Research Article

Crossbred Cows Respond Differently from Holstein Frisian and Bos Indicus to Heat Stress Under Various Climatic Conditions

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Abstract | The ability to adapt to environment is of great importance to all living things.Heat stress response was determined in dairy cows,9 each from Holstein Frisian (HF), Crossbred-50% (HF × Sahiwal) and *Bos Indicus* (Sahiwal and Achai). Cows were sampled at environmental temperature 18, 32 and 42 °C in February, April and June, respectively. The physiological parameters recorded were rectal temperature (RT), respiration rate (RR) and pulse rate (PR) whereas biochemical parameters determined from blood serum were glucose, cortisol and Heat shock protein-70 (HSP-70). Thermal stress increased all physiological parameterssignificantly (P < 0.001). Holstein Frisian manifested maximumincrease in RT, RR and PR (3.33, 209.50 and 59.41%) than crossbred (0.59, 40.22 and 22.0%), Sahiwal (0.78, 42.54 and 34.31%) and Achai (0.78, 39.22 and 33.85%) respectively (P < 0.001).Thermal stress increased biochemical changes significantly (P < 0.001). The increase in serum glucose, cortisol and HSP-70 concentrations wasmaximum in HF (43.44, 119.06 and72.11%) andcrossbred (38.66, 159.61 and79.12%) and minimum inAchai (34.93, 123.59 and52.53%) and Sahiwal (27.26, 117.22 and65.41%)respectively (P< 0.01). Holstein Frisian appeared as the most heat sensitive breed exhibiting changes at 32 °C i.e. 5 °C higher than 27 °C (upper critical temperature, UCT). Sahiwal and Achai were the most heat tolerant breeds manifesting changes at 42 °C i.e. 15°C higher than UCT. The crossbred cows responded differently to heat stress and showed biochemical changesat 32 °C like HF and physiological changes at 42 °C like *Bos Indicus*. It appears that crossbred cows acquire some genes for low physiological response to thermal stress from *Bos Indicus* cows and acclimatization to the tropical conditions of Pakistan.Therefore, further study is needed on acquisition of genes from *Bos Indicus* for low physiological response in crossbred cows.

Received | September 14, 2015; **Accepted** | March 15, 2018; **Published** | April 28, 2018
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Citation | Khan. I., M.S. Quershi, S. Akhtar, I. Ali and G. Ullah 2018. Crossbred cows respond differently from holstein frisian and bos indicusto heat stress under various climatic conditions. *Sarhad Journal of Agriculture,* 34(2): 301-310.
DOI | http://dx.doi.org/10.17582/journal.sja/2018/34.2.301.310
Keywords | Thermal stress, Cattle breeds, Physiological, Biochemical, HSP-70

Introduction

Dairy cattle change their behavior to reduce heat load in hot weather as defensive response to stressful conditions. The ability to adapt to environmental stress is of great importance to all living organisms including livestock (Haque et al., 2012). The climate of Pakistan, on the whole is dry and extreme, ranging from tropical to temperate. The summers are extremely hot and winters are extremely cold. Pakistan is a home tract of some of the finest breeds of *Bos Indicus* cattle including Sahiwal and Achai.

Bos Indicus (Zebu) also called the humped cattle are well adapted to the tropical environment of Pakistan due to high degree of heat tolerance (associated with low metabolic rate and low productivity), low nutritional requirements (due to low metabolic rate and small size), a considerable degree of resistance to ticks and rich genetic support (Farooq er al., 2010). During the last decades there was extensive urbanization due to which dairy industry has evolved towards intensive milk production systems. The *Bos Indicus* breeds could not respond positively due to their limited genetic potential. Therefore, high producing cattle breeds mainly Holstein-Friesian and Jersey belonging to temperate areas were imported. Cattle upgradation through use of exotic semen was initiated in 1954 at Lahore and through crossbreeding program in 1973 – 74 at Bahadurnagar under the supervision of Pakistan Agricultural Research Council (PARC). The sole objective was to increase the production potential of local cattle. Besides production efficiency, crossbred cattle developed unique adaptive characteristics to the local environment (Qureshi et al., 2000).

Dairy cattle are homeotherm and maintain their body temperature within a close defined thermoregulatory limit, regardless of the external environment variations. Normal homeostasis is disturbed when temperature exceeds 27 °C that leads to lower production and reproduction in high productive dairy cows (Petrujkić et al., 2009). Heat stress has been defined by Bernabucci et al. (2010) as "a physiological condition when the core body temperature of a given species exceeds its range specified for normal activity, which results from a total heat load (internal production and environment) exceeding the capacity for heat dissipation and this prompts physiological and behavioral responses to reduce the strain". The variation in upper critical temperatures (UCT) depends upon age, breed, level of production and the degree of acclimatization (Shearer and Beede, 1990).

Dairy cattle change their behavior to reduce heat load in hot weather. Different physiological traits (rectal temperature, respiration rate, pulse rate and alteration in hormonal regulations) are adversely influenced by the climatic conditions (Habeeb et al., 2007). However, the physiological changes to heat stress vary in the intensity and duration in relation to the animal genetic make-up and environmental factors (Haque et al., 2012). Activation of the hypothalamo-pituitary-adrenal (HPA) axis and the consequent increase in plasma glucocorticoid concentrations are two of the most important responses of the animals to heat stress. Cortisol is essential for stress response, glucose and electrolyte balance, blood pressure and general body condition (Forslund et al., 2010). Cell responds to heat stress with the synthesis of HSPs that can be transcribed and translated in high ambient temperatures. The biological role of HSPs is to restore protein homeostasis to the pre-stress state by refolding and repairing damaged proteins. Heat Shock Protein-70 (HSP-70) is one of the most studied and inducible HSP families under stressful conditions which is often used as stress marker and adaptation in a variety of physiological systems (Beckham et al., 2004).

In the current study, we investigated heat stress response in crossbred, HF and *Bos Indicus* breeds through physiological and biochemical changes under subtropical conditions of Peshawar, Pakistan. We also investigated the temperature threshold for induction of physiological and biochemical changes in these breeds.

Materials and Methods

The present study was conducted at Livestock Research and Development Station Surezai, District Peshawar, Pakistan, 34°N and 72°E. The research study was conducted under the approval of the ethics committee, department of Livestock management, Faculty of animal husbandry and veterinary sciences, The University of Agriculture Peshawar, Pakistan. The manuscript was designed according to the guidelines of the Helsinki Declaration as revised in 1975. All efforts were taken to minimize pain and discomfort to the animal while conducting the experiments.

Selection of animals

A total of 36 early lactating dairy cows, 9 each from HF, *Bos Indicus* (Sahiwal and Achai) and Crossbred-50% (HF × Sahiwal) were selected. The cows were at days open and physiologically sound. Average daily milk production of HF cows was 18.77 ± 0.11 Kg, crossbred 9.36 ± 0.49 Kg, Sahiwal 8.23 ± 0.43 Kg and Achai 6.26 ±0.29 Kg. All the cows were placed under similar managemental conditions practiced at the farm. The experimental animals were kept in sheds with adjacent open paddock. Drinking water was provided ad libitum. Seasonal green fodder with wheat straw and concentrate were fed to animals according to the local Farm management practices.

Sampling

Sampling was conducted when average ambient temperature was 18 °C (thermoneutral for dairy cattle and was used as control), 32 °C (thermal transitional period) and 42 °C (heat stress) in February, April and June respectively.

Physiological parameters

Physiological parameters were recorded at the farm level. Respiration rate was recorded by observing and counting per minute expansion of chest and flank region (mov/min) during inspiration. Rectal temperature was recorded by putting digital thermometer into the rectum of individual cow for one minute and then the thermometer reading was recorded. The pulse rate was calculated from coccygeal artery beat in one minute (bpm).

Biochemical parameters

Approximately 10 ml blood samples were collected for biochemical analysis on scheduled dates from jugular vein puncture in sterile vacutainer tubes without anticoagulant for serum separation. Immediately, the tubes were brought to the laboratory of Department of Livestock Management, The University of Agriculture Peshawar. Then serum was separated from blood by centrifugation at 3000 rpm for 20 min. The separated serum was stored at -20 °C till further analysis.

The serum cortisol concentrations were determined through ELISA kit (CALBIOTECH, USA), serum glucose level was determined through spectrophotometry using single reagent kit (MDSS GmbH, Germany) and serum HSP-70 concentrations were determined through ELISA kit (CUSABIO BIOTECH CO., LTD. China) according to the standard protocol provided by the manufacturer.

Statistical analysis

SPSS 16.0 for windows was used for statistical analysis. The data were analyzed statistically by two-way analysis of variance. Temperature and breed were used as independent variables while physiological and biochemical parameters were used as dependent variables. Means were compared using Least Significant Difference (LSD) test. Statistical significance was set at $P < 0.05$.

Results

Changes in physiological parameters

Results demonstrated that temperature and breed were significant ($P < 0.001$) factors affecting the RT (Table 1). Maximum increase (3.33%) in RT was recorded in HF cows. Sahiwal, Achai and crossbred cows showed minimum increase in RT i.e. 0.78, 0.78 and 0.59% respectively. The lowest RT was observed in Sahiwal cows (101.5 °F) at 18 °C in February while highest RT (105.4 °F) was observed in HF cows at 42 °C in June ($P < 0.001$) (Figure 1). No significant difference in RT was observed in February (18 °C). However, HF cows exhibited increased RT with the rise in ambient temperature to 32 °C, going towards worst in June at 42 °C. Crossbred cows along with two *Bos Indicus* breeds showed slight increase in RT at 42 °C in June.

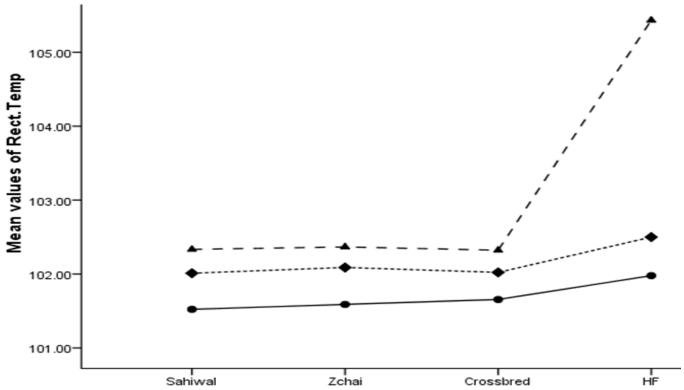

Figure 1: *Effect of various ambient temperatures on rectal temperature in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

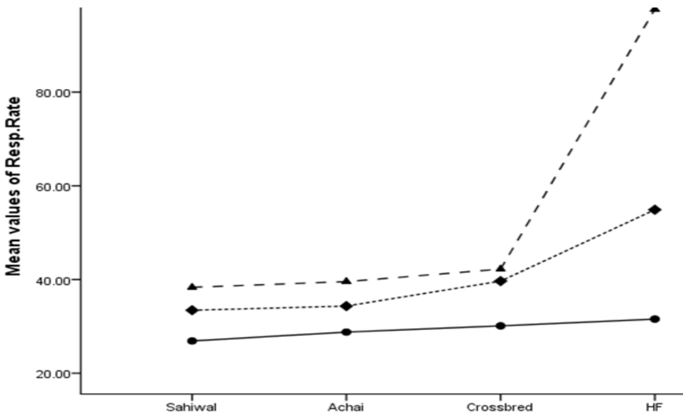

Figure 2: *Effect of various ambient temperatures on respiration rate in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

Table 1 shows that ambient temperature and breed significantly affected the RR ($P < 0.001$). The RR was lowest (26.89 mov/min) in Sahiwal in February (18 °C) and was highest (97.66 mov/min) in HF in June(42 °C) (Figure 2). The results indicated that HF cows manifested maximum increase (209.5%) in RR than the other cows of crossbred, Sahiwal and Achai breeds (40.22%, 42.54% and 39.22% respectively). The RR was almost similar in February (18 °C) in all the dairy cows (Figure 2). The two *Bos Indicus* breeds (Sahiwal and Achai) and crossbred cows responded similar to heat stress by showing no notable change in RR in April (32 °C) and slight increase in June (42 °C).However, the HF cows showed moderate increase in RR in April (32 °C) that became more sever in June (42 °C).

Table 1: *Mean value of biochemical parameters as affected by ambient temperature and breed*

Independent variables		Respiration Rate	Pulse Rate	Rectal Temp
Temperature (°C)	18	29.33[c]	44.25[c]	101.69[c]
	32	40.58[b]	55.06[b]	102.16[b]
	42	54.44[a]	60.52[a]	103.11[a]
	P- Value	< 0.001	< 0.001	< 0.001
Breed	Sahiwal	32.89[c]	51.33[b]	101.96[b]
	Achai	34.22[c]	50.18[b]	102.01[b]
	Crossbred	37.33[b]	5129[b]	102.0[b]
	HF	61.37[a]	60.29[a]	103.3[a]
	P- Value	< 0.001	< 0.001	< 0.001

[a, b, c] Means with different superscripts are different with in the column at P < 0.05

Ambient temperature and breed showed significant (P < 0.001) affect on PR (Table 1). Sahiwal cows showed lowest PR (42.44 bpm) at 18 °C in February while HF cows showed highest PR (73.33 bpm) at 42 °C (Figure 3). HF cows showed maximum increase (59.41%) in PR than crossbred (22.0%), Sahiwal (34.31%) and Achai (33.85%) (P < 0.001). All the cows followed almost similar pattern for PR like other physiological parameters showing increasing tendency with increase in ambient temperature (Figure 3). HF cows manifested moderate increase in PR at 32 °C that became more severe at 42 °C. However, the crossbred and *Bos Indicus* cows showed little increase in PR at 42 °C.

The crossbred and the two *Bos Indicus* breeds maintained physiological parameters within the normal range more efficiently than the HF cows during thermal stress condition. When the ambient temperature increased to 32 °C which was 5 °C higher than 27 °C (UCT), a moderate rise in physiological parameters was observed in HF cows that became more severe at 42 °C in June. However, the crossbred and the two *Bos Indicus* breeds maintained their physiological parameters at 32 °C in April and showed slight increase at 42 °C which was 10 and 15 °C higher than the HF and UCT respectively.

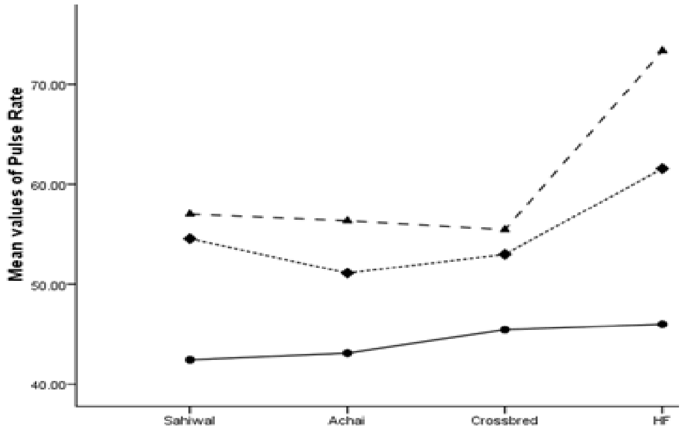

Figure 3: *Effect of various ambient temperatures on pulse rate in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

Changes in biochemical parameters

The mean values of biochemical parameters are given in Table 2. In general, the biochemical parameters examined were significantly affected by ambient temperature and breed (P < 0.001). However, the interaction effect of T × B was not significant (P > 0.05) for any of the biochemical parameters meaning that the breeds behaved independently of ambient temperature.

Table 2: *Mean values of biochemical parameters as affected by ambient temperature and breed*

Independent variables		Glucose (mg/dl)	Cortisol (ng/ml)	HSP-70 (ng/ml)
Temperature (°C)	18	29.32[b]	66.64[c]	1.83[c]
	32	33.25[b]	98.32[b]	2.64[b]
	42	39.98[a]	42.79[a]	3.08[a]
	P- Value	< 0.01	0.001	0.001
Breed	Sahiwal	31.71[b]	80.60[c]	2.17[c]
	Achai	31.23[b]	99.74[b]	1.98[c]
	Crossbred	32.95[b]	119.48[a]	2.64[b]
	HF	40.85[a]	121.19[a]	3.26[a]
	P- Value	<0.01	0.001	0.001

[a, b, c] Means with different superscripts are different with in the column at P < 0.05

Ambient temperature and breed showed significant (P < 0.01) effect on serum glucose level (Table 2). Lowest serum glucose level (27.24 mg/dl) was observed in crossbred cows at 18 °C in February while highest (46.72 mg/dl) level was observed in HF cows at 42 °C in June (Figure 4). There was no significant difference in glucose concentrations at 18° C (February) in all the breeds. However, asambient temperature increased to 32°C (April) and 42°C (June), the glucose level also started to increase. The two *Bos Indicus* breeds of Sahiwal and Achai expressed little changes in glucose concentrations than HF and crossbred cows with rise in ambient temperature (Figure 4). Maximum manifestation in serum glucose level was observed in HF (43.44%) and crossbred (38.66%) cows while Sahiwal and Achai cows showed minimum manifestations i.e. 27.26 and 34.93% respectively. The results showed that crossbred cows expressed glucose changes like HF and showed more glucose concentrations than the *Bos Indicus* breeds with rise in ambient temperature.

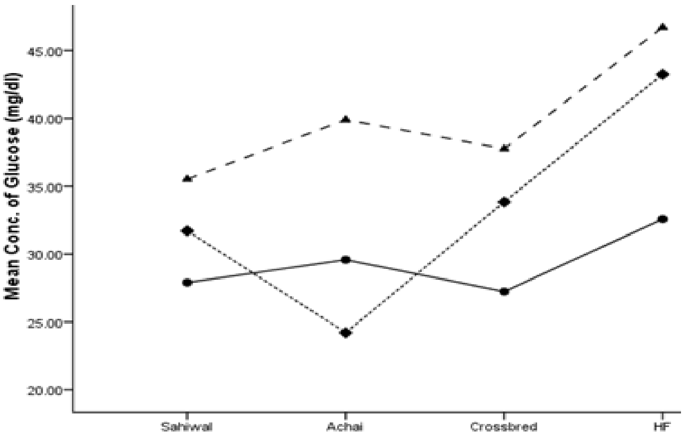

Figure 4: *Effect of various ambient temperatures on serum glucose level in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

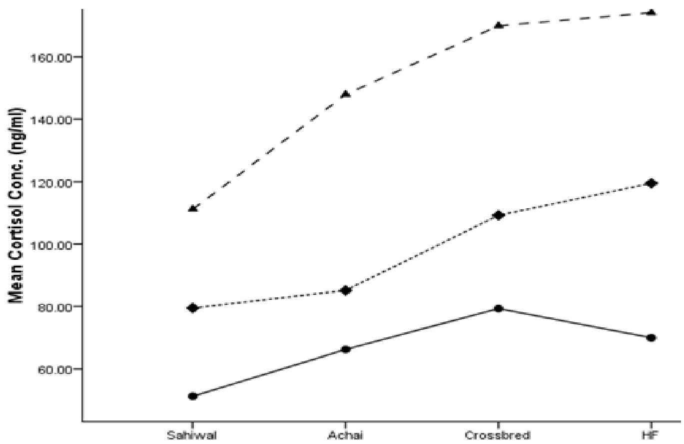

Figure 5: *Effect of various ambient temperatures on serum cortisol concentrations in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

The concentrations of serum were significantly (P < 0.001) affected by ambient temperature and breed (Table 2). Serum cortisol concentrations varied from 51.16 ng/ml (Sahiwal) to 174.15 ng/ml (HF) at 18 and 42 °C respectively (Figure 5). The overall increase in cortisol concentrations were 117.22, 123.59, 159.61 and 119.06% in Sahiwal, Achai, crossbred and HF cows respectively. The increase in cortisol concentrations started to appear at 32°C (April) which became more severe at 42°C (June) in HF and crossbred cows (Figure 5). The *Bos Indicus* breeds tolerated heat stress up to 32°C (April) by maintaining cortisol concentrations within the control range and then showed minute increase in cortisol concentrations at 42°C in June, more prominent in Achai than Sahiwal.

The concentrations of HSP-70 increased significantly (P < 0.001) with increase in ambient temperature (Table 2). Minimum HSP-70 concentration (1.58 ng/ml) was observed in Achai cows at 18 °C and while HF cows showed maximum concentrations (4.01 ng/ml) at 42 °C in June (Figure 6). HF, crossbred, Sahiwal and Achai cows showed 72.11, 79.12, 65.41 and 52.53% increase in HSP-70 concentrations. The *Bos Indicus* breeds showed more tolerance to heat stress and maintained the HSP-70 concentrations within the range of 1.5-2.5 ng/ml at all the three temperatures in February (18°C), April (32°C) and June (42°C). However, the HF and crossbred cows showed significant increase in HSP-70 concentrations at 32 and 42°C in April and June respectively (Figure 6).

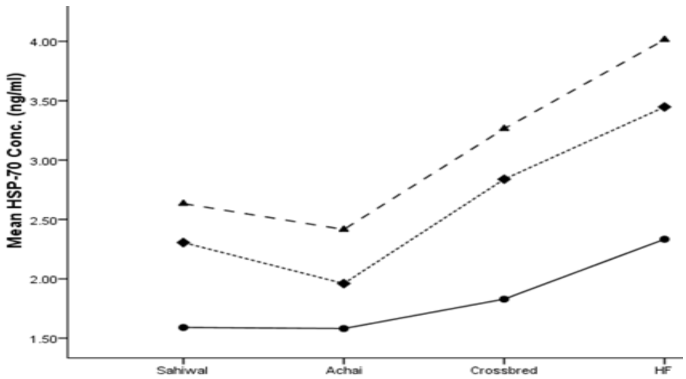

Figure 6: *Effect of various ambient temperatures on HSP-70 concentrations in various dairy cattle breeds (● 18 °C, ♦ 32 °C, ▲ 42 °C).*

The results of biochemical response revealed that crossbred and HF cows showed almost similar biochemical response to heat stress which was different from *Bos Indicus* cows. The crossbred and HF cows started to show increase in biochemical response pa-

Table 3: *Relationship (coefficient of correlation) of various studied parameters of different dairy cattle breeds (n= 108).*

Parameters	RR	PR	RT	Glucose	Cortisol	HSP-70
Temp (P-Value)	0.545 (0.000)	0.727(0.000)	0.544 (0.000)	0.347 (0.000)	0.686 (0.000)	0.576 (0.000)
RR	-	0.842 (0.000)	0.892 (0.000)	0.388 (0.000)	0.548 (0.000)	0.653 (0.000)
PR	-	-	0.782 (0.000)	0.422 (0.000)	0.592 (0.000)	0.672 (0.000)
RT	-	-	-	0.390 (0.000)	0.511 (0.000)	0.586 (0.000)
Glucose	-	-	-	-	0.299 (0.002)	0.390 (0.000)
Cortisol	-	-	-	-	-	0.561 (0.000)

rameters at 32°C in April which reached to peak level at 42°C in June. However, the *Bos Indicus* cows tolerated heat stress at 32°C ambient temperature in April and showed minor increase in biochemical response parameters at 42°C in June.

Relationship among studied parameters

Relationship between various parameters was determined through Pearson's coefficient of correlation analysis (Table 3). All the behavioral and biochemical parameters showed positive correlations with ambient temperature ($P < 0.001$). The studied parameters were also positively correlated with each other ($P < 0.001$).

Discussion

Changes in physiological parameters

The intensity of changes in physiological parameters was highest in HF than *Bos Indicus* and crossbred cows. The variations in physiological parameters in response to heat stress indicated that the ability of ruminants to regulate physiological response is species and breed dependent (Bernabucci et al., 2010). The HF cows exhibited physiological changes at 32 °C in April that was 5 °C greater than the UCT (27 °C) for the dairy cattle (Petrujkić et al., 2009). However, the crossbred and *Bos Indicus* cows tolerated heat stress more efficiently in April and showed minute physiological changes at 42 °C in June that was10 and 15 °C greater than HF and UCT respectively. Hence, the results revealed that *Bos Indicus* and crossbred cows could tolerate more heat stress than HF cows ($P < 0.001$). Several other research workers also reported variations in physiological changes among different breeds and age groups (Lallawmkimi, 2009; Haque et al., 2012).

In general, the *Bos Indicus* and crossbred cows tolerated more heat stress than HF due to acquisition of some genes through the process of acclimatization, responsible for behavioral, hormonal and metabolic changes to survive in a subtropical environmental condition (McManus et al., 2009). Several research workers found that animals with darker skin color are more susceptible to heat stress than animals having lighter skin colors (Correa-Calderon et al., 2004) because the temperature sensitive neurons are present throughout the surface of animal's body. These neurons after exposure to thermal stress send information through to hypothalamus, which invokes several physiological or behavioral changes to help in maintaining heat balance and homeostasis (Curtis, 1983). Therefore, the darken skin color of the HF cows in this study made it more susceptible to heat stress than *Bos Indicus* (Sahiwal and Achai) having lighter brown skin color.

Rectal temperature is considered representative of core body temperatures, usually accepted between 38 to 39.5°C (Du Preez, 2000). HF cows in this study were high milk yielders (20±1 Kg/day) than crossbred (12±1 Kg/day) and *Bos Indicus* (Sahiwal 8±1 Kg/day and Achai 6±1 Kg/day), thus producing more metabolic heat. Consequently, the generation of more metabolic heat coupled with high ambient temperature was the possible reason for higher body temperature (Bernabucci et al., 2010). The higher magnitude of increase in rectal temperature (3.4 °C) in HF indicated the inability of heat dissipation mechanism to maintain homeothermia (Mota, 1997) while the lower magnitude in *Bos Indicus* (Sahiwal, 0.8 °C; Achai, 0.8 °C) and crossbred (0.6 °C) indicated the higher capacity of these breeds to prevent increase in rectal temperature during heat stress (McManus et al., 2009).

Normal respiration rate in adult cattle is between 24-36 respiratory movements per minute. Increased respiration is the first visible sign of heat stress and is most pronounced at temperatures over 29 °C (McDowell, 1972). The magnitude of increase in respiration rate in HF cows (66.11mov/min) was higher than the crossbred (12.11mov/min) and *Bos Indicus* (Sahiwal, 11.44 mov/min; Achai, 11.33 mov/min) (P< 0.001). The higher magnitude of increase in respiration rate in HF cows might be to increase heat loss potential and to supply more oxygen needed by the tissues in stressful condition (Haque et al., 2012). The lower magnitude of increase in respiration rate in crossbred and *Bos Indicus* cows suggests that these breeds were less affected by heat stress than exotic HF cows (Srikandakumar et al., 2003).

The pulse rate is the expansion and contraction of arteries per minute in response to the heart. This rate is exactly equal to the heartbeat or the rate of heart contractions per minute. Therefore, taking the pulse is a direct measure of heart rate (MacGill, 2015).Normal pulse rate in dairy cattle varies between 48- 84 beats per minute. In all cows, Pulse rate was within the reference values for this species varying between 42.44 bpm (Sahiwal) and 73.33 bpm (HF), although the differences were observed significant for breed, temperature and T × B (P< 0.001). The magnitude of increase in pulse rate was higher in HF (27.33 bpm) showing more heat susceptibility than Sahiwal (14.56 bpm), Achai (14.67 bpm) and crossbred (10.0 bpm). These results are in agreement with the results of (McManus et al., 2009) which showed that increase in heart rate was more prominent in *Bos Taurus* than *Bos Indicus*. The animal depends on increased heart rate when heat loss is not controlled by evaporation to lose heat and prevent an increase in body temperature. However, this increase in heart rate with a compensatory fall in blood pressure caused by peripheral vasodilation in cattle is not efficient due to the high body mass of the animals (Silva, 2000). It suggests that the increase in pulse rate is more likely to supply oxygen-rich blood to deliver more oxygen and energy to the tissues in stressful conditions (MacGill, 2015).

Changes in biochemical parameters

Serum glucose, cortisol and HSP-70 concentrations were determined to find out biochemical changes with heat stress. We hypothesized that cows suffering from heat stress have elevated blood glucose levels due to an increase in serum cortisol concentrations. Increased serum glucose level was observed with increase in ambient temperature (P< 0.001) more substantial in HF followed by crossbred than *Bos Indicus* breeds (P< 0.01). Several other researchers have also reported increase in glucose concentrations in response to heat stress in cattle (Hoehn and Marieb, 2010)and sheep (Srikandakumar et al., 2003).

The higher magnitude of increase in glucose concentrations in HF (14.15 mg/dl) followed by crossbred (10.53 mg/dl) than Sahiwal (7.61 mg/dl) and Achai (10.33 mg/dl) again indicated that the HF and crossbred cows were less tolerant of heat stress than *Bos Indicus* cows. The higher level of cortisol observed in this study, increased glucose level through gluconeogenesis by aiding in fat, protein and carbohydrate metabolism (Hoehn and Marieb, 2010). These physiological modifications also alter nutrient partitioning and

may prevent heat-stressed lactating cows from recruiting glucose-sparing mechanisms (Bernabucci et al., 2010). It suggests that blood glucose needed for milk synthesis remain in higher concentrations in blood serum in response to increased energy demand associated with higher respiration rate in HF and crossbred than Sahiwal and Achai cows.

Cortisol is the main hormone in stress. Increased level of cortisol was observed in this study with rise in ambient temperature (P< 0.001). The results indicated that the intensity of changes was more prominent in HF and crossbred than *Bos Indicus* (P< 0.001). Our results are consistent with the results of (McManus et al., 2009) showing higher level of cortisol in heat stressed cows more severe in *Bos Taurus* than *Bos Indicus*. Certain environmental stressors have the potential to activate the hypothalamo-pituitary-adrenal cortical axis (HPA) and sympatho-adrenal medullary axis (Minton, 1994) which ultimately results into the increased production of cortisol (Sapolsky et al., 2000). The increased concentrations of cortisol play role in heat loss through vasodilation to the surroundings and in providing energy to the animal's body through proteolysis and lipolysis to compensate the reduction of feed intake (Cunningham and Klein, 2007). Hence, the increase in cortisol level was due to hyper pitutarism resulting into increase production of adrenocorticotrophic hormones (ACTH) by the hypothalamus, stimulated by the thermo receptors of the skin.

An increase in serum HSP-70 concentrations was found with increase in ambient temperature (P< 0.001). The increase in HSP-70 was observed in HF and crossbred cows at 32 °C and in *Bos Indicus* at 42 °C (P< 0.001). The concentration of HSP-70 of *Bos*

Indicus cows at 42 °C was almost equal to HF cows at 18°C indicating that these local breeds could tolerate more heat stress than the exotic and crossbred cows. These results are in consistent with the findings of (Patir and Upadhyay, 2010; Mishra et al., 2011).

Thermal stress duration and magnitude can affect the concentrations of HSP-70 (Wang et al., 2003).Currie and White, 1983 found that the production of HSPs can be stimulated by temperature of 42 °C for 20-60 min. Heat shock proteins are chaperones that protect cell from heat damage by preventing protein denaturation (Kregel, 2002). Cells produce certain non-native proteins in response to thermal stress which is harmful to cells. Therefore more induction of HSP-70 is needed to contest this stressed condition (Haque et al., 2012). Several studies confirmed genetic differences in thermo tolerance at the physiological and cellular levels in *Bos Indicus* and *Bos Taurus* (Lacetera et al., 2006; Farooq et al., 2010). The breed differences might be due to the destabilizing protein that exists in heat shocked non-thermo tolerant cells, which interacts with HSP-70 mRNA to decline its induction, while the interaction is protected in thermo tolerant cells (Theodorakis et al., 1999). It suggests that a higher HSP-70 concentration is associated with a lower tolerance of severe heat stress.

Conclusions and Reccomendations

In conclusion, the physiological and biochemical changes, appeared at 32 °C (thermo transitional, April) in HF cows were 5°C greater than 27°C (upper critical temperature). The *Bos Indicus* breeds maintained itself up to 32 °C (thermo transitional, April) and the changes were observed at 42 °C (thermal stress, June) that was 10 °C and 15 °C greater than the HF and UCT respectively. The crossbred cows responded differently and showed physiological changes like *Bos Indicus* and biochemical response like HF. The biochemical changes in crossbred cows started to appear at 32 °C like HF, however, they did not exhibit physiological changes at this temperature. It suggests the acquisition of some genes for low physiological response to thermal stress from *Bos Indicus* cows and acclimatization.

Acknowledgements

The completion of this research study was supported by Higher Education Commission (HEC) of Pakistan grants under Indigenous PhD scholarship Program.

Author's Contributions

IK completed the study as PhD Scholar including the manuscript drafting, MSQ designed the study, SA analyzed the data statistically, IA helped in laboratory analysis, while GU performed animal experiments. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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