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Effect of Spring and Summer Seasons on Some Bio-physiological Markers in Buffaloes

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ABSTRACT

Background: The purpose of this study was to ascertain the effects of the hot, dry spring and summer weather on the endocrinological and biochemical properties of buffaloes with the help of physiological as well as biochemical analysis.

Methods: Like plasma glucose using GOD-POD kits, total plasma protein using Autospan kits, Blood Urea Nitrogen using MBK Urea kits DAM assay, cortisol hormone by bovine cortisol enzyme linked immunosorbent assay kit *etc*.

Result: During the summer season, there was a significant (P<0.01) reduction in plasma glucose level (mg/dl), blood urea nitrogen level (mg/dl) and plasma total protein level (g/dl) in comparison to the spring season, but there was a significant (P<0.01) increase in plasma cortisol level in the summer in comparison to the spring season in all three districts. From the current study, it can be inferred that heat stress negatively affects the adaptability and metabolic reactions of buffalo.

Key words: Adaptability, Biochemical analysis, Buffalo, Cortisol, Physiological parameter.

INTRODUCTION

The buffalo (*Bubalus bubalis*) is referred to as the “black diamond” among Asian countries because of its valuable milk and meat output, as well as its use as a source of energy, fuel and for the production of leather. India, a tropical country, experiences warm, humid summers and mild winters. Eastern Uttar Pradesh experiences hot weather in March and April that is uncomfortable for nursing animals; these months are known as the spring season, while June features hot and muggy weather that is considered to be the summer season. During the summer (May-June), when the atmospheric temperature reaches 45°C during the day and 30°C at night, the photoperiod increases to 12-14 hours. In extreme environmental conditions, buffaloes are physiologically less adaptable than cattle. This is because the black skin coat absorbs the solar radiation and is less efficient at dissipating the heat from the body. In addition to these factors, their inability to sweat well results in a less effective heat-draining cooling mechanism. Animal production and reproduction are generally affected by many factors, especially the environment and nutrition. However, at high ambient temperatures, this has a negative effect on appetite, slowing growth and impairing reproduction (Marai *et al*., 2006; Marai *et al*., 2002).

Buffaloes decrease their performance at high ambient temperatures due to their black skin colour and fewer sweat glands (Upadhyay *et al*., 2010). Buffaloes cause various physiological changes in their body during hot conditions that include less feed consumption, inconvenience in the metabolism of energy, protein and water, stability of minerals, secretion of endocrine glands, action of enzymes and metabolites of blood. That physiological change causes deterioration in the reproduction and production of animals (Das *et al*., 2014). To protect the buffaloes from the heat stress, ensure that they have proper housing for protection against direct solar radiation. However, thermal shock due to high wind speeds and rapid temperature variation can produce heat stress, which increases susceptibility to diseases. Summer stress initiates a series of biological and physiological changes like depression of food intake, variation in hormonal secretions and increased respiration rate (RR), pulse rate (PR), rectal temperature (RT) and sweating in buffaloes (Das *et al*., 2016; Bombade *et al*., 2017).

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Stress is an automatic reaction of animals in adverse environments and causes unfavourable sequelae ranging from discomfort to death. A change in the climate is one of the major threats to the existence of various species of animals, ecosystems and livestock production systems across the globe, especially in tropical and temperate areas of the world. As a result, thermal stress in a homoeothermic animal can vary depending on species, breed and productivity to some extent. Several previous studies concluded that native breeds survive and perform better under tropical environmental conditions than non-native breeds and their crosses, which may be due to non-native genes' inability to express or adjust under tropical conditions.

A better understanding of the changes in the buffaloes' physiology during extreme environmental conditions can help in preventing heat stress-related economic loss and improving their welfare. The goal of this study was to determine how the hot, dry weather of the summer and spring affected the buffaloes' biological performance in terms of their endocrinological and biochemical characteristics.

MATERIALS AND METHODS

In the year 2021, the trials were conducted for a period of four weeks in Ayodhya, Mau and Bhadohi districts during the spring (March) and summer (June) seasons. In the spring (March) and summer, every climatic variable was noted twice daily, from 7:30 a.m. to 2:30 p.m. (June). To define ambient conditions, the temperature and humidity index are used. THI was calculated from the formula (NRC, 1971). Eighteen lactating Murrah breed buffaloes were randomly selected for this study, one group in each district. The Murrah buffaloes were maintained under standard management conditions. The animals were maintained in an open area made up of a brick floor and an asbestos roof. Inside the house, provisions were made so that the animals could move freely to get to the feed and water. The animals were given food and water *ad libitum*.

All the animals were fed on a ration consisting of a concentrated mixture and roughages (berseem, maize, or jowar fodder as per availability) and fed as per the standard feeding practises followed. The concentrated mixture consisted of mustard cake, maize, wheat bran, rice bran, a mineral mixture and salt. During the summer season, proper shade facilities are available at various farm and field conditions. At Livestock Farm Complex, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya and also from different private dairy farms and field conditions in Mau and Bhadohi districts.

In the spring and summer seasons, blood samples (10 mL/buffalo) were collected in sterile tubes at weekly intervals with EDTA in the afternoon at 1:30 pm to 2:30 pm from jugular vein punctures, causing minimal disturbance to the animal. The collected blood samples were taken for plasma collection and biochemical analysis like plasma glucose, total plasma protein, blood urea nitrogen (BUN), cortisol hormone, *etc*. n the spring and summer seasons, blood samples (10 mL/buffalo) were collected in sterile tubes at weekly intervals with EDTA in the afternoon at 1:30 pm to 2:30 pm from jugular vein punctures, causing minimal disturbance to the animal. The collected blood samples were taken for plasma collection and biochemical analysis like plasma glucose, total plasma protein, blood urea nitrogen (BUN), cortisol hormone, *etc*.

Physiological parameters

The physiological parameters, including rectal temperature, pulse rate and respiration rate, were recorded at weekly intervals during the study period in the spring and summer seasons.

The rectal temperature was recorded by a digital electronic thermometer, the respiration rate was recorded by observing the flank movement and pulse rate and the coccygeal artery was palpated beneath the root of the tail**.** Meteorological parameters like maximum and minimum environmental temperatures and relative humidity were recorded in both seasons during the study period.

Biochemical analysis of plasma cortisol

Cortisol was determined in the plasma of buffaloes by a "Bovine Cortisol ELISA kit" (Catalog No. E0110Bo) from the Bioassay Technology Laboratory, 501 Changsheng South Road, Nanhu Dist., Jiaxing, Zhejiang, China, as per standard protocol. The optical density (OD value) was measured at 450 nm wavelength for each well using an ELISA reader within 10 minutes after adding the stop solution.

Glucose

Glucose was estimated in plasma samples from buffalo by using GOD-POD kits from Span Diagnostics Ltd. (Kaplan, 1984 and Trinder, 1969).

Urea analysis

According to Roberts *et al*., 2008, blood Urea was estimated in buffalo plasma samples using MBK Urea kits DAM Assay from Arkray Healthcare Pvt. Ltd.

Total protein

Total protein was estimated in plasma samples from buffalo by using Autospan kits from Arkray Healthcare Pvt. Ltd. The principle of the assay is described by Roberts *et al*. (2008).

Statistical analysis

A statistical analysis of the data was carried out to find the mean±SE. A paired "t-test" was done to find significant differences between groups of experiments and their interaction using Prism-5 software. The correlations among the various parameters were also calculated by Snedecor and Cochran (2004).

RESULTS AND DISCUSSION

Physiological parameter

Rectal temperature

The value of the rectal temperature (oF) of buffaloes is presented in Table 1, 2 and 3. Rectal temperature was

significantly (P<0.01) higher in the summer season in comparison to the spring season; similar observations of rectal temperature have been reported by Lemrele and Goddard (1986) and Kaubkova *et al*. (2002). The increase in rectal temperature during summer is probably due to an increase in the temperature-humidity index (more than 80) during the summer season (Lemrele and Goddard 1986).

Pulse rate

The result of pulse rate (per minute) in the spring and summer seasons of buffaloes is presented in Table 1,2,3.

In buffaloes from all three districts, the pulse rate was significantly (P<0.01) higher in the summer than in the spring. This transient increase in pulse rate in the present study is probably due to heat stress in response to an increase in THI (Aggarwal and Singh, 2008). Similar effects of heat stress on pulse rate have been reported by Ganaie *et al*. (2013) in buffaloes.

Respiration rate

The value of respiration rate (per minute) has been presented in Table 1, 2, 3. It was found that the respiration rate of buffalo significantly (P<0.01) increases in the afternoon in all three districts. The increase in respiration rate may be attributed to the animal's heat load exceeding the animal's body's capacity to dissipate heat and (Aggarwal and Singh, 2006) the significant variation in respiration rate in buffaloes coincides with the finding of Kumar *et al*. (2018).

Temperature humidity index (THI)

The Mean±SE of THI was 78.21±0.86, 79.62±0.96 and 77.39±0.70 in Ayodhya, Bhadohi and Mau districts during the spring season. During the summer season Mean±SE of THI was 83.98±0.44, 85.28±1.12 and 83.07±1.28 in Ayodhya, Bhadohi and Mau districts, respectively. The THI value of the summer season was higher than the spring season. A similar observation of THI was reported by Li *et al*., (2020).

Biochemical changes

Plasma glucose

The results of plasma glucose level (mg/dl) in buffaloes during the spring and summer seasons were presented in Table 4, 5, 6. In all three districts, there was a significant (P<0.01) reduction in plasma glucose levels during the

Table 1: Mean±SE of rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute) in morning and afternoon during spring and summer season in buffaloes in Ayodhya district of Uttar Pradesh.

Week	Parameters	Rectal temperature (°F)		Pulse rate (Per minute)		Respiration rate (Per minute)	
	Season	Spring	Summer	Spring	Summer	Spring	Summer
First	Morning	101.17±0.07	101.23±0.03	41.67±0.21	40.67±0.42	20.67±0.33	21.50±0.22
	Afternoon	101.38±0.06	101.47±0.05	43.00±0.37	44.67±0.49	22.17±0.31	24.17±0.17
Second	Morning	101.12±0.03	101.28±0.09	41.33±0.21	41.00±0.26	20.67±0.21	20.67±0.21
	Afternoon	101.40±0.14	101.52±0.14	42.67±0.21	46.00±0.45	22.33±0.33	24.83±0.44
Third	Morning	101.12±0.03	101.15±0.04	41.50±0.22	41.00±0.37	20.83±0.31	20.33±0.33
	Afternoon	101.27±0.02	101.40±0.04	43.17±0.31	46.17±0.48	22.50±0.22	25.50±0.56
Fourth	Morning	101.13±0.04	101.22±0.05	41.50±0.22	42.00±0.26	20.83±0.31	21.33±0.21
	Afternoon	101.37±0.12	101.47±0.04	43.17±0.31	47.33±0.33	22.33±0.21	26.67±0.49
Mean±SEM (Morning)		101.13±0.04	101.22±0.05	41.50±0.22	41.92±0.33	20.75±0.29	20.96±0.24
Mean±SEM (Afternoon)		101.35±0.08	101.46±0.07	42.96^{a}±0.29	46.04^{b}±0.44	22.33^{a}±0.27	25.29^{b}±0.41

Notes: Means bearing different superscripts differ significantly (P<0.01).

Table 2: Mean ±SE of rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute) during spring and summer season in buffaloes in Bhadohi district of Uttar Pradesh.

Week	Parameters	Rectal temperature (°F)		Pulse rate (Per minute)		Respiration rate (Per minute)	
	Season	Spring	Summer	Spring	Summer	Spring	Summer
First	Morning	101.08±0.03	101.23±0.03	41.50±0.22	40.83±0.31	20.83±0.31	21.33±0.21
	Afternoon	101.28±0.05	101.47±0.049	43.17±0.31	45.00±0.37	22.17±0.31	26.67±0.49
Second	Morning	101.08±0.03	101.23±0.08	41.50±0.22	41.17±0.17	20.83±0.31	20.33±0.33
	Afternoon	101.27±0.07	101.45±0.096	43.17±0.31	45.67±0.76	22.67±0.21	25.50±0.56
Third	Morning	101.08±0.03	101.15±0.04	41.33±0.21	40.83±0.40	20.67±0.21	20.67±0.21
	Afternoon	101.23±0.03	101.40±0.037	42.67±0.21	45.67±0.56	22.00±0.37	24.83±0.40
Fourth	Morning	101.15±0.04	101.22±0.05	41.67±0.21	41.00±0.26	20.83±0.31	21.50±0.22
	Afternoon	101.37±0.12	101.47±0.042	43.00±0.37	47.33±0.33	22.50±0.22	24.17±0.17
Mean±SEM (Morning)		101.10±0.03	101.21±0.05	41.50±0.22	40.96±0.28	20.79±0.28	20.96±0.24
Mean±SEM (Afternoon)		101.29^{a}±0.07	101.45^{b}±0.06	43.00^{a}±0.30	45.92^{b}±0.50	22.33^{a}±0.28	25.29^{b}±0.41

Notes: Means bearing different superscripts differ significantly (P<0.01).

summer season compared to the spring season. The reason for the decrease in plasma glucose may be attributed to an increase in respiration rate in a hot climate that causes more utilization of glucose by the respiratory muscle Shafferi *et al*. (1981).

Similar observations of a reduction in plasma glucose levels in buffaloes during the summer are reported by Ronchi *et al*. (1995) and Verma *et al*. (2000).

Blood urea nitrogen (BUN)

The results of blood urea nitrogen levels (mg/dl) in buffaloes during the spring and summer seasons were presented in Table 4, 5, 6. In all three districts, there was a significant (P<0.01) reduction in blood urea nitrogen levels during the summer season compared to the spring season.

The decrease in blood urea nitrogen level may be attributed to increased resorption of urea nitrogen from the blood to the rumen to compensate for lower ammonia-nitrogen concentrations as a result of reduced feed consumption (Yousef, 1990) and digestible nitrogen utilization. Similar observations of a reduction in blood urea nitrogen levels during summer are reported by Ronchi *et al*. (1995) and Verma *et al*. (2000) in buffaloes and Kamal *et al*. (1989) in Friesian calves.

Table 3: Mean±SE of rectal temperature (°F), pulse rate (Per minute) and respiration rate (per minute) during spring and summer season in buffaloes in Mau district of Uttar Pradesh.

Week	Parameters	Rectal temperature (°F)		Pulse rate (Per minute)		Respiration rate (Per minute)	
	Season	Spring	Summer	Spring	Summer	Spring	Summer
First	Morning	101.15±0.04	101.22±0.05	41.50±0.22	40.67±0.42	21.00±0.37	21.50±0.22
	Afternoon	101.40±0.11	101.47±0.04	43.17±0.31	44.67±0.49	22.33±0.21	24.33±0.21
Second	Morning	101.12±0.02	101.15±0.04	41.33±0.21	41.00±0.26	20.83±0.31	20.67±0.21
	Afternoon	101.35±0.14	101.40±0.04	42.83±0.31	46.00±0.45	22.50±0.22	24.83±0.40
Third	Morning	101.12±0.04	101.28±0.09	41.33±0.21	41.00±0.37	20.50±0.22	20.33±0.33
	Afternoon	101.28±0.05	101.52±0.14	42.67±0.21	46.17±0.48	21.83±0.40	25.50±0.56
Fourth	Morning	101.15±0.07	101.23±0.03	41.67±0.21	41.00±0.26	20.83±0.31	21.33±0.21
	Afternoon	101.32±0.08	101.47±0.05	43.00±0.37	47.33±0.33	22.17±0.31	26.67±0.49
Mean±SEM (Morning)		101.13±0.04	101.22±0.05	41.46±0.21	40.92±0.33	20.79±0.30	20.96±0.24
Mean±SEM (Afternoon)		101.34±0.09	101.46±0.07	42.92^{a}±0.30	46.04^{b}±0.44	22.21^{a}±0.29	25.33^{b}±0.42

Notes: Means bearing different superscripts differ significantly(P<0.01).

Table 4: Mean±SE of plasma glucose level (mg/dl) and blood urea nitrogen (mg/dl) during spring and summer season in buffaloes in Ayodhya district of Uttar Pradesh.

Week	Plasma glucose level (mg/dl)		Blood urea nitrogen (mg/dl)	
	Spring	Summer	Spring	Summer
First	74.62±0.35	72.09±0.25	17.02±0.19	14.71±0.17
Second	74.39±0.35	72.09±0.30	16.95±0.19	14.56±0.16
Third	74.22±0.44	72.14±0.31	16.91±0.25	14.85±0.19
Fourth	74.41±0.31	72.10±0.17	16.86±0.26	14.61±0.19
Mean±SEM	74.41^{a}±0.36	72.10^{b}±0.26	16.93^{a}±0.22	14.68^{b}±0.18

Notes: Means bearing different superscripts differ significantly (P<0.01).

Table 5: Mean±SE of plasma glucose level (mg/dl) and blood urea nitrogen (mg/dl) during spring and summer season in buffaloes in Bhadohi district of Uttar Pradesh.

Week	Plasma glucose level (mg/dl)		Blood urea nitrogen (mg/dl)	
	Spring	Summer	Spring	Summer
First	74.40±0.30	72.20±0.24	16.55±0.27	14.64±0.14
Second	74.14±0.45	72.00±0.30	16.58±0.25	14.85±0.19
Third	74.58±0.39	72.10±0.19	16.47±0.25	14.80±0.18
Fourth	74.77±0.38	72.14±0.31	16.31±0.25	14.62±0.20
Mean ±SEM	74.47^{a}±0.38	72.11^{b}±0.26	16.47^{a}±0.25	14.72^{b}±0.18

Notes: Means bearing different superscripts differ significantly (P<0.01).

Plasma total protein

The results of plasma total protein level (g/dl) in buffaloes during the spring and summer seasons were presented in Table 7, 8, 9. The reason for the decrease in plasma total protein level may be attributed to a decrease in consumption of dietary protein during heat stress due to more THI in the summer season. In prolonged dietary protein deficiency, total plasma protein synthesis is significantly reduced (William, 2005).

Similar observations of a reduction in total plasma protein level during heat stress are reported by Habeeb *et al.* (2007) in buffalo calves and Verma *et al.* (2000) in Murrah buffaloes. Rasooli *et al*. (2004) found a significant increase in total plasma protein in Holstein heifers during the hot summer season, but their findings do not agree with our findings in buffalo during the summer season; this could be due to differences in environmental and management conditions.

Plasma cortisol

The concentration of plasma cortisol (ng/ml) has been presented in Table 7, 8, 9. In all three districts, plasma cortisol levels increased significantly (P<0.01) during the summer season compared to the spring season.

The reason for the increase in plasma cortisol level may be attributed to a stressful condition. The hypothalamic-pituitary-adrenal axis is activated, causing plasma cortisol levels to rise. Plasma cortisol helps in the physiological adjustments of animals during stressful conditions (Christison and Johnson, 1972). Lakhani *et al*. (2018) reported a similar increase in plasma cortisol level during stressful conditions in Murrah buffalo, Liu *et al*. (2020) in Nili-Ravi buffalo and Chaudhary *et al*. (2015) in Surti buffalo.

Table 6: Mean±SE of plasma glucose level (mg/dl) and blood urea nitrogen (mg/dl) during spring and summer season in buffaloes in Mau district of Uttar Pradesh.

Week	Plasma glucose level (mg/dl)		Blood urea nitrogen (mg/dl)	
	Spring	Summer	Spring	Summer
First	74.05±0.28	71.95±0.22	16.72±0.16	14.65±0.18
Second	74.19±0.43	71.96±0.30	16.68±0.23	14.56±0.17
Third	74.60±0.34	72.04±0.21	16.50±0.16	14.73±0.14
Fourth	74.81±0.42	72.04±0.31	16.38±0.16	14.54±0.16
Mean ±SEM	74.41^{a}±0.37	72.00^{b}±0.26	16.57^{a}±\0.18	14.62^{b}±0.16

Notes: Means bearing different superscripts differ significantly (P<0.01).

Table 7: Mean±SE of plasma total protein (g/dl) and plasma cortisol level (ng/ml) during spring and summer season in buffaloes in Ayodhya district of Uttar Pradesh.

Week	Plasma total protein (g/dl)		Plasma cortisol level (ng/ml)	
	Spring	Summer	Spring	Summer
First	7.47±0.06	6.52±0.08	4.80±0.27	12.68±0.18
Second	7.70±0.10	6.63±0.08	4.79±0.18	12.22±0.16
Third	7.53±0.13	6.49±0.14	4.58±0.19	12.17±0.25
Fourth	7.58±0.06	6.68±0.09	4.53±0.19	11.68±0.24
Mean±SEM	7.57^{a}±0.09	6.58^{b}±0.10	4.67^{a}±0.21	12.19^{b}±0.21

Notes: Means bearing different superscripts differ significantly(P<0.01).

Table 8: Mean±SE of plasma total protein (g/dl) and plasma cortisol level (ng/ml) during spring and summer season in buffaloes in Bhadohi district of Uttar Pradesh.

Week	Plasma total protein (g/dl)		Plasma cortisol level (ng/ml)	
	Spring	Summer	Spring	Summer
First	7.63±0.08	6.53±0.03	4.45±0.17	12.07±0.37
Second	7.54±0.13	6.74±0.11	4.44±0.14	12.03±0.31
Third	7.59±0.06	6.53±0.11	4.34±0.15	11.78±0.35
Fourth	7.43±0.12	6.63±0.08	4.31±0.17	11.47±0.32
Mean±SEM	7.55^{a}±0.10	6.60^{b}±0.08	4.38^{a}±0.16	11.84^{b}±0.34

Notes:Means bearing different superscripts differ significantly(P<0.01).

Table 9: Mean±SE of plasma total protein (g/dl) and plasma cortisol level (ng/ml) during spring and summer season in buffaloes in Mau district of Uttar Pradesh.

Week	Plasma total protein (g/dl)		Plasma cortisol level (ng/ml)	
	Spring	Summer	Spring	Summer
First	7.67±0.08	6.57±0.11	5.61±0.28	12.37±0.24
Second	7.62±010	6.61±0.10	5.43±0.27	12.25±0.16
Third	7.74±0.08	6.66±0.07	5.26±0.26	12.09±0.25
Fourth	7.57±0.05	6.65±0.15	5.10±0.26	11.67±0.23
Mean±SEM	7.65^{a}±0.08	6.62^{b}±0.11	$5.3.5^{a}$±0.27	12.09^{b}±0.22

Notes: Means bearing different superscripts differ significantly(P<0.01).

CONCLUSION

Buffaloes showed better thermal adaptability during spring season as compared to summer season. The stress hormone (cortisol) level were more during summer season as compared to spring season. Biochemical estimation revealed total protein, plasma glucose and Blood Urea Nitrogen reduced in summer as compared to spring season. In present study it can be concluded that heat stress has unfavorable effect on adaptability and biochemical responses of buffalo.

Conflict of interest: None.

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