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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 1491-1495

www.thepharmajournal.com
Received: 18-04-2022
Accepted: 21-05-2022

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Impact of seasonal variation on physiological and haematological attributes in murrah buffalo

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Abstract
Present study was designed to investigate the changes in physiological and haematological attributes during different seasons in Murrah buffaloes. This study revealed a substantial seasonal impact on all physiological and haematological attributes. Physiological indicators such as rectal temperature, respiration rate and pulse rate had greater values in the hot-humid season than winter season. Physiological attributes were recorded prior to blood sample collection during both season. The rectal temperature (RT), respiration rate (RR) and pulse rate (PR) was measured with electronic thermometer inserting into rectum, through nostrils during normal respiration and through a point on the underside of the base of the tail (middle coccygeal artery), respectively. Blood samples were collected aseptically from jugular vein using 18 gauge needles in blood vacutainer BS EDTA K3 vial (2ml) and then mix each blood sample. The haematological attributes were determined by Spincell 5 Compact Vet Mode Automated Blood Analyzer. The mean±SE values of Total Erythrocyte Count /RBC count, Neutrophil (%) showed a significantly ($P \leq 0.01$) higher level during hot-humid than winter season while Lymphocyte (%) was significantly ($P \leq 0.01$) higher in winter as compared to hot-humid season. The mean±SE values of Total leukocyte count/WBC count were significantly ($P \leq 0.05$) elevated during winter than hot-humid season whereas a non-significant ($P > 0.05$) effect of hot-humid and winter season was observed on the mean±SE values of Monocyte (%), Eosinophil (%), Basophil (%). This study showed effect of winter and hot-humid seasons on the health of Murrah buffalo in terms of variation in different physiological and haematological attributes which are important indicators of health status in animals.

Keywords: Murrah buffalo, physiological, haematological, RBC, WBC, respiration rate

Introduction
Buffalo plays a crucial role within the agricultural economy in numerous developing countries, providing draught power, meat and milk. Among all the farm animals, the Asian buffaloes hold the best promise and potential for production. Murrah buffalo has the best genetic material for milk production within the world.

Buffalo's productivity is stricken by climate which is one among the most important factors (Marai and Haeeb, 2010) [20]. Stressors can be physical, chemical, physiological, psychological and environmental, out of which, heat stress has the best negative impact (Singh *et al.*, 2012) [26]. Buffaloes combat several environmental and physiological stresses every day and affecting life by disturbing their production and this might be the rationale for substantial economic losses. The thermoregulatory capacity of buffaloes is poor compared to cattle. They display distress signs after exposure to high environmental conditions. High environmental stress contributes to sequences of variation in buffalo's biological functions. This contains food intake depression, oxidative stress and variation in hormonal and blood biochemistry (Bombade *et al.*, 2017) [4].

The variation of haemato-biochemical parameters is foremost marker for physiological and pathological states of the animal (Hassan *et al.*, 2012; Mamun *et al.*, 2013) [14, 18]. Summer and winter stress causes rigorous changes within the blood biochemical and hormonal concentration and thereby reducing the assembly performance of the animals (Ganaie *et al.*, 2013) [10]. Haematological values are considered nearly as good indicators of stress because variation within the level of haematological markers could be a sign of adaptation to unfavorable environmental conditions. Blood examination is performed as a screening procedure to gauge the overall health. Looking to the impact of seasonal variation on animal health and productivity, this study was undertaken to gauge the changes in physiological and haematological parameters owing to seasonal variation in Murrah buffalo.

Materials and Methods

Selection, feeding and maintenance of animals

The present study was carried out in Murrah buffaloes at Livestock farm complex (LFC) at Post Graduate Institute of Veterinary Education and Research, (PGIVER), Jaipur (Rajasthan), situated at an altitude of 1417 feet above mean sea level, latitude and longitude position being 26.9°N and 75.8°E respectively, with ambient temperature in hot-humid as high as 35 °C and in winter as low as 8 °C. The experiment was conducted on ten Murrah buffaloes (about 36 months) selected from LFC herd. The experimental animals were maintained as per standard feeding and management practices followed at Livestock farm complex, PGIVER, Jaipur. This consists of feeding ad libitum roughages and water; concentrates mixture as per Kearl (1982) feeding standard. Concentrate mixture composited of mustard cake, maize, wheat bran, rice bran and mineral mixture and salt.

Estimation of physiological parameters

The rectal temperature (RT), respiration rate (RR) and pulse rate (PR) was measured with electronic thermometer inserting into rectum, through nostrils during normal respiration and through a point on the underside of the base of the tail (middle coccygeal artery), respectively.

Estimation of haematological parameters

Blood samples were collected aseptically from jugular vein using 18 gauge needles in blood vacutainer BS EDTA K3 vial (2ml) and then mix each blood sample. The haematological attributes [Total erythrocyte count (TLC), Total leukocyte count (TLC) and Differential leukocyte count such as Neutrophils (%), Lymphocyte (%), Monocyte (%), Eosinophils (%), Basophils (%)] were estimated by using Spincell 5 Compact Vet Mode Automated Blood Analyzer in the Department of Veterinary Physiology and Biochemistry, P.G.I.V.E.R., Jaipur. Blood sample from each animal was taken on any single day of the month/season, to estimate the haematological parameters in Murrah buffalo under seasonal variation. The haematological parameters were estimated from whole blood.

Statistical analysis

The results were presented as Mean±SE. The data was analyzed statistically as per Snedecor and Cochran (1989) using t- test in Microsoft excel 2007. Paired to Samples for Means and results were interpreted.

Results and Discussions

Respiration rate (RR)

Respiration rate was 17.90±0.38 and 14.20±0.25 breath/minute during hot-humid and winter, respectively (Table 1). The mean±SE value of respiration rate was found higher during hot-humid in comparison to winter season and the effect was highly significant (*P*≤0.01). Elevated respiration is a strive to increase heat loss by evaporative cooling. These results were in conformation with Das *et al.* (1999) [6]; Gudev *et al.* (2007) [13]; Korde *et al.* (2007) [15]; Chandra Bhan *et al.* (2012) [5]; Singh *et al.* (2014) [27] in buffaloes and Sailo *et al.* (2017) [24] in cattle who also reported increased respiration rate (per minute) during summer as compared to winter season. This increased respiration rate in summer might be due to the increased demand of oxygen by the tissues in stressed condition.

Respiration rate increased during hot-humid to increase loss of heat through skin and respiratory evaporation. The response ensures direct heat stimulation of peripheral receptors which transmit nervous impulse to the heat centre in the hypothalamus. The cardio-respiratory center is stimulated to send impulses to respiratory activity.

Pulse rate (PR)

Pulse rate was 66.20±0.49 and 57.30±0.47 beats/ minute during hot-humid and winter, respectively (Table 1). The mean±SE value of pulse rate was noticed at highly significant higher (*P*≤0.01) level in hot-humid compared to winter season. These results were in conformation with Das *et al.* (1999) [6]; Naik *et al.* (2013) [22] and Chandra Bhan *et al.* (2012) [5]; Kumar *et al.* (2017) [16] in Hariana and Sahiwal cattle; Singh *et al.* (2018) [25] in crossbred cattle who reported that increased pulse rate during summer season than winter season in the buffaloes and cows, respectively. PR was significantly higher in Murrah buffalo during hot-humid season compared to winter which might be an acclimation process to dissipate extra heat by this breed. Pulse rate increased during hot-humid was mainly due to direct temperature effect on Murrah buffaloes leading to high blood flow from core to periphery to maintain the body temperature as a result of heat loss by conduction, convection, and radiation.

Rectal temperature (RT)

Rectal temperature was 101.55±0.11 and 99.56±0.13 ºF during hot-humid and winter, respectively (Table 1). The mean±SE value of rectal temperature was highly significant (*P*≤0.01) in hot-humid as compared to winter season. These results were in conformation with Das *et al.* (1999) [6] in Murrah buffaloes; Srikandakumar *et al.* (2004) [29] in Holstein, Jersey and Australian Milking Zebu Cows; Chandra Bhan *et al.* (2012) [5] in growing and adult Sahiwal cattle; Sailo *et al.* (2017) [24] in Sahiwal and Karan Fries cows and Singh *et al.* (2018) [25] in crossbred cattle recorded that the RT values were significantly (*P*<0.05) higher during summer season as compared to winter and spring seasons. Rectal temperature was considered as a good index of body temperature even though there was a considerable variation in different parts of the body core at different times of the day. Under higher environmental temperatures, animals maintain their body heat balance by dissipating the excess heat from the body to the surrounding environment with the help of convection, conduction, and radiation. Increased physiological references (RT, RR and PR) with increase in THI during hot-humid season indicated that the animals were in heat-stressed condition i.e. mild to moderate in the present study.

RBC count (Total erytrocyte count)

RBC count (10^6/µl) was 5.74±0.17 and 4.93±0.12 during hot-humid and winter, respectively (Table 2). The mean±SE value of red blood cell count (10^6/µl) was significantly (*P*≤0.01) increased in hot-humid as compared to winter season.

Similar findings to the present study were noticed by Al-Saeed *et al.* (2009) [2] in cattle; Farooq *et al.* (2017) [9] in bulls; Parmar *et al.* (2013) [23] and Enculescu *et al.* (2017) [8] in buffalo who observed that the RBC count were significantly (*P*<0.05) higher during summer season as compared to winter season. During heat stress, the demand of oxygen is increased by tissues to meet their physiological need; the level of erythrocytes is increased in blood. Heat stress may also lead

to dehydration in animals which may cause haemoconcentration during hot /hot-humid season.
Present results were contrary to the findings of Giri *et al.* (2017) [12] and Chandra Bhan *et al.* (2012) [5] in cattle; Babeker *et al.* (2013) [3] in camel; Aggarwal *et al.* (2016) [1]; Manjari *et al.* (2016) [19] and Dayal *et al.* (2017) [7] in buffalo. The rise in haematological parameters in buffalo may be an adaptive response. The physiological mechanism of body to produce a greater number of RBCs either due to splenic contraction or increased number of erythropoiesis cells. Because of the fact that blood vessels in the skin dilate during endurable hot weather in summer, primarily, to bring body heat to the skin for dissipation by radiation and convection.

WBC count (Total leukocyte count)
WBC count (10^3/µl) was 8.59±0.56 and 9.82±0.93 during hot-humid and winter, respectively (Table 2). The mean±SE value of white blood cell count was significantly ($P \leq 0.05$) higher in winter as compared to hot- humid season.
The increased TLC levels in present study during winter season was in accordance with Kuralkar *et al.* (2013) [17] and Aggarwal *et al.* (2016) [1] who reported that the total leukocyte count (TLC) was higher in winter season as compared to hot-humid and summer season in Purnathadi strain of Nagpuri buffalo and adult Murrah buffaloes, respectively. Present results were Similar with the findings of Al-Saeed *et al.* (2009) [2] and Chandra Bhan *et al.* (2012) [5] in cattle and Ghosh *et al.* (2013) [11] in goat. The observation of the present study might be due to higher levels of parasitic infection during the winter season.
Present findings were contrary to the findings of Parmar *et al.* (2013) [23]; Enculescu *et al.* (2017) [8]; Dayal *et al.* (2017) [7] and Manjari *et al.* (2016) [19] in buffalo; Farooq *et al.* (2017) [9] in bulls and Babeker *et al.* (2013) [3] in camel and Giri *et al.* (2017) [12] in dairy cattle who reported that the WBC count was higher in summer season as compared to winter season. WBC in animals' changes under the influence of the central nervous system as induced by an unconditional and conditional refectory changes. Leukocyte profile is particularly useful in the field of physiological adaptation because they are altered by stress and can be directly related to stress hormone (cortisol) levels.

Lymphocyte (%)
Lymphocyte (%) was 35.11±4.52 and 62.40±5.62 during hot-humid and winter season, respectively (Table 3). The mean±SE values of lymphocyte (%) were significantly ($P \leq 0.01$) higher in winter season as compared to hot-humid season.
Similar findings to the present study were reported by Parmar *et al.* (2013) [23] and Dayal *et al.* (2017) [7] in Murrah buffaloes; Kuralkar *et al.* (2013) [17] in Nagpuri buffalo and Manjari *et al.* (2016) [19] in Tarai buffaloes. It might be due to increased stress hormone (cortisol) levels under hot-humid condition which leads to decrease in lymphocyte percentage.
Present results were contrary to the findings of Babeker *et al.* (2013) [3] in camel; Ghosh *et al.* (2013) [11] in goat; Farooq *et al.* (2017) [9] in bulls and Giri *et al.* (2017) [12] in cattle. Lymphocytes are involved in protection of the body from viral infection. Elevated levels may indicate an exhausted immune system. They may get infected with several season-dependent diseases during the months of winter condition. This variation might be due to species, breed, environmental and physiological conditions of the animal.

Monocyte (%)
Monocyte (%) was 13.00±2.48 and 16.08±3.79 during hot-humid and winter season, respectively (Table 3). The mean±SE values of monocyte percent were non-significantly ($P>0.05$) increased in winter season as compared to hot-humid season.
Similar findings to the present study were suggested by Kuralkar *et al.* (2013) [17]; Parmar *et al.* (2013) [23]; Manjari *et al.* (2016) [19] and Dayal *et al.* (2017) [7] in buffaloes who reported higher values during winter season as compared to summer season.
Present results were contrary to the findings of Babeker *et al.* (2013) [3] in camel; Ghosh *et al.* (2013) [11] in goats; Farroq *et al.* (2017) [9] in bulls and Giri *et al.* (2017) [12] in dairy cow who reported that the monocyte values were higher in the summer season as compared to winter season. Monocytes are helpful in fighting severe infections and are considered the bodies' second line of defense against infection and the largest cells in the blood stream. Increased levels of monocytes were associated with an increase in cell-mediated immunity and antibody-mediated immunity.

Neutrophil (%)
Neutrophil (%) was 46.79±4.39 and 16.39±4.64 during hot-humid and winter season, respectively (Table 3). The mean±SE value of neutrophil percent showed highly significant ($P \leq 0.01$) decrease in winter season as compared to hot- humid season.
Similar findings to the present study were investigated by Parmar *et al.* (2013) [23] and Dayal *et al.* (2017) [7] in Murrah buffaloes; Kuralkar *et al.* (2013) [17] in Purnathadi buffaloes; Manjari *et al.* (2016) [19] in Tarai buffaloes; Babeker *et al.* (2013) [3] in camel and Giri *et al.* (2017) [12] in cattle. It might be due to corticosteroids which accelerate the release of neutrophils and bands from a large storage pool within the bone marrow and impair the migration of neutrophils from the circulation into tissues.
Present results were contrary to the findings of Ghosh *et al.* (2013) [11] in goats and Farroq *et al.* (2017) [9] in bulls. This variation might be due to different species, breed, environmental and physiological conditions of the animal.

Eosinophil (%)
Eosinophil (%) was 4.93±1.04 and 4.89±0.87 during hot-humid and winter season, respectively (Table 3). The mean±SE value of eosinophil percent was non-significantly ($P>0.05$) lower in winter as compared to hot humid season.
Similar findings to the present study were measured by Giri *et al.* (2017) [12] in dairy cow and Babeker *et al.* (2013) [3] in dromedary camel.
Present results were contrary to the findings of Kuralkar *et al.* (2013) [17], Parmar *et al.* (2013) [23] in goat who noticed that eosinophil percent was higher in the winter season as compared to summer season. Eosinophilia has been reported to be proportional to the degree of antigenic stimulation or parasitic burden in helminthes infections. This is normally linked to antigen-antibody reaction which occurred when the sensitivity to the protein of the parasites has developed or when the secretory products were released within the blood associated with cell-mediated immunity.

Basophil (%)
Basophil (%) was 0.17±0.05 and 0.24±0.05 during hot-humid and winter season, respectively (Table 3). The mean±SE

value of basophil percent was non-significantly (P>0.05) increased in winter season as compared to hot-humid.

Similar findings to the present study were observed by Parmar *et al.* (2013) [23] in Murrah buffaloes who observed higher basophils percent during winter season.

Present results were contrary to the findings of Giri *et al.* (2017) [12] in dairy cow; Manjari *et al.* (2016) [19] and Dayal *et al.* (2017) [7] in buffaloes who estimated that the basophil percent were higher in summer compared to winter season. It might be due to variation in age, sex, physiological and environmental condition. Basophils are necessary for the immune system's natural response to invaders. The body response to allergens also involves basophils. When a potentially harmful allergen enters the body, the immune system responds by trying to isolate and eliminate the allergen.

Table 1: Effect of Hot-humid and winter season on Mean±SE values of RR, PR and RT in Murrah buffalo (N=10)

Parameter	Season	Mean±SE	Observation
RR (Breaths/Minute)	Hot-humid	17.90±0.38	**
	Winter	14.20±0.25	
PR (Beats/ Minute)	Hot-humid	66.20±0.49	**
	Winter	57.30±0.47	
RT (°F)	Hot-humid	101.55±0.11	**
	Winter	99.56±0.13	

N = No. of Animals
NS = Non-significant (P>0.05)
** = Highly Significant (P≤0.01)
* = Significant (P≤0.05)
RT: Rectal Temperature; RR: Respiratory Rate; PR: Pulse Rate

Table 2: Effect of Hot-humid and winter season on Mean±SE values of WBC and RBC count in Murrah buffalo (N=10)

Parameter	Season	Mean±SE	Observation
WBC (10^3/µl)	Hot-humid	8.59±0.56	*
	Winter	9.82±0.93	
RBC (10^6/µl)	Hot-humid	5.74±0.17	**
	Winter	4.93±0.12	

N = No. of Animals
NS = Non-significant (P>0.05)
** = Highly Significant (P≤0.01)
* = Significant (P≤0.05)
RBC: Red blood cell count; WBC: White blood cell count

Table 3: Effect of Hot-humid and winter season on Mean±SE values of LYM%, MON%, NEU%, EOS% and BASO% in Murrah buffalo (N=10)

Parameter	Season	Mean±SE	Observation
LYM%	Hot-humid	35.11±4.52	**
	Winter	62.40±5.62	
MON%	Hot-humid	13.00±2.48	NS
	Winter	16.08±3.79	
NEU%	Hot-humid	46.79±4.39	**
	Winter	16.39±4.64	
EOS%	Hot-humid	4.93±1.04	NS
	Winter	4.89±0.87	
BASO%	Hot-humid	0.17±0.05	NS
	Winter	0.24±0.05	

N = No. of Animals
NS = Non-significant (P>0.05)
** = Highly Significant (P≤0.01)
* = Significant (P≤0.05)
LYM%: Lymphocyte percent; MON%: Monocyte percent; NEU%: Neutrophil percent;
EOS%: Eosinophils percent; BASO%: Basophils percent

Conclusion

The buffalo does not adapted to seasonal weather fluctuations in the environment it was found in research. The animals demonstrated a high ability to maintain a constant monocyte percent, eosinophil percent and basophil percent while slightly raising their total leukocyte count during winter and highly increasing their total erythrocyte count during hot-humid, lymphocyte percent during winter and neutrophil percent during hot-humid. Murrah buffaloes exhibited a highly elevated rectal temperature, pulse rate and respiration rate during hot-humid, as well as a large capacity for heat dissipation. They, on the other hand, respond quickly to cooler temperatures, increasing their heat-producing capability. Physiological and haematological properties alter during season variation, such as metabolism slowing during heat stress and speeding up during cold stress. As a result, proper assessment of the adaptive profile necessitates taking into account animals' physiological and haematological parameters to environmental conditions.

Acknowledgment

Authors thankfully acknowledge Hon'ble Vice chancellor RAJUVAS Bikaner, Dean, CVAS, Post Graduate Institute of Veterinary Education and Research, Jamdoli, Jaipur (Rajasthan) for providing necessary facilities.,

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