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Integrated effects of lactation stages and seasons on milk somatic cells and some haematological entities in healthy Tarai buffaloes

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ABSTRACT

Tarai buffalo is a dual purpose indigenous buffalo breed of Uttarakhand state. The present study was conducted to investigate the integrated effect of lactation stages and seasons on milk somatic cells and some haematological entities in healthy Tarai buffaloes. Blood TLC value was significantly ($p < 0.05$) higher in early lactation stage while PCV, TEC and Hb values remained unaffected due to lactation stages. Both TLC and PCV values were significantly higher during summer and rainy seasons. Milk SCC was significantly higher in early and late lactation stage and during summer to rainy seasons. Climatic conditions during summer and rainy seasons were at higher (THI $\leq$74 and 74 <THI <79). Pearson correlation coefficient found significant and positively correlation ($r_p = 0.984$, $p < 0.05$) between THI and milk SCC ($r_p = 0.995$, $p < 0.01$) and between milk SCC and blood TLC. It was concluded that increased number of milk SCC and blood TLC during lactation stage and hot-humid seasons might be an indication of immuno-protective mechanism against various infectious agents. THI can be used as climatic variant in predicting climatic environmental stress to Tarai buffaloes as significant correlation with increased seasonal milk SCC was found.

Keyword: Blood; lactation; milk; season; Tarai buffalo; temperature-humidity index

INTRODUCTION

Tarai buffalo, dual purpose buffalo breed native to tropical Tarai region of Uttarakhand state, is reared by the marginal and small landholding farmers of 'Van Gujjar' living in the Tarai forests which are located at the foothill of Himalaya and Siwalik hills of northern India. This breed is known for excellent draught purpose rather than for milk purpose having high resistant to many tropical diseases. The unique physical appearance and physiology of this breed are its adaptation to adverse climatic conditions of Tarai region (Manjari et al 2015, 2016). They require less external inputs and survive mostly on natural lush grasses and forest products available round the year with milk production capabilities in the average milk yield of 1054.08 ± 1957 kg during the lactation cycle of 291.19 ± 3.63 days (http://www.buffalopedia.cirb.res.in/index.php?option=com_content&view=article&id=75& Itemid=80&lang=en).

Mammary gland during lactation is made up of remarkably sensitive alveolar tissues which synthesize and secrete milk in normal and abnormal conditions. The number of milk somatic cells is associated with inflammation and udder health (Bansal et al 2007) and this is accepted as the international standard for measurement of milk quality (Sharif and Muhammad 2008) and intra-mammary infections (Barkema et al 1999). Tarai buffalo requires further knowledge for its improvement and utilization as better dual purpose indigenous buffalo breed. Considering its significant importance in providing livelihood to the small and landless farmers living here, the present study was conducted to investigate the integrated effect of lactation stages and seasons on milk somatic cells and some haematological entities in healthy Tarai buffalo.

MATERIAL and METHODS

The study area was located in and around Lalkaun area of Udham Singh Nagar district lying in northern upper Gangetic plains of Tarai region of Uttarakhand. It is located at 344 m amsl (79° E longitude, 29° N latitude). The climatic conditions of Tarai region are known for hot and humid during summer and rainy seasons with intense cold during winter and short spring seasons.

Animal ethical approval

The experiments on animals including all procedures of this study were conducted with approval from Institutional Animal Ethics Committee (Registration Number: 330/ GO/Ere/SL/01/CPCSEA).

Selection of experimental animal

Thirty six healthy Tarai buffaloes (280-350 ± 10 kg BW, parity 2nd to 4th) for one lactation cycle (291.19 ± 3.63 days) were selected from four Gujjar farms and were divided into four groups of nine buffaloes per group as early (30 to 90 ± 15 days), mid (100 to 180 ± 15 days) and late (200 to 280 ± 15 days) lactation stages and another group of dry buffaloes. Selected buffaloes were usually provided with minimal concentrate input which mainly depended on abundant access availability of water and natural forest products (grasses and fodders) round the year in Tarai region. The animals were normally maintained in open housing system with roof made of locally available materials (thatch and dry perennial grasses) which provided shelter mainly for the night and during summer to rainy seasons.

Climatological variants recorded during different seasons

Seasons studied were winter (1 December to 30 January), spring (15 February to 31 March), summer (15 April to 15 June) and rainy (15 July to 15 September). Climatic variants recorded were environment air temperature (°C) and relative humidity (%) in frequency of twice daily at 7.00 AM and 02.00 PM for each month season-wise. Temperature-humidity index (THI) was calculated according to McDowell et al (1976):

$$THI = 0.72 (td + tw) + 40.6$$

where td= Dry bulb temperature in °C, tw= Wet bulb temperature in °C

THI values commonly used to assign heat stress levels were categorized as <70 as comfortable, ≤74 as mild stress or alert, 74 <THI <79 as stressful or danger, 79 <THI <84 as severe stress or emergency and >86 as lethal (Anon 1970).

Milk and blood samples collected for various parameters

Milk yield of each selected buffalo was collected from farmers. Both blood and milk samples were collected for each season and lactation stage in selected buffaloes. All the analysis works of collected samples were done in the Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Prior to milk sampling, the mammary udder was washed with lukewarm water and the teat was dip into the dipping solution (0.5% iodine or 4% hypochlorite) for at least 20-30 seconds before milking after which the entire mammary udder was wiped dry by using a clean muslin cloth. Milking was done by manual full hand milking technique and samples of morning were collected in a sterile vial without any preservative. Prior to that initial few streaks of milk were discarded after which collection was done. Fresh milk samples were taken for enumeration of milk SCC which was done by direct method (Schalm et al 1971) and stained using modified Newmen-Lampert staining technique (Marshall 1992).

Blood samples (3 ml/animal) were collected from jugular vein aseptically using vacutainer tube containing disodium EDTA @ 1 mg/ml (Wittgenstein 1953) as anticoagulant for various haematological estimations. Blood sampling was done once in every season and during different lactation stages. Haematological analysis for estimation of hemoglobin (Hb) concentration was done by the method of Hawk and Oser (1965), packed cell volume (PCV) by Dacie and Lewis (1994), total erythrocytic count (TEC) and total leukocytic count (TLC) by Schalm et al (1975).

Statistical analysis

Effects of seasonal variation and lactation stage on hematological entities and milk SCC were analyzed by one-way ANOVA and Pearson correlation coefficient was done using IBM SPSS Version 20 software.

RESULTS and DISCUSSIONS

The total lactation yield was reported around 812.55 ± 23.42 kg with highest around mid-lactation (415 ± 23.21 kg). The peak yield (3.12 ± 0.04 kg/day) was seen around 212 ± 7.39 days. The average milk SCC was 1.05 ± 0.06 X 10^5/ml throughout the lactation period. The milk yield recorded in the present observations was lower as compared to the earlier report (http://www.buffalopedia.cirb.res.in/index.php?option=com_content&view=article&id=75&Itemid=80&lang=en). The total lactation yield of Tarai buffaloes was comparable with Nagpuri buffaloes, another dual purpose breed of India with less peak lactation yield (Panicker et al 2016).

Hematological parameters during different lactation stages and seasons have been given in Fig 1. No alterations were found in the hematological parameters during lactation stages except TLC which was significantly ($p < 0.05$) higher during early lactation stage and slightly higher in lactation stages than in dry cows. Hematological parameters of Tarai buffaloes were within the normal range (Manjari et al 2016). The results revealed stability in the PCV, Hb and TEC values during different stages of lactation cycle. The finding corroborated with the earlier reports in Indian buffaloes (Hagawane et al 2012, Das et al 2016).

Higher TLC during early lactation stage recorded was similar to the earlier reports of Meglia et al (2001) which could be due to higher levels of glucocorticoids around parturition that facilitated increased neutrophil outputs from bone marrow (Lee and Kehrli 1998). Seasonal variation was found affecting hematological parameters of PCV and TLC values which were found significantly ($p < 0.05$) higher during summer to rainy seasons while decreased in winter and spring seasons. This could be due to increased stress load in lactating buffaloes when exposed to stressful climatic condition. Stressful climatic condition increases blood leukocytes which act as active immunity to protect lactating buffaloes and increased hematocrit to meet their cellular respiration.

Milk somatic cell count (SCC) alteration during different lactation stages and seasons is presented in Fig 2. Milk SCC showed significant ($p < 0.01$) difference during different lactation stages and lowest milk SCC was reported during mid-lactation period. Milk SCC of Tarai buffaloes reported in the present investigations was lower than that reported by Singh and Ludri (2001) but within the range as reported by Dang et al (2010).

The pattern of alterations of milk SCC during different lactation stages is also corroborated with the earlier reports of Singh and Ludri (2001) in Murrah buffaloes. Normally buffalo milk SCC is less than the dairy cows; the number is abnormally increased in response to udder injury and mastitis (Anon 2009). Increased milk SCC in early lactation has been linked with innate immune response during parturition and to the onset of lactation. Migration of leukocytes into the uterus and mammary gland during the transition period is essential to prevent inflammations (metritis and mastitis) as there is every chance of infectious agents invasion. Increased milk SCC was also observed with resumption of mammary gland functional activity after a long dormant period of gestation (Schalm et al 1971) and during the end of lactation period (Singh and Ludri 2001) due to excessive desquamation of alveolar epithelial cells.

Another study suggested that the increased number of milk somatic cells during lactation was normally contributed mostly by neutrophils and lesser lymphocytes cells (McDonald and Anderson 1981). Neutrophils or polymorphonuclear (PMN) perform active phagocytosis of bacteria, viruses and cellular remnants at the sites of the inflammation and restrain their proliferation. Seasonal variations were found to affect the number of milk somatic cells as it showed significantly ($p < 0.05$) higher during summer to rainy seasons while it remained at lowest count during winter and spring seasons. The present findings are similar to the findings in Murrah buffaloes (Khate and Yadav 2010, Singh and Ludri 2001).

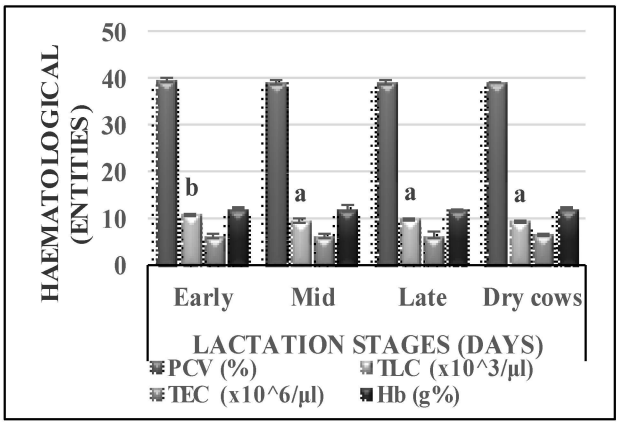
Increased number of milk SCC during these seasons, reflects the immune response to infection with high temperature and relative humidity which make favorable for bacteria to grow in the bedding material of housed stock or surrounding environment (Harmon 1994).

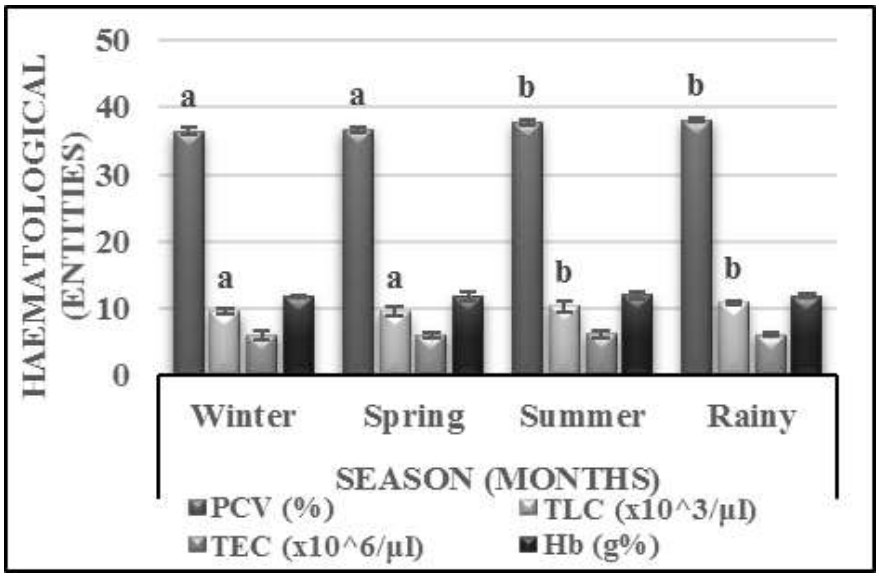
In this study highly significant and positive correlation was found ($r_p = 0.995$, $p < 0.01$) between milk SCC and blood TLC. This indicates that higher blood leukocytes may contribute to increased milk SCC as blood leukocytes (neutrophils, macrophages, lymphocytes) contributed about 75 per cent of milk somatic cells and the rest 25 per cent were of alveolar epithelial cells from the lining of the mammary alveoli which were normally shed during the course of normal

Table 1. Climatic variants recorded round the year in Tarai region

Month	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)	Season
	Maximum	Minimum	0712	1412		
Jul	32.0	25.7	89	72	79.372	Rainy
Aug	31.8	25.5	89	73	79.228	Rainy
Sep	31.8	23.9	89	68	77.212	Rainy
Oct	31.2	16.1	88	44	69.148	Autumn
Nov	27.2	9.9	90	37	61.876	Autumn
Dec	21.3	7.15	91	55	57.322	Winter
Jan	21.3	7.1	91	55	57.304	Winter
Feb	22.8	9.9	90	56	59.572	Spring
Mar	29.1	13.5	89	44	66.736	Spring
Apr	35.0	17.6	68	26	69.616	Summer
May	38.9	23.4	62	32	76.708	Summer
Jun	33.2	25.7	82	64	79.084	Summer

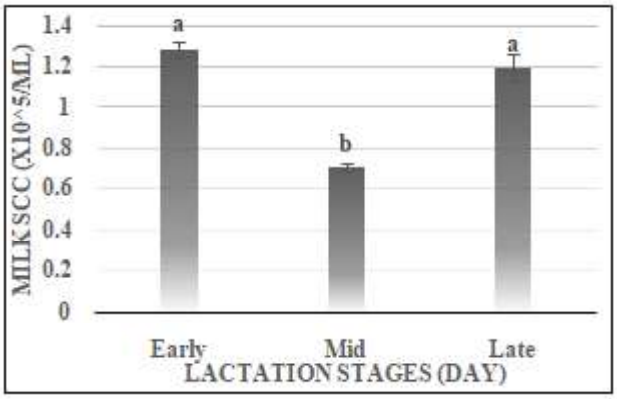
Note: Tarai region is with prolong summer and rainy seasons as compared to other seasons

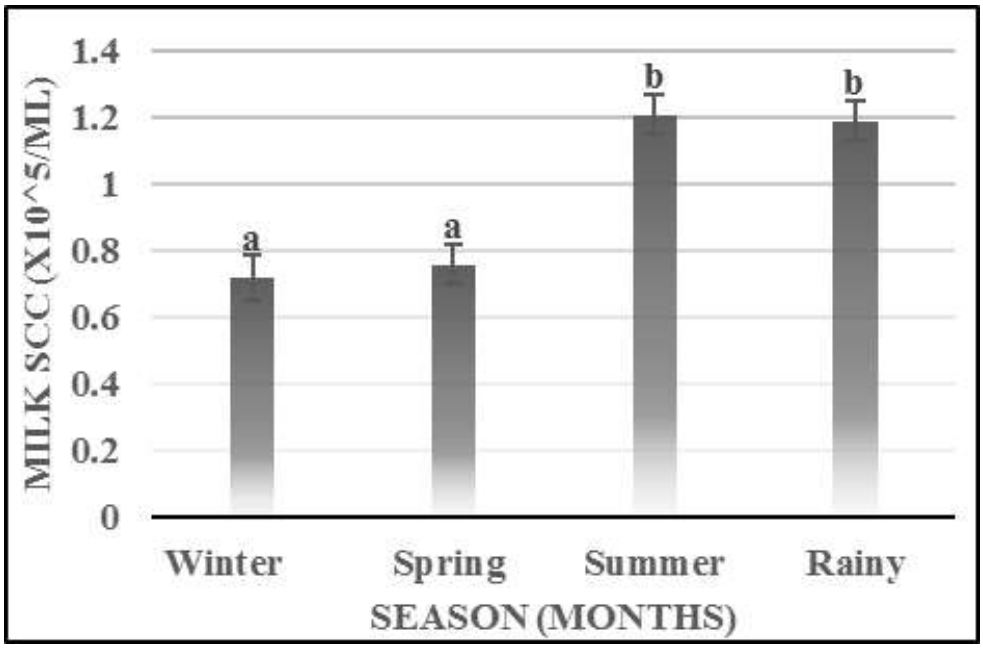




Mean ± SE values and different alphabetical superscripts (a, b) are significantly ($p < 0.05$) different

Fig 1. Some haematological entities of Tarai buffaloes during different lactation stages and seasons





Mean ± SE values and different alphabetical superscripts (a, b) are significantly ($p < 0.01$) different

Fig 2. Milk somatic cell count of Tarai buffaloes during different lactation stages and seasons

milking (Harmon 1994). Increased leukocytes count perform an important function at the mammary gland in giving protection against infectious agents causing inflammation (mastitis) and assist in the repair of damaged mammary tissue with increase in the milk yield.

Climatological variants of environmental air temperature and relative humidity were recorded daily month–wise and calculated THI for each selected season is presented in Table 1. Climatic condition during peak summer to rainy seasons in sub-tropical Tarai region was found to have high air temperature (>30°C) and relative humidity (>70%) which may cause distress to producing buffaloes. Since buffaloes are prone to environmental stress due to scanty hairs on their skin surface and less sweat glands thus gain more heat from the environment due to their body colour. So during these seasons of environmental discomforts, buffaloes would prefer wallowing to maintain their body heat balance and to avoid getting heat stress (Marai and Haeeb 2010). Based on environmental climatic conditions during summer to rainy seasons in tropical Tarai region, the THI calculated from the recorded climatological variants was found on higher side ie THI $\leq$74 and 74 <THI <79. THI values commonly used to assess and predict the risk of heat stress levels according to categorized level are <70 comfortable, $\leq$74 mild stress or alert, 74 <THI <79 stressful or danger, 79

<THI <84 severe stress or emergency and >86 is lethal (Anon 1970). This indicates that the lactating Tarai buffaloes can be affected by environmental heat stress which may even become severe during summer to rainy seasons. Higher THI values during summer and rainy seasons were found to affect the number of milk somatic cells and they showed significant and positive correlation ($r_p = 0.984$, $p < 0.05$).

CONCLUSION

Healthy lactating Tarai buffaloes were affected during different lactation stages and hot-humid seasons with alterations in milk SCC and blood leucocytes. Blood total leukocytes highly contributed to increase in the number of milk SCC and were positively correlated. Increased milk SCC in healthy udder is an immune response in protecting the udder during different lactation stages and seasons from various infectious agents. THI values were found valuable in predicting climatic environmental stress to Tarai buffaloes and were found to have significant correlation with increased seasonal milk SCC.

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