.0055 \times RH) \times (1.8 \times T - 26)]$$

Where: T = Air temperature (°C)

RH = Relative humidity (%)

Rectal Temperature (Tr) and Respiration Rate (RR): The measurements of Tr of cows were made to the nearest $\pm 0.1^\circ\text{C}$ using a certified digital thermometer. The thermometer was inserted into the rectum to a depth of approximately 8 cm for one minute before the reading was obtained. RR was measured visually by counting the flank movement with the aid of a stopwatch. The value was taken for one minute of regular breathing with the animal standing quietly.

Blood Collection and Analysis: Blood samples were collected aseptically by venipuncture from the jugular vein using disposable syringes and were immediately transferred to test tubes containing Na₂-EDTA as anticoagulant. The samples were used for haematological measurements using standard methods [26]. The packed cell volume (PCV) was measured in plain capillary tube using a microhaematocrit centrifuge (Hettich-Germany). The haemoglobin concentration (Hb) was determined by cyanmethaemoglobin technique using Drabkin's solution. The total leukocyte count (TLC) was performed in an improved Neubauer haemocytometer using Turk's solution dilution fluid.

Statistical Analysis: The experimental data obtained for 18 weeks covering two seasons (summer and winter) at different stages of pregnancy in dairy cows have been subjected to standard methods of statistical analysis. The mean values and standard deviations (Mean $\pm$ SD) were calculated and the analysis was performed using General

Linear Methods (GLM) procedure of Statistical Analysis System [27]. Analysis of variance (ANOVA) test was used to evaluate the effects of season and stage of pregnancy on thermoregulation and haematological parameters. The differences are considered statistically significant at P value < 0.05 .

RESULTS

Climatic Conditions: The ambient temperature (Ta), relative humidity (RH) and temperature-humidity index (THI) prevailing during the experimental period are shown in Table 2. The data indicate that the highest mean values of Ta ($^{\circ}\text{C}$) were measured in June in dry summer while the minimum mean value was recorded during December in winter. The minimum mean value of RH (%) was measured in June, during dry summer, whereas higher mean value was recorded in March during winter. The THI value during summer (80.92) was markedly higher compared to the value obtained in winter (65.75).

Thermoregulation: Table 3 shows the effects of season and stage of pregnancy on rectal temperature (Tr) in dairy cows. Tr was significantly higher during summer for both non pregnant ($P<0.05$) and pregnant cows at different stages of pregnancy ($P<0.001$). During summer, cows in mid-and late pregnancy had significantly ($P<0.001$) higher Tr values compared to early pregnancy and non pregnant cows. However, during winter, there was no significant increase in Tr with the advance of pregnancy.

Table 2: The mean values of ambient temperature, Ta ($^{\circ}\text{C}$), relative humidity, RH (%) and temperature-humidity index (THI) during the experimental period

	Ta ($^{\circ}\text{C}$)			RH (%) (Mean)	THI (Mean)
	Max.	Min.	Mean		
Summer	40.3 $\pm$ 3.0	25.7 $\pm$ 2.3	32.9 $\pm$ 1.7	43.4 $\pm$ 19.8	80.92
Winter	27.6 $\pm$ 2.5	14.8 $\pm$ 1.9	21.3 $\pm$ 1.9	33.1 $\pm$ 5.3	65.75

Table 3: Effects of season and stage of pregnancy on rectal temperature, T_r ($^{\circ}\text{C}$) in dairy cows

Group	Season		LS
	Summer	Winter	
A	^A 38.35 $\pm$ 0.31 ^a	^A 38.13 $\pm$ 0.67 ^b	*
B	^A 38.48 $\pm$ 0.24 ^a	^A 38.19 $\pm$ 0.48 ^b	***
C	^B 38.71 $\pm$ 0.46 ^a	^A 38.17 $\pm$ 0.64 ^b	***
D	^B 38.81 $\pm$ 0.46 ^a	^A 38.30 $\pm$ 0.48 ^b	***
LS	***	NS	

A: Empty cows; B: Early pregnancy; C: Mid pregnancy; D: Late pregnancy

^{A,B}: Mean values within the same column with different superscripts (capital) are significantly different.

^{a,b}: Mean values within the same row with different superscripts (small) are significantly different.

*: $P<0.05$ ***: $P<0.001$ NS: Not significant

Table 4: Effect of season and stage of pregnancy on respiratory rate, RR (breaths/min) in dairy cows

Group	Season		LS
	Summer	Winter	
A	^A 40.92 $\pm$ 4.90 ^a	^A 33.83 $\pm$ 2.44 ^b	***
B	^A 41.33 $\pm$ 5.30 ^a	^A 34.23 $\pm$ 2.24 ^b	***
C	^A 42.83 $\pm$ 5.90 ^a	^A 34.03 $\pm$ 2.43 ^b	***
D	^B 45.37 $\pm$ 6.20 ^a	^B 35.03 $\pm$ 2.42 ^b	***
LS	***	*	

A: Empty cows; B: Early pregnancy; C: Mid pregnancy; D: Late pregnancy

^{A,B}: Mean values within the same column with different superscripts (capital) are significantly different.

^{a,b}: Mean values within the same row with different superscripts (small) are significantly different.

*: $P<0.05$ ***: $P<0.001$

Table 5: Effect of season and stage of pregnancy on PCV (%) in dairy cows.

Group	Season		LS
	Summer	Winter	
A	^A 28.54 $\pm$ 3.79 ^a	^A 30.96 $\pm$ 4.13 ^b	**
B	^B 32.27 $\pm$ 3.45 ^a	^B 31.87 $\pm$ 3.08 ^a	NS
C	^B 34.25 $\pm$ 2.26 ^a	^B 33.88 $\pm$ 4.79 ^a	NS
D	^C 35.56 $\pm$ 2.89 ^a	^C 36.08 $\pm$ 4.33 ^a	NS
LS	***	***	

A: Empty cows; B: Early pregnancy; C: Mid pregnancy; D: Late pregnancy

^{A,B,C}: Mean values within the same column with different superscripts (capital) are significantly different.

^{a,b}: Mean values within the same row with different superscripts (small) are significantly different.

** : $P<0.01$ ***: $P<0.001$ NS: Not significant

Table 6: Effect of season and stage of pregnancy on haemoglobin concentration, Hb (g/dL) in dairy cows

Group	Season		LS
	Summer	Winter	
A	^A 11.39 $\pm$ 2.00 ^a	^A 11.41 $\pm$ 2.84 ^b	*
B	^A 11.99 $\pm$ 2.04 ^a	^A 11.48 $\pm$ 1.17 ^a	NS
C	^B 12.99 $\pm$ 1.56 ^a	^B 12.61 $\pm$ 1.90 ^a	NS
D	^C 13.46 $\pm$ 1.32 ^a	^C 13.36 $\pm$ 1.41 ^a	NS
LS	***	***	

A: Empty cows; B: Early pregnancy; C: Mid pregnancy; D: Late pregnancy

^{A,B,C}: Mean values within the same column with different superscripts (capital) are significantly different.

^{a,b}: Mean values within the same row with different superscripts (small) are significantly different.

*: $P<0.05$ ***: $P<0.001$ NS: Not significant

Table 7: Effect of season and stage of pregnancy on total leukocyte count, TLC ($\times 10^3/\mu\text{L}$) in dairy cows.

Group	Season		LS
	Summer	Winter	
A	$^{\text{A}}8.41 \pm 1.30^{\text{a}}$	$^{\text{A}}8.79 \pm 2.11^{\text{a}}$	NS
B	$^{\text{A}}8.42 \pm 1.27^{\text{a}}$	$^{\text{B}}9.69 \pm 2.17^{\text{b}}$	***
C	$^{\text{A}}8.66 \pm 1.37^{\text{a}}$	$^{\text{A}}8.63 \pm 2.38^{\text{a}}$	NS
D	$^{\text{A}}8.25 \pm 1.37^{\text{a}}$	$^{\text{A}}7.92 \pm 2.989^{\text{a}}$	NS
LS	NS	**	

A: Empty cows; B: Early pregnancy; C: Mid pregnancy; D: Late pregnancy

^{A,B}: Mean values within the same column with different superscripts (capital) are significantly different.

^{a,b}: Mean values within the same row with different superscripts (small) are significantly different.

: $P < 0.01$ *: $P < 0.001$; NS: Not significant

Table 4 shows that the cows maintained significantly ($P < 0.001$) higher respiration rate (RR) during summer for all physiological states investigated. The cows had significantly higher RR values during late pregnancy in summer ($P < 0.001$) and winter ($P < 0.05$).

Haematological Values: Tables 5 and 6 show that non pregnant cows had significantly lower PCV (< 0.01) and Hb concentration (< 0.05) values in summer. In both seasons, there was a significant ($P < 0.001$) increase in PCV and Hb concentration with the advance of pregnancy. Table 7 shows that the cows had significantly ($P < 0.01$) higher total leukocyte count (TLC) in winter only for early pregnancy state.

DISCUSSION

This study evaluated the effects of seasonal change in thermal environment and pregnancy on thermoregulation and some haematological parameters in crossbred dairy cows. The climatic data (Table 2) indicate that the cows were exposed to higher thermal load during tropical summer. The THI value during summer (80.92) induced high heat stress; the value during winter (65.75) is considered to be comfortable to dairy cows. The THI is usually used as an indicator of thermal stress in animals. Heat stress in dairy cows is considered to be cool when THI values are lower than 72. Index values higher than 72, 78, and 88 reflect the potential for mild, high and severe levels, respectively [6]. Kendall *et al.* [28] indicated that the THI threshold for lactating dairy cows indicating the first physiological response to thermal stress was THI: 67. Although THI is simplistic, it is currently the best heat

stress indicator for cattle, and can account for up to 78% of an animal's response to the climatic environment [29]. The high THI reported during summer in the current study may compromise the success of pregnancy in cows. The pregnancy rate of dairy cows declined from 55 to 10% when the THI increased from 70 to 84 [30]. Other studies [31, 32] reported that in dairy cows, the conception rates were lower during summer months compared to inter.

Seasonal change in thermal environment influenced thermoregulation in dairy cows. The effects of hot environment are associated with change in heat balance, and accordingly higher values of Tr and RR were measured under hot summer conditions (Tables 3 and 4). In line with the present results, other researchers have reported that the body temperature of cows in summer was higher than that in winter [13, 15, 33]. Berman *et al.* [34] suggested that the upper limit of ambient temperature at which Holstein cattle may maintain a stable body temperature is 24-26°C and that above 26°C, practices should be instituted to minimize the rise in body temperature. The thermal load on cows subjected to heat stress is the sum of heat gained from the environment and heat generated by metabolic processes. Previous studies indicate that respiratory rate (RR) is positively correlated with ambient temperature [35] and THI [36], so that RR may be used as an indicator of heat stress in cattle [37]. However, Kadzere *et al.* [38] noted that the body temperature of dairy cattle shows great susceptibility to thermal load and it is considered as a sensitive indicator of thermal stress.

The results indicate that pregnancy influenced the thermal responses of dairy cows. The increase in rectal temperature (Tr) and respiration rate (RR) with the advance of pregnancy, particularly during summer (Tables 3 and 4) is associated with increase in metabolic heat production and confirms findings reported by other researchers [39,40]. Furthermore, prior studies also indicated that extra heat production that occurs during pregnancy is primarily foetal in origin and the foetus loses its heat via foetal circulation and uterine wall [41,42]. Hormones play a major role in thermoregulation during pregnancy; secretion of adrenocortical hormones and sex hormones may increase basal metabolic rate (BMR) during mid-and late pregnancy, which influences thermoregulation of the mother [43]. Secretion of progesterone during pregnancy increases the sensitivity of respiratory centre to CO_2 which influences pulmonary ventilation. The increase in RR during pregnancy could also be related to the fact that pregnancy leads to limited excursion of the diaphragm; this is compensated by

increase in respiratory frequency. The more pronounced pattern of thermal responses of cows with advance of pregnancy in summer indicates additive effects of gestation and environmental temperature.

The PCV and Hb concentration showed significantly higher values in winter for empty cows. However, no significant differences were observed between summer and winter in different stages of pregnancy (Tables 5 and 6). This finding is in general agreement with Toharmat and Kume [44]; they did not find significant difference in PCV and Hb between hot and cool environment. The current result, however, disagrees with the findings reported by Toharmat *et al.* [20] which demonstrated increases in PCV and Hb concentration in summer. The observed decrease in PCV and Hb concentration in non-pregnant cows during summer could be attributed to haemodilution and is in general agreement with the results of Lee *et al.* [45]. Heat stress influences energy and water metabolism [46] and it may induce increases in plasma and extracellular fluid volume in proportion to the thermoregulatory requirement [38]. The decline in PCV and Hb in heat-stressed animals could be attributed to haemolysis, haemadilution and /or to reduction in cellular O₂ requirements to minimize metabolic heat production [47]. The decrease in haematological indices of empty cows during summer could also partly be associated with a decline in food intake.

The haematological indices were influenced by pregnancy and thermal environment. Pregnancy influenced PCV and Hb concentration in dairy cows. In both seasons, the PCV and Hb concentration showed increase with the advance of pregnancy (Tables 5 and 6). In Holstein-Friesian cows, Hb decreased with ongoing lactation and pregnancy, but increased several weeks before parturition [18]. Our results for crossbred cows are in general agreement with other reports in cows in the tropics [48,49]. Similar results were also reported in sheep [50,51]. The increase in Hb concentration during pregnancy may be associated with erythrocyte release from the spleen due to the stress of pregnancy [52]. Also it could be related to changes in erythrocyte stimulating factor (ESF) release which is controlled by the relationship between oxygen demand of tissues and the amount of oxygen carried by blood [26].

The irregular pattern of total leukocyte count (TLC) observed in the current study (Table 7) could be attributed to the fact that the TLC is affected by many internal and external factors which may not induce similar effects under farm conditions. However, previous studies

in sheep [51, 53] reported an increase in TLC at parturition associated with neutrophilia and lymphopenia. For early pregnancy data, the TLC was significantly higher in winter compared to respective summer value (Table 7). However, previous reports [45,54, 55] indicated that high ambient temperature evoked leukocytosis in cattle.

In conclusion, the results indicate that crossbred dairy cows are exposed to climatic stress during tropical summer conditions. Although cattle may have the ability to adapt physiologically and cope with environmental stress, necessary measures should be adopted to minimize the adverse effects during summer.

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