Effect of different ambient management interventions on milk production and physiological performance of lactating Nili-Ravi buffaloes during hot humid summer

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Abstract

Ambient environment has direct effect on the productive performance and welfare of dairy animals and it causes significant changes in their biological functions. Study was conducted to evaluate the productive, physiological and behavioral differences of Nili-Ravi buffalo reared in different ambient management strategies in humid hot environment of Pakistan. Fifteen (15) lactating multiparous Nili-Ravi buffalos were divided into three treatments, comprising of 5 animals in each and animals were housed in semi-open barn. Nili-Ravi buffaloes of approximately similar weight, age and same parity (3rd) were randomly allotted to three different treatments included;1) provision of only roof shade, Controlled, designated as S, 2) provision of shade assisted with fan (SF), 3) provision of shade, fan and sprinklers (SFS). Three animal groups were kept in separate sheds under tied sloped floor system. The buffaloes were fed on iso-caloric and iso-nitrogeneous diet during the experimental period. The animal's individual milk production was measured daily. Respiration rate, rectal temperature, total time spent in feeding, drinking, rumination, standing, lying and locomotion were measured during the trial period. The physiological parameters were recorded twice daily on 12:00 and 18:00 h. Behavioral observations were recorded visually from morning milking till next morning milking on fortnightly basis. The data were analyzed using completely randomized design (CRD) through SAS 9.1.3. Significant differences *(p<0.05)* were observed in milk yield (kg/d) 5.66Â±0.23, 7.27Â±0.20, 10.45Â±0.79 in buffaloes under S, SF and SFS treatment, respectively. Rectal temperature was 102.06Â±0.07, 101.69Â±0.08, 101.05Â±0.9 (^{o}F) in buffaloes under S, SF and SFS treatment, respectively, and respiration rate were 26.16Â±1.12, 33.8Â±0.82 and 38.48Â±0.84 breaths/min were observed in buffaloes under S, SF and SFS treatment, respectively. Significant differences *(p<0.05)* were observed in behavioral responses regarding total time feeding, rumination, lying and locomotion activities between different treatment groups. It is concluded that combined effect of shade, fan and sprinklers causes beneficial effect on milk production, physiological and behavioral responses in lactating Nili-Ravi buffalo animals during humid hot season.

Key Words: *behavior, environment, fan, physiology, production, shade, shower*

Introduction

Buffalo is the second most important species in the world in terms of milk production, after dairy cows (Coroian et al 2013). Pakistan holds second position among buffalo-milk producing countries in the world (Hussain et al 2010). Nili-Ravi buffalo is regarded as one of the best dairy animals in the tropics. Their home tract is canal irrigated areas of central Punjab Province of Pakistan, where abundant green fodder is available (Hussain et al 2006).

Profitability of dairy animals is influenced by the herd's level of milk production and reproduction (Baharizadeh 2012; Rehman and Khan 2012). Annual milk yield is an index and it reflects the intensity of lactation and it combines the milk yield and reproductive efficiency of dairy animals (Katok and Yanar, 2012). Management conditions have pronounced effect on milk production and reproduction (Kellogg et al 2001; El-Awady 2013). Climatic conditions affect body temperature, which lead to lower milk production (West 1999). Ambient housing management minimizes the heat stress effects in dairy animals (Bucklin et al 1991; Valtorta and Gallardo 2004).

Physiological responses such as respiration rate, pulse rate, skin temperature, and body temperature are good indicators of heat stress (Ganaie et al 2013). Physiological responses also measure the comfort and adaptability to an environment. Metabolic intermediate products cumulate in blood and hemoconcentration rises with a plasma volume decrease. The hyperthermic stress influence decreased the milk yield, reduced feed intake, worsens the health condition and affects the behavior of dairy animals. Fat and protein content in milk decrease, respiratory rate, body temperature, drinking frequency and partially water intake increase in dairy cows (Koubkova et al 2002). Several environmental practices have been used to mitigate heat stress and improve performance of farm animals in hot and humid regions (Abdoun et al 2014). The present study was designed with the aim to decrease the negative effects of thermal stress and to promote the performance of lactating Nili-Ravi buffalos reared under hot environmental conditions through ambient management interventions.

Materials and methods

The present study was carried out at Dairy Animals Training and Research Centre, Ravi Campus, University of Veterinary and Animals Sciences, Lahore, which is located 31.02 latitude and 73.85 longitudes, Punjab, Pakistan. The elevation of the area is about 194 m above sea level (Ali et al 2016). The average minimum and maximum temperature during July to September were 27 to 36, 27 to 35, and 25 to 35^{o}C. Trial was conducted during the hot humid summer, July to September. During the whole period the weather was hot and humid throughout the experimental period.

Selection of Animals

Fifteen lactating Nili-Ravi buffaloes of same lactation were selected from the dairy herd maintained at the University livestock farm and randomly divided into three groups, comprising of 5 animals in each. Animals of S treatment were kept just under the shade considered, while the animals in SF were provided by shade alongwith fan. Animals in SFS treatment were provided shade, fan and sprinklers during the hot humid day hour i.e 10:00 to 18:00 h/d.

Management of Animals

Lactating Nili-Ravi buffaloes were kept within the shade during the hot humid day hours from 06:00 to 18:00hr daily. After that animals were kept open in the loose housing system within the paddock to trigger off the heat load with environment to co-op up the harsh environment of the next day. Animas were housed in head to head housing system. All groups of experimental animals were provided similar feeding but different ambient management conditions. Silage of oat grass was provided to experimental animal *adlibitum* while the fixed quantity of concentrate was given at rate of 6 kg per animal per day.

Recording of Parameters

Drenching in lactating Nili-Ravi animals to control the internal parasites were carried out at the start of experiment. Experimental animals in each group were provided measured quantity of silage *adlib* in the morning at 8 AM daily while fixed amount of concentrates was provided two times daily, firstly 3 kg/animal, at morning feeding alongwith silage at 8 AM, and 3kg concentrate before the evening milking at 4 PM. In the next morning, the leftover silage was weighed to calculate the actual intake of each animal before the next feeding. Dry matter intake was measured 3 times in a week. The oat grass silage and concentrate were analyzed in the University Animal Nutrition laboratory for its chemical composition (AOAC 2000). The concentrate prepared on the University livestock farm was provided to the animals during the whole study period. Animals were given measured quantity of water *adlib* 4 times and it was measured thrice weekly. Total water intake was measured by subtracting the water left over from the water offered. Water was provided in a specially designed water tubs. The shed air temperature and relative humidity were measured by the LCD Digital Temperature Humidity thermometer HTC-2 at 12:00 and 18:00 hour daily to calculate the THI. The milk production was recorded on daily basis at morning and evening milking (05:00 and 17:00 h/day) throughout the study period. Impact of high temperature on milk quality was determined. Morning and evening milk was mixed thoroughly and milk composition was estimated through Lacto scan, Funke Gerber (Lacto star type, year 2012, Ser. No. 3510-144703, voltage frequencies 230 V, 50-60 Hz, Power input/current- 0.4 KW/2A) on weekly basis. The physiological parameter (skin temperature (ST), rectal temperature (RT), respiration rate (RR), and pulse rate (PR) were recorded thrice weekly. The RR were measured by observing the flank movement after one complete inward and outward movement through the hand. The PR (beats/10 sec) was recorded from the coccygeal artery. The skin temperature was recorded by the 1.2" LCD Non-contact Digital Infrared Laser Temperature Gun Sensor (-50 ^{o} C$^{?}$~ 380 ^{o}C) whereas the RT was observed by the help of digital thermometer in the rectum and waited till the buzzer. Hematological parameters were analyzed through hematological analyzer (Stac Abacus Junior 5 Hematological Analyzer, STAC Medical Science & Technology Co., Ltd. China), on fortnightly basis. The blood was taken from the juggler vein and serum was extracted from the blood by centrifuging at 3000 rpm for 5 min and the blood serum was frozen at -20^{o}C till further analysis. The blood serum was analyzed through commercially available kits, serum total protein was determined by Zaia et al (2005), albumin Tietz (1995), Glucose by Burtis and Bruns (2008), Cholesterol by Magnarri et al (2015) and globulin was calculated by subtracting albumin from total protein.

Statistical Analysis

Data of mean THI, DMI, WI, milk production, avg RR, PR, ST and RT on weekly basis, whereas, blood hematology, and blood serum biochemistry, under different treatments were calculated on fortnightly basis. One-way ANOVA was used to test the difference between the three groups. Multiple comparisons among means were carried out through LSD test (Steel et al 1997). All the statistical analysis was performed by using the statistical software SAS 9.1.3.

Results

Meteorological Parameters

Daily mean ambient temperature of shed A, B and C was 85.41, 83.07 and 81.75, respectively. Significant difference was observed between shed A and shed C during the hot humid season of tropical region. Daily mean relative humidity of shed A, B and C was 64.76, 62.40 and 69.37, respectively. Daily mean THI of shed A, B and C was 85.41, 83.07 and 81.75, respectively. Greater difference observed in THI between the shed A and shed C. No difference was observed between shed A and shed B and similarly, no difference was observed between shed B and shed C.

Dry Matter Intake, Water Intake, Milk Production and Composition

During the hot humid months of summer, the daily mean milk production of buffaloes was 5.66 kg/d, 7.27 kg/d and 10.45 kg/d of S, SF and SFS treatment, respectively. Highest milk production was noticed in SFS followed by SF than S. Milk composition showed greater differences in fat%, protein% and SNF%. Highest milk fat (%) observed in SFS (6.77) followed by SF (6.41) than S (6.02). Milk protein (%) was found highest ($p<0.05$) in SFS than S, while, no difference observed between SFS (3.85) and SF (3.70). However, greater difference observed between SF (3.70) and S (3.52). SNF (%) of milk of S, SF and SFS was 8.73, 8.97 and 9.11, respectively. SFS is greater than S in SNF. While, no difference was observed between SFS and SF. No difference in milk lactose contents were observed in S, SF and SFS treatment (4.71, 4.69 and 4.80, respectively).

DMI (kg/d) of S, SF and SFS were 13.24, 14.56 and 14.73, respectively during the hot humid summer. Increased difference was observed between SFS and S, while lesser difference between SFS and SF. Highest DMI (kg/d) was observed in SFS and lowest DMI (kg/d) was found in buffaloes under S treatment. Water intake (lit/d) of S, SF and SFS was 139.38, 122.61 and 112.74, respectively. Greater differences ($p<0.05$) were observed between different treatment groups during hot humid summer period.

Physiological Parameters

Increased RT was observed in controlled treatment (S) followed by fan (SF) than sprinkler SFS treatment, 102.06, 101.69 and 101.05, respectively. Greater skin temperature was observed in buffaloes under S, SF and SFS treatment (34.66, 33.55 and 32.38^{o}C), respectively. The RR was observed higher in S treatment during the hot humid summer. Highest RR (breaths/min) was observed in controlled treatment (38.45), followed by fan treatment (33.80) than sprinkler treatment (26.15), respectively. Pulse rate also showed increased difference in heat stressed buffaloes (69.80), followed by SF (64.72) and sprinkler treatment (54.30) during the hot humid summer.

Blood Hematology

While blood cells (WBCs, 10-3/Âµl) count were higher in buffaloes in only shade group (8.99) followed by fan grouped animals (7.76) than sprinkler group (7.49). Red blood cells (RBCs, 10-6/Âµl) count were higher in buffaloes in sprinkler group (8.23) than fan group (8.19) and only shade group (7.48). Hemoglobin (Hb) level was highest in SFS than SF and SFS, 10.47, 9.74, and 8.99 (g/dl) respectively. Hematocrit (HcT) contents were higher in SFS, followed by SF than S, 42.3, 38.25, and 35.63%, respectively. Greater values in WBCs, RBCs, Hb, and HcT, were observed in different treatment groups.

Blood Metabolites

The total protein was estimated higher *($p<0.05$)* in shower group (SFS) than SF and S treatment during the hot humid summer months (10.70, 7.24, 7.11 mg/dl, respectively). Albumin and globulin levels were observed lower in buffaloes under SFS, followed by SF than S treatment (35.89, 42.92, 46.61 and 39.50, 39.24, 25.20 mg/dl, respectively). No difference in globulin concentration was found between shower (SFS) and fan treatment (SF) but albumin levels were observed higher than controlled treatment (S). In present study, the serum glucose concentration was lower in controlled (S) than SF and SFS treatment (75.89, 78.79, 86.98 mg/dl, respectively). While, serum glucose concentration was observed high in SFS than other treatments (S and SF). Blood serum cholesterol concentration of lactating Nili-Ravi buffalo during hot humid season showed difference between treatment groups. The highest circulating cholesterol concentration was observed in control treatment (226.64 mg/dl) followed by SF (211.46 mg/dl) and SFS treatment (184.06 mg/dl) during the hot humid summer season of subtropical region.

Behavioral Parameters

Total time spent in feed intake (min/24h) was 246.33, 280.33 and 309.50 of S, SF and SFS, respectively. Greater values were found between different treatment groups. Highest total time spent in feed intake was observed in SFS followed by SF than S treatment. Total time spent in water intake (min/24h) of S, SF and SFS were 24.67, 22.50 and 19.50, respectively. Greater differences were observed between SFS and S but no difference was observed between S and SF treatment. Time spent in rumination in buffaloes under S, SF and SFS treatment was 360.83, 385.17 and 399.00 min/24h, respectively. Greater differences were observed between SFS and S treatments. While, no difference was observed between SFS and SF. Total time spent in sitting showed higher difference between SFS and A, while, no difference was observed between SFS and SF. Time spent in lying showed greater value in SFS than S. No difference was observed in S and SF treatment. Time spent in locomotion (min/24h) was 92.50, 76.33 and 68.67 of S, SF and SFS, respectively. Greater difference was observed between S and SF in time spent in locomotion. No difference was observed in SF and SFS. No difference was observed in total time spent in reproductive behaviors between different treatment groups.

Discussion

Results of the meteorological data of ambient environment are illustrated in Table 1. The data obtained represents that the buffaloes were exposed to a higher ambient temperature higher and higher relative humidity (RH) in controlled shed (A) during the humid summer compared to treatment shed SF and SFS. By incorporating the effect of ambient temperature and RH together, THI is calculated, which is more commonly used to quantify the discomfort level or it quantify the degree of thermal stress on animals (Bohmanova et al 2007; Vitali et al 2009). In present study, the calculated mean THI values showed higher difference between different treatment. THI values were observed with mean of 85.41**Â±**1.09, 83.07 **Â±**2.32, 81.75**Â±**0.8 in S, SF and SFS, respectively, which were well above the comfort value of THI (<72).

Results of present study showed that lactating buffaloes were under thermal stress during the hot humid season, based on the findings that calculated mean THI values exists during the hot humid season surpass the THI threshold well above, at which the most animals enter in the state of severe thermal stress (Marai et al 2007). Babor et al (2010) reported that internal and external body heat gradients under TNZ drive the heat flux from the body to the environment while the reverse will be the case in heat stressed animals. In present study, the body heat gradient of lactating buffaloes exhibited the noticeable reduction in controlled animals group, which proved that buffaloes tried to constrict the heat gradient in between their body and the surrounding environment to regulate their normal body temperature. This is in line with the study of Samara et al (2012) in goats, Al-Haidary et al (2013) in camel, Singh et al (2014) in Murrah buffaloes who reported THI has great impact on animal milk production. The use of sprinkling and fan system proved to be more useful to relief the buffalos from heat stress by lowering THI of the shed.

Table 1. Meteorological data of microclimate of Nili-Ravi in hot humid summer

Parameter	**S**	**SF**	**SFS**
THI	85.41Â±1.09a	83.07Â±2.32ab	81.75Â±0.8b
Ambient Temperature (^{o}C)	33.34Â±0.89a	32.01Â±1.79ab	30.46Â±0.7b
Humidity (%)	64.76Â±2.19ab	62.4Â±2.22b	69.37Â±2.81a

Means of triplicate analysis, different letters within the same row indicates significantly different result ($p<0.05$), n= 5/group
S: Shade only (control), SF: Shade and Fan ,SFS: Shade, Fan and Shower

Results of milk production of water buffaloes during the hot humid summer of subtropical region are illustrated in Table 2. Higher difference was observed in the milk production between different treatment groups of lactating Nili-Ravi buffaloes during the hot humid season. The difference in milk production was highest during the months of July and August, which are the important months for the assessment of impact of increased THI on milk production. Thereafter, the difference in milk production between treatments was although higher but lesser in extent, this difference persists until October. Harsh effects of increased THI, leads to lower DMI, increased water intake and decreased metabolism during the hot humid summer. The difference in milk production was primarily caused by the high temperature and humidity. Results of present study showed positive effect of sprinklers on buffalo milk production which was more apparent when environmental temperature increased. Therefore, higher milk production was observed in cooled group (SFS) followed by fan group (SF) then controlled group (AS), respectively. This might have been observed due to the higher ability of animals provided with the shower to thrive in hot humid season. Effect of heat stress on milk production is extensively studied (Novak et al 2009; De Rosa et al 2009; Sahin et al 2016).

Zewdu et al (2014) reported that climatic condition during hot summer months resulted in lower milk production in dairy animals. Moreover, increased THI was also found as the major factor for lower milk production during hot summer (Zewdu et al 2014). Abubakar (2012) suggested that higher environmental temperature has a negative correlation with milk yield, health and behavior of dairy animals but this can be overcome by providing shade, fan or some other cooling strategies.

Milk composition of buffaloes during hot humid summer is shown in Table 2. The sprinkler plus fan group of animals showed greater milk fat, milk protein and SNF contents as compared to SF and S animals. Increased milk fat, protein and SNF might be better microclimate environment as compared to SF and S animals, respectively. Milk production and constitutes decreases when ambient temperature increases well above the comfortable limits (Bailey et al 2005). Milk fat (%) decreased in heat stressed animals due to increase water intake, thus, the more water intake decreases the milk fat (%), protein (%) and SNF (%). Environmental variation in buffalo milk composition was greater in between treatment groups. Results of present study are in line with the study of Bailey et al (2005) in Holstein cows, Adin et al (2008) in cows, and Yadav et al (2013) in Murrah buffalo, who showed significantly decreased milk constitutes during hot humid summer as compared to winter.

Greater differnce was observed in DMI among three different groups of lactating buffaloes (Table 2). This might be due to the animal being exposed to different ambient environments and have lower value of THI (Khongdee et al 2011). Buffalo tends to increase feed intake as the THI decreases with the decrease in environmental temperature at the end of hot humid season. In dairy animals, effective heat dissipation might induce more efficient energy utilization and high ambient temperature associated with decreased dry matter intake, decreased metabolic rate which consequently decreased milk production, which represent the strategies to maintain their normal body temperature (Kadzere et al 2002). However, not only the high environmental temperature but high relative humidity in combination with increased environmental temperature made higher values of THI which plays important negative role on effect of heat stress on nutrient intake (Holter et al 1996). Previously studies showed negative correlation between THI and DMI (Farooq et al 2010). However, genetic and individual difference might exist for heat tolerance in dairy animals because of sweating capacity and reduced metabolic rates (Blackshaw and Blackshaw 1994). Decreased feed intake and thereafter, heat generated within the animal body during ruminal fermentation and body metabolism process, helps in maintaining heat balance (Aggarwal and Upadhyay, 2013). Results of our study are in line with the study of Farooq et al (2010) and Khongdee et al (2011) who reported decreased dry matter intake in heat stressed animals. Similarly, Pereira et al (2008) in Murrah buffaloes and Chaiyabutr et al (2008) in Holstein cattle reported 2% decrease in feed intake during hot summer season. Aggarwal and Singh (2010) and Singh et al (2014) also reported increased DMI in water cooled animals compared to other groups during hot humid season.

Water inside the body maintains hoemeothermy in heat stressed animals by the process of thermoregulation by dissipating accumulated heat through the process of sweating and panting. Water intake in experimental animals was higher in controlled group, followed by fan S and sprinkler group, respectively. The mean daily water intake in S, SF and SFS was 139.38, 122.61 and 112.7 liter/ day (Table 2). Increased water loss in heat stressed animals through skin and respiratory evaporation occurred which was an attempt to dissipate heat which disturbs the body water level, especially within vascular and extracellular components. The altered heat stress, changes the water intake within the dairy animal's ability to maintain blood pressure and osmotic balance, although increase water loss during thermal stress period due to sweating and panting can increase the chances of cardiovascular dysfunction and animal's inability euthermia (Abdel-Fattah 2014). Thermal stress in dairy animal influences the water metabolism by increasing body plasma and volume of extracellular fluid in response to thermoregulatory requirements (Silanikove 1992). Singh et al (2014) reported an increased in water intake in heat stressed animals.

Table 2. Productive performance of Nili-Ravi buffaloes during hot humid summer

Parameter	**S**	**SF**	**SFS**

Milk Production (Kg/d)	5.66Â±0.23c	7.27Â±0.2b	10.45Â±0.79a
Fat (%)	6.02Â±0.062c	6.41Â±0.06b	6.77Â±0.10a
Protein (%)	3.52Â±0.089b	3.70Â±0.025a	3.85Â±0.04a
SNF (%)	8.73Â±0.033b	8.97Â±0.07a	9.11Â±0.03a
Lactose (%)	4.71Â±0.03a	4.69Â±0.04a	4.80Â±0.06a
DMI (Kg/d)	13.24Â±0.45b	14.56Â±0.22a	14.73Â±0.35a
Water Intake (Liter/d)	139.38Â±2.23a	122.61Â±0.73b	112.74Â±4.58c

Different letters within the same row indicate significantly different result ($p<0.05$). For the detail of groups see Table no.1.

Results of Rectal temperature (RT), skin temperature (ST), respiration rate (RR) and pulse rate (PR) were in lactating buffaloes during hot humid season are presented in Table 3. Increased RT during hot humid season in controlled group might illustrate the insufficient evaporative heat dissipation from animal’s body. During the hot humid summer season, body temperature is relative higher due to increased environmental temperature with increased humidity. Increased RR was due to increase in RT, which might be directly proportional to RR. Increase in respiration rate during hot humid season suggested the adjustment of respiratory function by increasing evaporative heat loss (Korde et al 2007). Whereas, Singh et al (2014) reported that RR is associated with the environmental temperature, solar radiation, wind velocity, air temperature and relative humidity. Increased RR in heat stressed animals in hot humid summer might be due to increased demand of oxygen by the different body tissues in stress condition (Singh et al 2014). PR increases with high blood volume (Guyton 2001) which might be the reason of increased pulse rate during hot humid season in buffaloes.

Greater values were observed in skin temperature in different groups during hot humid season. Increased difference in skin temperature was observed due to the lower ambient temperature and due to the effect of water on animal which cooled the animals through conduction process. Results showed that lactating buffaloes were more comfortable in sprinkling with water animals compared to fan and controlled animals. Present study results are in agreement with Sigh et al (2005) in Nili-Ravi buffalo, Aaggarwal and Singh (2008) in Murrah buffaloes, (Rahangdale et al 2010) in Murrah buffalo, Das et al (2011) in Nili-Ravi calves, and Singh et al (2014b) in Murrah buffalo, who reported significant decreased in RR, PR, ST and RT when buffalo animals were subjected to water splashing during hot summer. In different researches, the physiological parameters were decreased with the increase in cooling facilities compared with the non-cooled buffaloes during the hot summer.

Table 3. Physiological parameters of Nili-Ravi buffaloes during hot humid season

Parameter	**S**	**SF**	**SFS**
Rectal Temperature (^{o}F)	102.06Â±0.07a	101.69Â±0.08b	101.05Â±0.09c
Skin Temperature (^{o}C)	34.66Â±4.77a	33.55Â±0.04b	32.38Â±0.15c
Pulse Rate (beats/min)	69.80Â±1.52a	64.72Â±3.96a	54.30Â±1.09b
Respiration rate (breath/min)	38.45Â±0.84a	33.80Â±0.82b	26.15Â±1.12c

n **=** *5; different letters within the same row indicate significantly different result (p<0.05)*

Higher values in WBCs, RBCs, Hb, and HcT were observed between the treatment groups (Table 4). Increase in RBCs, Hb and HcT contrary to WBCs might be due to the increase hemoconcentration during hot humid period. Hb concentration might be reduced in heat stressed group due to thermal radiation in buffaloes. It may be due to the fact that dairy animals reduce their metabolic rate which in turn decreases their metabolic heat production which results in decreased Hb concentration during hot humid summer season (Das et al 2016). Increased mean values of RBCs count, Hb, and HCT during the hot humid summer could be the result of improvement in DMI in sprinkler SFS compared to SF and S. The decline in RBCs counts Hb and HCT in hot humid summer period might be in relation to the decrease in thyroid secretion associated with lowered erythropoiesis. Thyroid hormones enhance the development rate of erythroid progenitors (Popovic et al 1977), which increase the production of erythropoietic growth factors (Fandrey et al 1994). Adjustment in thyroid response is slow and as a result during hot summer thyroid activity is decreased (Habeeb et al 1992). Result of present study, during hot humid summer, the RBCs counts, Hb and HCT in Nili-Ravi buffaloes, are in harmony with the findings of Bhatti et al (2009) in Nili-Ravi heifers, Abdelatif et al (2009) in goats, and Chaudharyet al (2015) in Surti buffaloes who reported decreased counts of RBCs, Hb and HCT during hot humid season. Hematological profile is an important physiological stress indicator in animals (Kumar and Pachaura, 2000). RBCs indicate the adaptation of animals to the ambient environment. Hb concentration determines the acclimatization into the adverse environment. These values used to determine the stress and welfare in animals (Anderson et al 1999). Sareedhar et al (2013) reported non-significant difference in HCT in crossbred Sahiwal cattle calve during hot summer period. Whereas, present study results with respect to WBCs are in accordance with the study of Das et al (2014) in lactating Nili-Ravi, and Abdelatif et al (2009) in goats, who reported significant increase in WBCs during hot humid summer. Olayemi et al (2009) reported higher WBCs, in African dwarf goats, due to the increased challenges from harsh environment. Contrary to study, Bhan et al (2012) found decreased level of WBCs in cattle during the hot summer months.

Table 4. Hematological values of Nili-Rvi buffalo during hot humid summer

Parameters	**S**	**SF**	**SFS**
WBC (10-3/Âµl)	8.99Â±0.23a	7.76Â±0.07b	7.49Â±0.05b
RBC (10-6/Âµl)	7.48Â±0.24b	8.19Â±0.25a	8.23Â±0.14a
Hb (g/dl)	8.99Â±0.20b	9.74Â±0.18ab	10.47Â±0.19a
HCT (%)	35.63Â±722.86c	38.25Â±0.65b	42.30Â±0.95a

n **=** *5 per treatment; different letters within the same row indicate significantly different result (p<0.05).*
WBC = White blood cells; RBC=Red blood cells; Hb=Hemoglobin; HCT=Hematocrite

Serum total protein level lowered in heat stressed animals during the thermal stress period (Gudev et al 2007). Thermal stress cause initial hemoconcentration followed by the hemodilution. Decreased level of serum total protein suggested that animals might be in the stage of hemodilution in heat stressed animals. Serum total protein, globulin and albumin concentration could be improved by cooling the microenvironment of animals (El-Khashab, 2010). Habeeb et al (2007) observed decreased concentration of total protein levels in heat stressed animals. Similar findings were observed by Hood and Singh (2010) in buffalo heifers that total protein concentration was lowered in heat stressed animals than cooled animals. Another experiment of Aboulnaga et al (1989) found that sprinkling increased the serum total protein concentration. Kumar et al (2011) illustrated that blood protein, globulin and albumin levels were greater between the cooled and non-cooled buffalo heifer groups. However, in the experiment of Koubkova et al (2002), total protein concentrations were found higher during the heat stress period followed by the significant drop in total protein level with cooling in summer season.

Decrease in glucose concentration occurred due to increase in basal insulin concentration in heat stressed animals (Singh et al 2012). Increase in serum protein level might be the physiologically attempt by the heat stressed animals to maintain increased plasma level (Rasooli et al 2004). Krober et al (2000) reported that increased plasma glucose level were found by providing protected protein. Abdel-Ghani et al (2011) found the increased level of glucose in the sheep, might be due to positive effect of protein on animal nutrition which increases glucose concentration in the blood of animal. Present study results are in agreement with the study of Add El-Latif (2003) in growing Friesian calves, Singh et al (2012) in buffalo heifers, Ranjan et al (2012) lactating Murrah buffaloes, who reported higher level of blood glucose in response to thyroid hormone which might increases the carbohydrate metabolism and it increases the plasma glucose level in blood of animals (Cole et al 1994).

Serum cholesterol concentration was increased in control group during the hot humid season due to the increased cortisol synthesis that occurs in heat stressed animals as the serum cholesterol acts as a precursor for the synthesis of steroid hormones within the body. The results of present study were in harmony with Kumar et al (2012) in Beetal goats, Sejian et al (2013) in Malpura ewes, Kalmath et al (2013) in Hallikar cattle and Das et al (2013) who reported increased serum cholesterol in animals during hot humid summer. In contrast to present study, Anand and Parkash (2008) in Murrah buffaloes, Cincovic et al (2011) in Hostein cows, Pandey et al (2012) in Marwari goats, who reported decreased level of cholesterol in heat stressed animals. During the heat stress period, the serum cholesterol level was tended to lower thyroid activity (Pandey et al 2012), which decreased the feed intake during the hot environment and consequently reduced intake of dietary cholesterol (Gudev et al 2007).

Table 5. Blood biochemical indices in lactating Nili-Ravi during hot humid summer

Parameters	**S**	**SF**	**SFS**
Total Protein (mg/dl)	7.11Â±0.35b	7.24Â±0.4b	10.70Â±0.22a
Albumin (mg/dl)	46.61Â±2.13a	42.92Â±2.53b	35.89Â±2.06c
Globulin (mg/dl)	25.20Â±1.85b	39.24Â±3.61a	39.50Â±1.64a
Glucose (mg/dl)	75.89Â±1.87b	78.79Â±3.52b	86.98Â±3.12a
Cholesterol (mg/dl)	226.64Â±3.01a	211.46Â±3.06a	184.06Â±2.81b

n=5; different letters within the same row indicate significantly different result (p<0.05)

Results of behavioral responses (mins/24h) of Nili-Ravi buffaloes during the hot humid summer of subtropical region are illustrated in Table 6. Buffalo had greater feed intake time in sprinklers cooled group (SFS) followed by SF than controlled treatment (S). SFS animals had spent 24.64%, while animals in SF spent 13.8% spent more time in feed intake as compared to S. The heat stress abatement strategies used might have persuaded lactating buffaloes to spent increased time in feeding during hot humid season.

Rumination time also differ significantly among different treatment groups. Total time spent in ruminating was 10.57 and 6.74% more time in SFS, and SF than heat stressed animals, respectively. This could be due to the increased feed intake and decreased metabolic heat within the body of animals. Cooled ambient temperature might influence the rumination activity of the animals. Total time spent in lying, differ in different treatment groups. Lactating buffalo in SFS spent 22.71% more time than treatment groups during hot humid season. This might be due to cooled ambient environment and cooled ground due to sprinkling effect and animals dissipate their accumulated heat via process of conduction. Increased difference were observed in locomotion in different treatment groups. Increased locomotion was observed in heat stressed animals (controlled group) than cooled animals during the hot humid summer season. Increased locomotion might be due to higher ambient temperature and higher metabolic accumulated heat which in turn did not allow animals to get rid of excessive heat load within the body of animals to the hotter environment. While cooled animals get rid of their excessive heat load through conduction process via cooled floor. If comfortable conditions are provided within the shed of animals, dairy animals spent more time lying down (Overton et al 2002). In agreement to literature, the effective of climatic variation on animal behavior indices is evident at increased THI values (Provolo and Riva 2009). Our results are in line with the study of Overton et al (2002) in cattle, Tucker and Wearsy (2004) in cows, and Cook et al (2007) in lactating cows, who reported increased lying time in cooled animals.

Table 6. Behavioral Response of Buffalo during Hot Humid Summer

Treatment	**S**	**SF**	**SFS**
Feed Intake	246.33Â±9.99c	280.33Â±3.85b	309.50Â±7.64a
Water Intake	24.67Â±0.84a	22.50Â±0.84a	19.50Â±0.62b
Rumination	360.83Â±8.41b	385.17Â±3.30a	399.00Â±4.72a
Sitting	357.17Â±9.26b	386.00Â±4.34a	398.50Â±8.96a
Standing	306.83Â±12.43	294.00Â±9.22	281.33Â±8.32
Lying	193.00Â±10.29b	197.67Â±10.49b	236.83Â±3.10a
Locomotion	92.50Â±4.83a	76.33Â±2.97b	68.67Â±1.74b
Reproductive Behavior	0.833Â±0.40	1.17Â±0.48	0.833Â±0.31

All parameters were recorded as minutes per 24 hours

Conclusion

Temperature humidity index based on temperature and humidity recorded during the trial period within the microclimate suggested that micro-environment for Nili-Ravi buffaloes was stressful as indicated by the drop in milk production, and alternation in animal physiological responses (rectal temperature, skin temperature, respiration rate, pulse rate), hematological parameters (WBCs, RBCs, Hb and HcT) and blood biochemical parameters (total protein, albumin, globulin, glucose and cholesterol) in control group, fan plus sprinkler group. Sprinkling with water plus fan for ventilation, proved to be more efficient as a cooling strategy during the hot humid summer of subtropical region by preventing alteration in physio-biochemical responses and drop in milk production.

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