

Postpartum endocrine activities, metabolic attributes and milk yield are influenced by thermal stress in crossbred dairy cows

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Abstract This study was conducted on 30 freshly parturated multiparous crossbred dairy cows possessing three levels of Holstein Friesian genetic makeup (62.5, 75.0, and 87.5%). Data on temperature humidity index (THI) were classified into comfortable (≤ 71), mild stress (72–79), moderate stress (80–89), and stressful (≥ 90) zone. Results showed that serum cortisol concentration increased significantly ($P < 0.05$) in cows during stressful condition irrespective of genetic makeup compared to the other zones. Daily milk yield (DMY) was significantly ($P < 0.05$) lower in cows during stressful condition. Triglyceride was significantly higher in cows with genetic makeup 87.5% compared to the others, while total serum protein was significantly ($P < 0.05$) higher in cows during both moderate and stressful conditions. The mean concentration of cortisol and protein increased linearly from comfort to the stressful condition, while mean serum triglyceride, glucose, progesterone (P_4), and luteinizing hormone (LH) decreased by moving from comfort to stressful conditions. Results also indicated that higher cortisol level in higher grade crossbred cows was adversely associated with LH concentration and milk yield under thermal stress conditions. Greater triglyceride in high-grade crossbred (87.5%) cows indicates higher fat mobilization reflecting a negative energy balance. We concluded that heat stress increased blood cortisol and protein, and reduced milk yield in dairy cows irrespective of the genetic makeup. In addition, there was no significant difference in blood

metabolites and daily milk yield in the different levels of genetic makeup cows.

Keywords Cows · Milk yield · Blood metabolites · Stress

Introduction

Under subtropical condition, dairy cows are exposed to stressful climatic conditions associated with extended period of high temperature and solar radiation. Crossbred cows have been exploited for blending the adaptability of tropical cattle with high milking potential (Bello et al. 2009). The adaptation of dairy cows to tropical environment has been reduced, as the local genetically adapted cattle are crossed with high-producing environmentally nonadopted Holstein Friesian breed of European origin (Kim and Rothschild 2014). Genetic improvement that enhances production traits might increase the susceptibility to high thermal loads as there is a close relationship between metabolic heat generation and the level of production (Kadzere et al. 2002).

Among the stresses, heat is one of the major concerns in tropical and subtropical countries (Silanikove et al. 1997). Different climatic factors such as temperature, humidity, and their combination pose a potential threat to the growth and production of domestic animals. High ambient temperatures accompanied by high humidity cause an additional discomfort and enhance the stress level, which in turn result in depression of the physiological and metabolic activities of the animals (Collier et al. 2006; Hansen 2009). In dairy farming, it is desirable to achieve a calf per year and obtain a maximum milk yield per life of a cow. This can be achieved by early resumption of ovarian activity during postpartum period; however, the exposure

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of dairy cows to the summer heat stress suppresses the ovarian activity (Wolfenson et al. 2000).

In Pakistan, most of the farmers are rearing crossbred dairy cattle having an exotic blood level of 50%, 75%, and above. These dairy cows are the major contributors towards the milk production in irrigated rural and periurban areas of Pakistan. The present study was, therefore, planned to find the effect of thermal stress on blood metabolites and milk yield in a subtropical environment of Pakistani in graded crossbred cows.

Materials and methods

This work was approved by The Departmental Committee of Ethics and Experimental Protocol, Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

Selection of animals

A total of 30 freshly parturated, clinically fertile, multiparous Holstein Frisian dairy cows possessing three levels of inheritance, i.e., 62.5, 75.0, and 87.5%, were selected for the study. Animals were fed seasonal fodders in two meals after routine milking in addition to casual grazing for a short period of 1–2 h. The basal ration of winter and spring included green berseem and/or oats mixed with wheat straw. In summer and autumn, the herd was fed green maize/sorghum-Sudan hybrid (Sadabahar) or Mott grass. Concentrate was provided at the rate of 1 kg per each 2 l of milk produced by a cow. The ingredients included cotton seed cake, wheat bran, and maize oil cake in equal ratios.

Animals were housed in barns during day-time and in open yard at night in summer and autumn. Contrarily, cows remain housed in barns at night and allowed free in open yards during day-time in winter. Free access to fresh drinking water was provided during all seasons. Cows were hand milked twice daily at equal intervals.

Temperature humidity index

The climatic data were compiled from the metrological station located approximately 3 km from the research station. The State Dairy Farm is located in rural areas of Peshawar valley (34.0117° N latitude, 71.5389° E Longitude) 306 m above the sea level and characterized as subtropical climate.

The effect of ambient temperature and relative humidity was expressed as temperate humidity index (THI). The relative humidity and air temperature varied over different months of the experimental period. The mean values of ambient temperature and relative humidity recorded during the experimental period were used to calculate the THI

according to the following formula (Ravagnolo and Misztal 2000).

$$\text{THI} = (1.8 \times T \pm 32) - [(0.0055 \times \text{RH}) \times (1.8 \times T - 26)]$$

where

T air temperature (C°)

RH relative humidity (%)

The THI from July to January was divided into four zones as suggested by Frank Wiersama (1990), Department of Agriculture Engineering, The University of Arizona, Tucson. Comfort = ≥ 71 ; mild stress = $\geq 72-79$; moderate = 80–89; stressful ≥ 90 . The changes in the pattern of THI are presented in Fig. 1.

To know the trend of the data in different zones, a trend line was added for different parameters as shown in Figs. 1, 2, 3, 4, 5, 6, and 7. The equation obtained for different parameters are as follows:

$$\text{Temperature humidity index: } y = -7.3036x + 111.36, R^2 = 0.976$$

$$\text{Blood cortisol: } y = 2.195x^2 - 3.991x + 48.365, R^2 = 0.9978$$

$$\text{Blood protein: } y = -0.0675x^2 + 0.8785x + 7.1375, R^2 = 0.9773$$

$$\text{Blood triglyceride: } y = 0.295x^2 - 2.891x + 80.575, R^2 = 0.9343$$

$$\text{Blood glucose: } y = 1.645x^2 - 12.415x + 100.79, R^2 = 0.9978$$

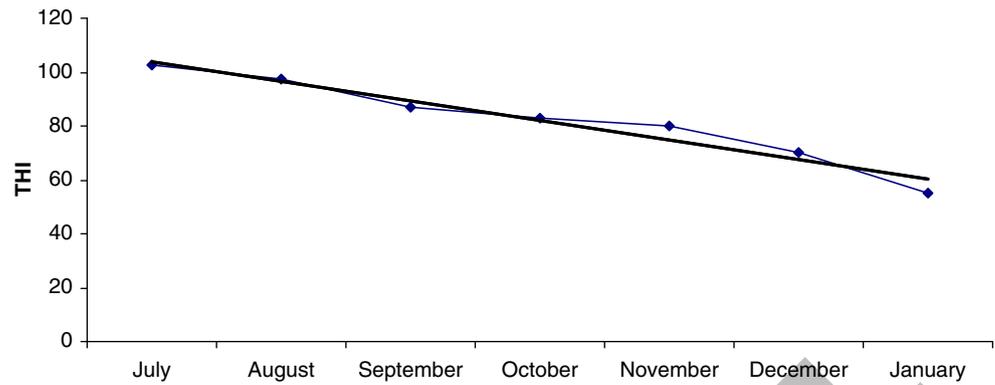
$$\text{Blood progesterone: } y = -0.02x^2 - 0.014x + 0.735, R^2 = 0.9762$$

$$\text{Blood luteinizing hormone: } y = -0.0025x^2 - 0.7045x + 5.7425, R^2 = 0.8067$$

Blood sampling and metabolites determination

Blood samples of 10 ml were collected aseptically from each cow from the jugular vein using disposable syringes during each period. The blood samples were immediately brought to the laboratory to separate serum through centrifugation at 2000 rpm for 15 min. The separated serum was stored at -10 °C for subsequent laboratory analysis. Serum progesterone (P_4), cortisol, and luteinizing hormone (LH) concentration were analyzed with the help of commercially available kits (Amzinx Microlisa TM kits) using an ELISA reader (IRMECO model, U2020). Serum glucose, triglycerides, and total protein concentration were determined through a spectrophotometer using kits (AMEDA Laboradiagnostik GmbH).

Fig. 1 Changes in temperature-humidity index (THI) during the trial period in various stress zones. A linear trend line has been inserted for mean THI values



Statistical analysis

Data was subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Statistical Analysis System Institute, Inc. (SAS 2002). Means of each treatment were compared by using least significant difference (LSD) and significance was determined at $P < 0.05$.

Results

Serum cortisol concentration in crossbred dairy cows under different zones is given in Table 1. Serum concentration was significantly ($P < 0.05$) high during stressful conditions in cows irrespective of their genetic makeup. The cows did not vary in cortisol concentration irrespective of zones (Table 2); however, the interaction effect of THI \times exotic blood level was found significant ($P < 0.05$) in crossbred cows (Table 3). Mean cortisol concentration in crossbred dairy cows increased linearly from comfort zone to stressful thermal zone (Fig. 2).

Mean values of serum concentration of protein in crossbred dairy cows at different levels of THI are presented in Table 1. Significant difference ($P < 0.05$) was found in protein concentration in crossbred dairy cows in different climate zones. The data showed an increasing trend in protein concentration when

the animals moved from comfort to the stressful condition (Fig. 3). The interaction effect of THI \times exotic blood level (Table 3) was found nonsignificant ($P > 0.05$).

Mean values of triglyceride under various THI zones are given in Table 1. The level of triglyceride concentration in blood decreased linearly (Fig. 4) from comfort to stressful zone but the decrease was statistically nonsignificant ($P > 0.05$). The level of triglyceride concentration was highly significant ($P = 0.001$) among different grades crossbred dairy cows (Table 2). However, the interaction effect of THI \times exotic blood level was found nonsignificant ($P > 0.05$) as shown in Table 3.

No significant effect of THI was observed on glucose concentrations ($P > 0.05$) in cows irrespective of their genetic makeup (Table 1). However, the glucose concentration was slightly higher in the comfort zone and decreased gradually from comfort to mild stress and stressful climatic condition (Fig. 5). The exotic groups also did not vary ($P > 0.05$) in glucose concentrations under various THI zones (Table 3). However, a nonsignificantly decreasing pattern was observed in glucose concentrations with the increasing THI level from comfort to stressful zone.

Mean values of serum progesterone concentration were not significantly different ($P > 0.05$) among different thermal zones (Table 1). However, the progesterone concentration at

Fig. 2 Overall changing pattern of serum cortisol concentrations (ng/ml) in crossbred dairy cows under various THI zones

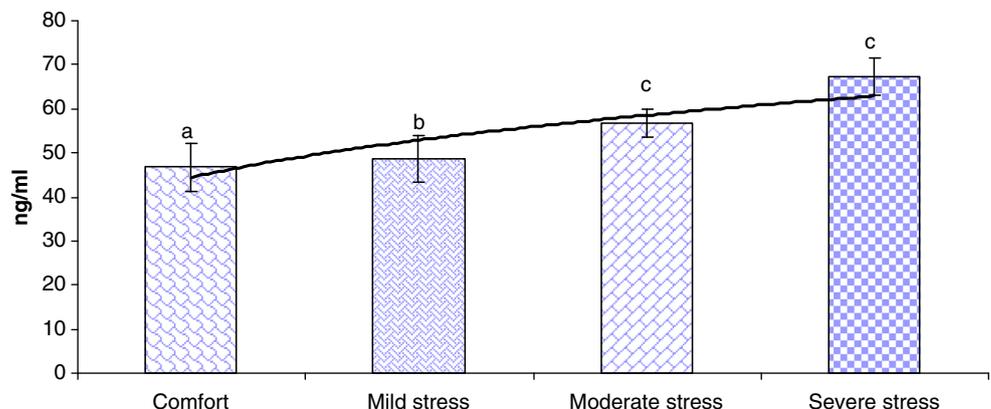
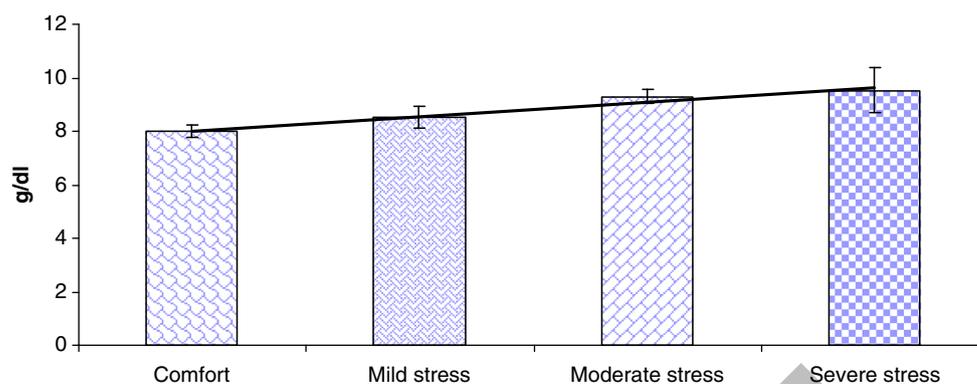


Fig. 3 Overall changing pattern of serum protein concentrations (g/dl) in crossbred dairy cows under various THI zones



comfort zone was markedly higher than stressful THI (Fig. 6). The progesterone concentration at mild stress and moderate THI zones were almost similar. Similarly, Tables 2 and 3 reveal that P4 concentration was not significantly different ($P > 0.05$) in different crossbred cows. However, P4 level in blood of HF 87.5% was quite higher as compared to HF 62.5%.

Mean LH concentration in blood of crossbred dairy cows is given in Table 1. The results showed that THI level has no significant effect on the serum LH concentration ($P > 0.05$). However, LH concentration linearly decreased from comfort zone to stressful zone (Fig. 7). Table 3 shows that LH concentration of crossbred cows was not statistically ($P > 0.05$) different; however, the maximum LH concentration was observed in HF 75% followed by HF 62.5%. This reveals a nonsignificant decrease in LH concentration from 62.5 to 87.5% group cows. The interaction effect of the THI \times exotic blood level was also found not significant (Table 3).

Mean value of daily milk yield (DMI) at different THI zones is given in Table 1. Daily milk yield varied significantly ($P < 0.05$) among different THI zones. The results showed that there was a continuous decrease in DMY with THI from comfort to stressful zone. The milk yield was higher but similar in comfort and mild stress. There was no significant ($P > 0.05$) difference in DMY among all exotic blood level groups indicating that cows inheriting different levels of exotic blood

were affected almost equally by heat stress (Table 1). The interaction effect of THI \times exotic blood levels was found significant ($P < 0.05$) (Table 3). The HF 87.5% genetic group produced more daily milk followed by HF 75% and HF 62.5% crossbred dairy cows in comfort and stressful climatic condition.

Discussion

Long-term stress, like thermal stress, during tropical and subtropical summer, results in an elevated level of corticotropin. This usually happens due to the increased hypothalamo-pituitary-adrenal axis activity, characterized by activation of corticotropin releasing factor (CRF) and arginine vasopressin (AVP) neuron in the para-ventricular nucleus and secretion of these neuropeptides into hypophyseal portal system (Tilbrook et al. 2000). The corticotrophs produce pro-opiomelanocortin including adrenocorticotrophic hormone (ACTH), endorphin, and melanocyte-stimulating hormone in response to heat stress (Engler et al. 1989). The ACTH acts on the cortex of adrenal gland and stimulates the secretion of glucocorticoids (cortisol) to produce higher concentration of cortisol in cows to maintain milk production (Abilay et al. 1975).

In the current study, blood cortisol level was significantly high during the stressful condition. The blood cortisol level is

Fig. 4 Overall changing pattern of serum triglycerides concentrations (mg/dl) in crossbred dairy cows under various THI zones

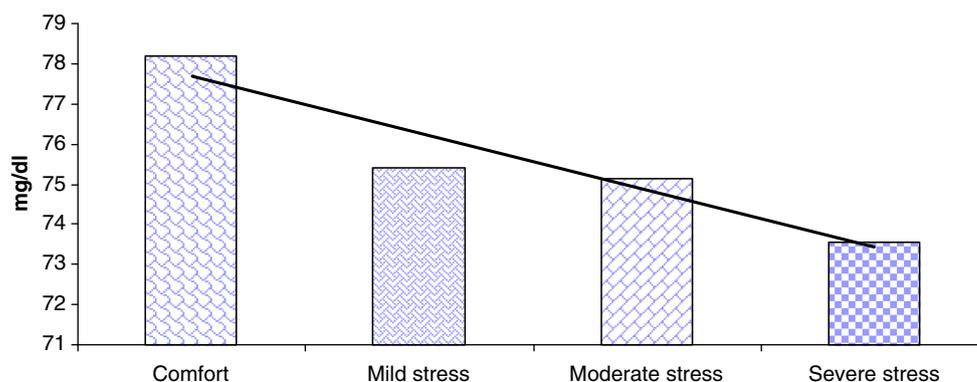


Fig. 5 Overall changing pattern of serum glucose concentrations (mg/dl) in crossbred dairy cows under various THI zones



generally considered as a reliable physiological index for determining animal response to stress as indicated by assessment of glucocorticoid under a variety of thermal conditions (Abilay et al. 1975). The rise in cortisol level during thermal stress in the present study is in agreement with the findings of Bouraoui et al. (2002) who reported higher plasma cortisol level in dairy cows in summer compared to the spring. Other research workers reported that heat stress was associated with an increase in blood cortisol level and ACTH (Dobson and Smith 2000; Silanikove 2000).

Different trials reported variable results in serum protein concentration in cows exposed to heat stress. Habib et al. (2007) reported that despite a significant increase in concentration of heat shock protein, the heat stress induced marked decrease in total protein concentration. Yousef (1990) and Elmasry and Marai (1991) noted an increase in protein level in colder part of the year. Chronic heat stress was claimed to be responsible for the significant decrease in protein concentration in Friesian cows during the experimental period. Similar results were found by Verma et al. (2000) in Murrah buffaloes. Marai et al. (1997) and Wolfenson et al. (2000) also reported a decrease in protein concentration during summer in lactating cattle. In contrast, Podar and Oroian (2003) reported that the elevated environmental stress is a potential cause of increased serum protein concentration in lactating dairy cows as shown in this experiment. Raghavan and Mullick (1961) reported little variation in serum protein concentration in buffalo during

spring and summer season. Rasooli et al. (2004) found a significant increase in total protein in the hot summer in nonpregnant Holstein Heifers. The increase in serum protein could be a physiological attempt to maintain extended plasma volume.

Our findings revealed an insignificant reduction in the serum triglyceride level in crossbred dairy cattle under different THI zones. The cows having higher levels of exotic blood were more affected by THI in this experiment. This response may relate to decrease in dry matter intake, or changes in endocrine status ultimately influence the lipid metabolism (Drackley et al. 2003). The lower triglyceride level could also be associated with reduced voluntary food intake during heat stress as reported by other research workers (Nardone et al. 1997). From the present findings of triglyceride, it can be inferred that cows having higher exotic blood level experience reduction in feed intake under subtropical environmental condition.

Unlike this study, several other workers found an increase in glucose concentrations. Nessim (2004) reported an increase in glucose concentration in buffaloes upon exposure to acute heat. Chaiyabuter et al. (1987) reported an increase of 20% in glucose concentration when exposed to acute heat. The response of heat stress in Friesian cows has a decreasing effect on plasma glucose under THI 85.9 for 10–18 h (Ronchi et al. 2001). The increase in plasma glucose in response to heat stress is probably due to the increased plasma water concentration and glucose mobilization. Thus, increased plasma

Fig. 6 Overall changing pattern of serum progesterone concentrations (ng/ml) in crossbred dairy cows under various THI zones

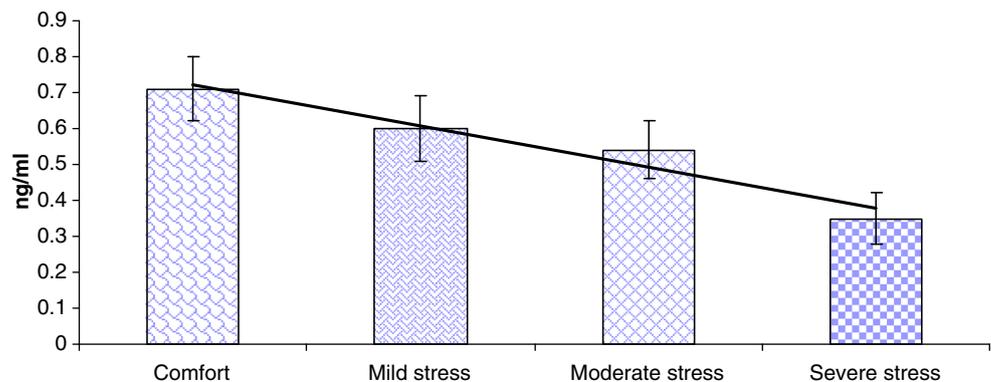
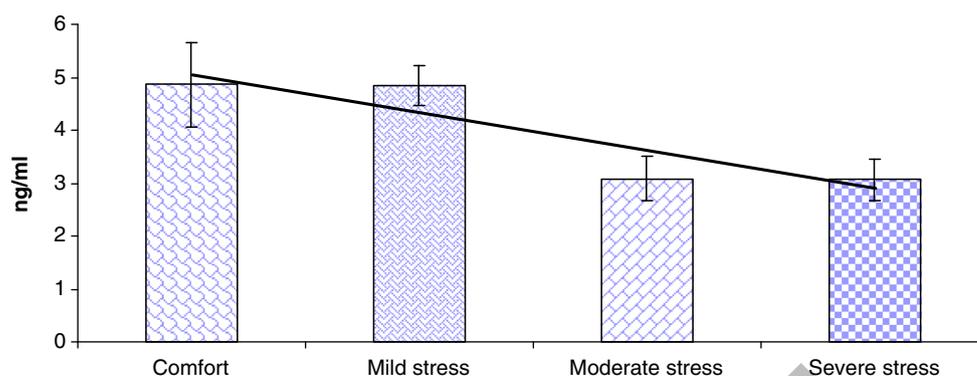


Fig. 7 Overall changing pattern of serum LH concentrations (ng/ml) in crossbred dairy cows under various THI zones



insulin level is the subsequent effect of elevated glucose concentration during heat stress. The decreased level of glucose concentration in blood in the thermally stressed animals is probably due to the increased respiration rate causing glucose combustion in the respiratory muscle. The significant decrease in feed intake is another contributing factor to the heat-induced drop in glucose contents. Furthermore, these may cause a hyperglycemic effect which results in hypersecretion of glucocorticoids. Glucose concentration in blood decreases during the hottest period of the day (Cincovic et al. 2010) mainly due to the reduced feed intake, altered gluconeogenesis, and increased insulin level in the blood (O'Brien et al. 2010; Rhoads et al. 2010).

Qureshi et al. (2002) reported a lower level of P_4 concentrations in the off season, particularly during summer month in buffaloes. Several other research workers also reported reduced P_4 concentration due to the heat stress (Howell et al. 1994; Ronchi et al. 2001). In contrast, an increased level of P_4 has been reported by others due to thermal stress (Vaught et al. 1977). A few other researchers reported no change in P_4 level when cows were subjected to heat stress (Guzeloglu et al. 2001). Serum concentration of P_4 decreases during summer month in dairy cows (Wolfenson et al. 2000) and goats (Ozawa et al. 2005). Progesterone concentration reduces under chronic heat

stress as evident by in vitro studies of the luteal cells (Ahmad et al. 1995). The drop in P_4 level is obvious from the above studies because the reproductive function is disturbed during the summer. According to Roth et al. (2001), the decrease in heat stress raises the P_4 level. Furthermore, the plasma P_4 level determined by the difference between the rate of luteal production and the rate of hepatic metabolism is affected by changes in dry matter intake (Khodaei-Motlagh et al. 2011). Therefore, the decrease in P_4 level in the present study may be attributed to small luteal secretion, higher hepatic clearance, and decreased feed intake.

The results of the present study revealed that the pattern of LH concentration was not significantly affected under different thermal zones. However, the pattern of LH concentration decreased linearly from the comfort to stressful zone in crossbred dairy cows. Similarly, the LH production was not affected significantly in various graded crossbred dairy cows at different THI. It seems that higher cortisol level adversely affected LH concentration under thermal stress. Similarly, response of cortisol is considered as an inhibitor of LH in the bovine species (Khodaei-Motlagh et al. 2011). Different research workers also reported low concentration of LH in peripheral blood circulation following heat stress in dairy cattle (Wise et al. 1988; Gilad et al. 1993; Lee 1993). In heat-stressed cows, decrease in LH pulse amplitude (Gilad et al.

Table 1 Mean \pm SE values of daily milk yield, blood metabolites, and reproductive and stress hormones at different climatic zones based on maximum THI

THI Zone	Cortisol (ng/ml)	Protein (g/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Progesterone (ng/ml)	LH (ng/ml)	DMY (l)
Comfort	46.74 \pm 5.39 c	7.99 \pm 0.25 b	78.17 \pm 2.02	90.12 \pm 7.83	0.71 \pm 0.13	4.86 \pm 1.06	9.64 \pm 1.02 a
Mild stress	48.65 \pm 8.38 c	8.50 \pm 0.68 b	75.40 \pm 5.47	82.22 \pm 4.44	0.60 \pm 0.09	4.85 \pm 0.36	8.98 \pm 0.44 a
Moderate stress	56.66 \pm 3.22 b	9.29 \pm 0.25 a	75.13 \pm 3.38	78.66 \pm 3.27	0.54 \pm 0.28	3.08 \pm 0.42	7.75 \pm 0.33 b
Stressful	67.35 \pm 8.44 a	9.53 \pm 0.85 a	73.54 \pm 1.62	77.34 \pm 2.33	0.35 \pm 0.13	3.06 \pm 0.39	5.07 \pm 0.94 c
<i>P</i> value	0.047	0.024	0.641	0.611	0.643	0.149	0.049

Mean values with different lowercase letters in a column differ significantly ($P < 0.05$). Comfort \leq 71 THI; mild stress = 72–79 THI; moderate stress = 80–89 THI; severe stressful \geq 90

DMY daily milk yield, LH luteinizing hormone

Table 2 Changes in cortisol, blood metabolites, and reproductive and lactation parameters with increasing exotic blood levels in dairy cows (mean \pm SE)

Exotic blood level	Cortisol (ng/ml)	Protein (g/dl)	Triglyceride (g/dl)	Glucose (g/dl)	Progesterone (ng/ml)	LH (ng/ml)	DMY (l)
62.5%	48.55 \pm 4.48	8.35 \pm 0.21	72.37 \pm 1.55 ^c	79.39 \pm 3.43	0.50 \pm 0.09	4.43 \pm 0.54	8.37 \pm 0.45
75%	48.61 \pm 3.80	8.38 \pm 0.11	77.33 \pm 2.67 ^b	78.89 \pm 2.90	0.61 \pm 0.13	4.50 \pm 0.38	8.84 \pm 0.37
87.5%	50.89 \pm 5.40	8.90 \pm 0.13	78.09 \pm 2.28 ^a	79.01 \pm 2.07	0.89 \pm 0.19	3.45 \pm 0.35	9.71 \pm 0.52
<i>P</i> value	0.379	0.435	0.001	0.862	0.338	0.675	0.285

DMY daily milk yield, LH luteinizing hormone

1993) and frequency (Wise et al. 1988) has been previously reported. It is inferred that during summer stress, the dominant follicle is developed under low blood LH, which results in reduced estradiol secretion leading to poor expression of estrus.

Cortisol is the major stress hormone and released in response to certain stress conditions such as environment (thermal, oxidative) nutrition, overcrowding, transportation, and rough handling which activate hypothalamus-pituitary adrenal cortical axis (HPA) (Minton 1994). Gluconeogenesis and activation of anti-stress and anti-inflammatory pathways are considered as the primary function of cortisol. However, elevated level of cortisol, if continues for a longer period, leads to proteolysis and loss of condition unlike other stresses.

Our results are in agreement with the findings of several other reports in which the crossbred dairy cows reduced milk production under subtropical environment (Barash et al. 1996; Kimothi and Ghosh 2005). The current data suggest that crossbred cows have probably partially adapted over a period

to thrive and perform normally under mild and moderate climatic conditions.

The reduction in feed intake is linearly and negatively correlated with thermal stress. The reduction in productivity due to thermal load is attributed to the loss of balance between metabolic heat production and heat loss. The result of the present data revealed that cows having more exotic blood were more sensitive to thermal stress. It is generally accepted that dairy cattle of *Bos taurus* are most sensitive to high ambient temperature than *Bos indicus* cows (Blackshaw and Blackshaw 1994). The primary manifestation of heat tolerance is the depressed metabolism and subsequently lower milk yield.

The adaptation of dairy cows to tropical and subtropical environment has been reduced since local cattle are crossed with high-producing nonadapted exotic breeds of European origin. The genetic improvement in local cows has enhanced the production traits; however, their susceptibility to high thermal stress has increased since there is an inverse relationship

Table 3 Difference in cortisol, blood metabolites, and reproductive and lactation parameters in different grades of crossbred cattle under varying levels of thermal humidity index (mean \pm SE)

THI zones	Blood level	Cortisol (ng/ml)	Protein (g/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Progesterone (ng/ml)	LH (ng/ml)	DMY (l)
Comfort ^a	62.5%	35.76 \pm 8.84 d	5.10 \pm 0.39	82.10 \pm 11.58	91.77 \pm 11.28	0.54 \pm 0.13	7.16 \pm 2.13	8.73 \pm 0.7 a
Mild stress	62.5%	40.20 \pm 4.15 c	8.17 \pm 0.37	78.59 \pm 4.06	90.52 \pm 9.18	0.48 \pm 0.14	4.86 \pm 0.76	7.35 \pm 0.67 b
Moderate	62.5%	44.00 \pm 3.25 b	8.17 \pm 0.36	74.56 \pm 2.41	79.21 \pm 5.08	0.46 \pm 0.31	2.90 \pm 1.39	7.28 \pm 0.66 b
Stressful	62.5%	48.17 \pm 10.39 a	10.18 \pm 0.96	54.89 \pm 2.08	75.10 \pm 4.77	0.20 \pm 0.01	2.87 \pm 0.69	6.00 \pm 0.64 c
Comfort ^a	75%	45.05 \pm 9.87 c	8.23 \pm 0.45	89.25 \pm 5.35	90.73 \pm 12.92	0.69 \pm 0.16	5.26 \pm 0.52	8.94 \pm 0.59 a
Mild stress	75%	45.48 \pm 7.32 c	8.36 \pm 0.39	75.24 \pm 2.75	84.11 \pm 5.91	0.64 \pm 0.20	3.58 \pm 1.15	7.59 \pm 1.71 b
Moderate	75%	51.19 \pm 5.14 b	8.73 \pm 1.0	70.85 \pm 2.00	79.49 \pm 15.80	0.38 \pm 0.65	2.65 \pm 1.35	6.74 \pm 0.45 c
Stressful	75%	64.25 \pm 4.55 a	9.01 \pm 2.37	68.42 \pm 5.75	74.95 \pm 3.41	0.23 \pm 0.10	2.58 \pm 0.15	6.50 \pm 1.51 c
Comfort ^a	87.5%	62.47 \pm 10.66 c	7.21 \pm 0.38	90.22 \pm 5.69	82.49 \pm 3.41	1.12 \pm 0.32	3.82 \pm 0.51	9.75 \pm 0.75 a
Mild stress	87.5%	74.00 \pm 3.70 b	9.28 \pm 0.56	86.95 \pm 11.88	80.92 \pm 2.95	0.65 \pm 0.41	3.27 \pm 1.15	9.41 \pm 0.94 a
Moderate	87.5%	73.81 \pm 6.65 b	9.82 \pm 0.8	73.63 \pm 2.48	80.15 \pm 2.93	0.62 \pm 0.19	2.91 \pm 0.76	6.40 \pm 0.71 b
Stressful	87.5%	80.93 \pm 12.47 a	11.34 \pm 2.74	71.51 \pm 2.83	72.73 \pm 4.44	0.60 \pm 0.30	2.65 \pm 1.35	4.00 \pm 1.16 c
<i>P</i> value		0.03	0.43	0.12	0.08	0.71	0.09	0.02

Mean values with different lowercase letters in a column differ significantly ($P < 0.05$)

DMY daily milk yield, LH luteinizing hormone

^a Comfort \leq 71 THI; mild stress = 72–79 THI; moderate stress = 80–89 THI; stressful \geq 90

between metabolic heat generation and the level of production (Kadzere et al. 2002). Selection of cows for milk yield reduces the thermoregulation ability during heat stress, which increases the depression in the productivity during heat stress (AL-Katanani et al. 1998; Chaiyabutr et al. 2008).

Cows in the subtropical region suffer the most during summer causing a drop in milk production, immunity, and reproductive efficiency (Cincovic et al. 2010). The heat stress adaptability of tropical cattle has been linked to their low feed intake and metabolic rates. The secondary factor is the heat dissipation mechanism, where the local cows sweat vigorously. The loose skin attachment and light reflection due to coat color and covering are considered another reason for the elevated heat tolerance.

Conclusion

We concluded that blood cortisol and protein were significantly high and milk yield were adversely affected during summer stress irrespective of the genetic makeup. In addition, there was no significant difference in blood metabolites and daily milk yield in cows with different levels of genetic makeup.

Compliance with ethical standards This work was approved by The Departmental Committee of Ethics and Experimental Protocol, Faculty of Animal Husbandry & Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

References

- Abilay TA, Johnson HD, Madam M (1975) Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycles. *J Anim Sci* 58:1836
- Ahmad N, Schrick FN, Butcher RL, Inskoop EK (1995) Effect of persistent follicles on early embryonic losses in beef cows. *Biol Reprod* 52:1129–1135
- Al-Katanani YM, Webb DW, Hansen PJ (1998) Factors affecting seasonal variation in non-return of lactating dairy cows. *J Dairy Sci* 81(Suppl):217 Abstract. 53–56
- Barash H, Silanikove N, Weller JI (1996) Effect of season of birth on milk, fat, and protein production of Israeli Holsteins. *J Dairy Sci* 79:1016–1020
- Bello AA, Rwuan JS, Voh AA Jr (2009) Some factors affecting postpartum resumption of ovarian cyclicity in dairy cattle. *Nig Vet J* 30:17–25
- Blackshaw JK, Blackshaw AW (1994) Heat stress in cattle and the effect of shade on production and behaviour: a review. *Aust J Exp Agric* 34:972–985
- Bouraoui R, Lahmar M, Majdoub A, Djemali M, Belyea R (2002) The relationship of temperature humidity index with milk production of dairy cows in a Mediterranean climate. *Anim Res* 51: 479–491
- Chaiyabutr N, Buranakarl C, Muangcharoen V, Loypetjra P, Pichaicharnarong A (1987) Effects of acute heat stress on changes in the rate of liquid flow from the rumen and turnover of body water of swamp buffalo (*Bubalus Bubalis*). *J Agric Sci* 77:549–553
- Chaiyabutr N, Champongsang S, Suadsong S (2008) Effect of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *Int J Biometeorol* 52: 575–585
- Cincovic MR, Belic B, Stevancevic M, Lako B, Toholj B, Potkonjak A (2010) Diurnal variation of blood metabolite in dairy cows during heat stress. *Contemp Agric* 59(4):300–305
- Collier RJ, Dahl GE, Vanbaale MJ (2006) Major advances associate with environmental effect on dairy cattle. *J Dairy Sci* 89:1244–1253
- Dobson H, Smith R (2000) What is stress and how does it affect reproduction. *Anim Reprod Sci* 60-61:743–752
- Drackley JK, Cicela TM, Lacount DW (2003) Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. *J Dairy Sci* 86:1306–1314
- El-Masry KA, Marai LFM (1991) Comparison friesians and water buffaloes in growth rate, milk production and some blood constituents, during winter and summer conditions Egypt. *J Anim port* 53:39–43
- Engler D, Pham T, Fullerton MJ, Ooi G, Funder JW, Clarke IJ (1989) Studies of the secretion of corticotrophin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. *Neuroendocrinology* 49:667–381
- Gilad E, Meidan R, Berman A, Graber Y, Wolfenson D (1993) Effect of heat stress on tonic and GnRH-induced gonadotropin secretion in relation to concentration of Oestradiol in plasma of cyclic cows. *J Rep Fertil* 99(2):315–321
- Guzeloglu A, Ambrose JD, Kassa T, Diaz T, Thatcher MJ, Thatcher WW (2001) Long term follicular dynamics and biochemical characteristics of dominant follicles in dairy cows subject to acute heat stress. *Anim Reprod Sci* 66:15–34
- Habib GM et al (2007) Glutathione protects cells against arsenate-induced toxicity. *Free Radic Biol Med* 42(2):191–201
- Hansen PJ (2009) Effects of heat stress on mammalian reproduction. *Philosophical Transaction Royal Soc London B Biol Soc* 364: 3341–3350
- Howell JL, Fuquay JW, Smith AE (1994) Corpus luteum growth and function in lactating Holstein cows during spring and summer. *J Dairy Sci* 77(3):735–739
- Kadzere CT, Murphy MR, Silanikove N, Maltz E (2002) Heat stress in lactating dairy cows: a review. *Livest Prod Sci* 77:59–91
- Khodaei-Motlagh M, Zare Shahneh A, Masoumi R, Derensis F (2011) Alterations in reproductive hormones during heat stress in dairy cattle. *Afr J Biotechnol* 10:5552–5558
- Kim E, Rothschild MF (2014) Genomic adaptation of admixed dairy cattle in East Africa. *Front Genet* 5:443
- Kimothi SP, Ghosh CP (2005) Strategies for ameliorating heat stress in dairy animals. *Dairy Year book*, p 371–377
- Lee CN (1993) Environmental stress effects on bovine reproduction. *Vet Clin North Amer Food Anim Prac* 9:263–273
- Marai IFM, Daader A, Am A-s, Ibrahim H (1997) Winter and summer effects and their amelioration on lactating freesian and holstein cows maintained under Egyptian condition international conference on animal. Poultry and rabbit production and health. Egyptian international center for agri, Dokki
- Minton JE (1994) Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *J Anim Sci* 72:1891–1898
- Nardone A, Lacetera N, Bemabucci U, Ronchi B (1997) Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *J Dairy Sci* 80: 838–844
- Nessim MG (2004) Heat induced biological changes as heat tolerance indices related to growth performance in bofflaoes. Ph.D thesis. Faculty of Agri: Ain Shums university, Cairo, Egypt
- O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH (2010) Metabolic adaptations to heat stress in growing cattle. *Domest Anim Endocrinol* 38:86–94

- Ozawa M, Tabayashi D, Latief TA, Shimizu T, Oshima I, Kanai Y (2005) Alterations in follicular dynamics and steroidogenic abilities induced by heat stress during follicular recruitment in goats. *Reproduction* 129(5):621–630
- Podar C, Oroian I (2003) The influence of high temperature on milk cows. *Buletinul-Universitatii de Stiinta-agricole-si-Medicina veterinara-Cluj-Napoca-seria-zoonehniei biotehnologie*, p 130–133
- Qureshi MS, Khan JM, Khan I, Chaudhry RA, Ashraf K, Khan BD (2002) Improvement in economic traits of local cattle through cross-breeding with Holstein Frisian semen. *Pak Vet J* 22:1–21–26
- Raghavan GV, Mullick DN (1961) Effect of air temperature and humidity on the metabolism of nutrient of buffalo. *Ann Biochem Exp Med* 21:277
- Rasooli A, Nouri M, Khadjeh GH, Rasekh A (2004) The influences of seasonal variations on thyroid activity and some biochemical parameters of cattle. *Ind J Vet Sci* 5(2):1383
- Ravagnolo O, Misztal I (2000) Genetic component of heat stress in dairy cattle, parameter estimation. *J Dairy Sci* 83:2126–2130
- Rhoads ML, Kim JW, Collier RJ, Crooker BA, Boisclair YR, Baumgard LH, Rhoads RP (2010) Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness. *J Dairy Sci* 93:170–179
- Ronchi B, Stradaoli G, Verini Supplizi A, Bernabuci U, Lacetera N, Accorsi PA (2001) Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17beta, LH, FSH, prolactin and cortisol in Holstein heifers. *Livestock Prod Sci* 68:231–241
- Roth Z, Arav Z, Bor A, Zeron Y, Braw-Tal R, Wolfenson D (2001) Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction* 122:737–744
- SAS (2002) Statistical analysis system proprietary software. Release 8.1. SAS Institute Inc., Cary, NC
- Silanikove N (2000) Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest Prod Sci* 67:1–18
- Silanikove N, Matiz E, Halevi A, Sinder D (1997) Metabolism of water, sodium, potassium and chloride by high yielding dairy cows at the onset of lactation. *J Dairy Sci* 80:949–956
- Tilbrook AJ, Turner AI, Clarke IJ (2000) Effects of heat stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *J Reprod Fertil* 5:105–113
- Vaught LW, Monty DW, Foote WC (1977) Effect of summer heat stress on serum LH and progesterone values in Holstein-Frisian cows in Arizona. *Am J Vet Res* 38:1027–1032
- Verma DN, Lal SN, Singh SP, Parkash OM, Parkash O (2000) Effect of season on biological responses and productivity of buffalo. *Inter J Anim Sci* 15:237–244
- Wise ME, Armstrong DV, Huber JT, Hunter R, Wiersma F (1988) Hormonal alterations in the lactating dairy cow in response to thermal stress. *J Dairy Sci* 71:2480
- Wolfenson D, Roth Z, Meidan R (2000) Impaired reproduction in heat-stressed cattle: basic and applied aspects. *Anim Reprod Sci* 60–61: 535–547
- Yousef HM (1990) Studies on adaptation of Friesian cattle in Egypt. PhD. Thesis, Faculty of Agri: Zagazig Uni: Zagazig. Egypt

FOR APPROVAL