Haematological Profiles of Lactating Nili-Ravi Buffaloes Under Heat Stress-Alleviated Conditions During Sub-tropical Summer Season

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Abstract: The experiment was conducted using forty two Nili-Ravi lactating buffaloes of Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha, Patiala, Punjab to investigate the effect of heat stress-alleviated conditions on haematological profile in Nili-Ravi buffaloes during hot-dry (HD) and hot-humid (HH) season. All buffaloes were uniformly divided into two groups of twenty one in each group considering their lactation number, stage of lactation, body weight, dam's milk yield and milk yield in current lactation. Out of which ten buffaloes from each group were selected for blood sampling considering those above factors. The control (T_0) group were kept in a separate shed without any extra nutrient supplementation and modification in microclimate and management. The treatment (T_1) group was supplemented with niacin@6 gm/buffalo/day, yeast@10 gm/buffalo/day and mustard oil @150gms/buffalo/day. They were provided curtains and mist fans in the shed. Feeding time, frequency and type of ration were also altered. During HD period, T_0 group exhibited higher ($P<0.002$, $P<0.05$) WBC (17.2×10^9 /L), and lymphocyte (12.52×10^9 /L) counts and lymphocyte per cent (69.46 %) than T_1 group (12.32×10^9 /L, 7.32×10^9 /L and 59.68%, respectively). Similar results were recorded during hot humid season in T_0 and T_1 group. The neutrophil per cent differed significantly between T_0 and T_1 group under HD ($P<0.001$) and HH ($P<0.006$) season. The study indicated that heat stress during sub-tropical summer months affected the haematology of lactating buffaloes and the stress could be reduced through the change (use of fans and curtains, nutritional supplementations, and feeding alterations together in the form of one package) of microclimate.

Keywords: Haematology, Heat stress-alleviation, Lactating buffalo.

INTRODUCTION

Buffaloes are considered as '*Black Gold*' of India. They contribute more than half of total milk production in the country. But, due to country's geographical location, buffaloes are exposed to severe heat stress resulting to the changes in their normal physiology. Amongst other categories, lactating buffaloes are badly affected by this kind of stress causing decrease in milk production [1]. Physiological variation in the blood parameters plays an important role in clinical haematology and animal production because, blood constituents are indicative of heat tolerance and environmental stress [2]. Many studies investigated the haematology of Murrah buffaloes [3-5], Jaffarabadi, Nagpuri and non-descript buffaloes [6] under heat stress conditions. As this type of thermal stress is totally unavoidable in the subtropical/tropical regions, various methods viz. nutrient supplementations, housing modifications and management alterations are adopted as a routine practice to combat the heat stress on buffaloes. The effect of individual cooling method on blood constituents in buffaloes during summer was also studied earlier [7]. But, literature related to the effect of heat stress alleviation using a complete package of practices on haematological profile in lactating Nili-Ravi buffaloes is very scanty. Therefore, in the present study, an effort was made to detect the potential changes in the blood constituents of heat stress-alleviated lactating Nili-Ravi buffaloes during summer months of sub-tropical region.

MATERIALS AND METHODS

Location of Study

This study was conducted at Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha (latitude, 30° 22′ 28″ N and longitude, 76° 8′ 54″ E), Patiala, Punjab, India during hot-dry season (HD; April to Mid June) and hot-humid season (HH; Mid June to August). The elevation of the area is about 250 m above mean sea level. The ambient temperature reaches lowest near 1°C in winter season and highest 45°C in summer. The average annual rainfall is around 700 mm.

Experimental Design

A total of forty two Nili-Ravi lactating buffaloes, kept at Central Institute for Research on Buffaloes, Regional Station-Bir Dosanjh, Nabha, Patiala, Punjab, were used to study the effect of heat stress alleviation on

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haematological profile in Nili-Ravi buffaloes during hot-dry season (HD; April to Mid June) and hot-humid season (HH; Mid June to August). Forty two buffaloes were uniformly divided into two equal groups taking into account their lactation number, stage of lactation, body weight, dam's highest milk yield and milk yield in current lactation. First group was considered as treatment (T_1) and the second as control (T_0). Ten buffaloes from each group were also uniformly selected for blood sampling considering the above factors.

Management of Experimental Animals

A similar but separate conventional tail-to-tail house containing concrete roof with wide opened window (height of wall was 4 feet from the ground level) shed was used for keeping both T_1 and T_0 group buffaloes. The shed height was 12 feet. Ceiling fans were fitted about 1.5 feet from the roof. The windows of T_1 group buffalo shed were covered with curtains to prevent entry of hot air. Additional mist fans (speed-about 1400 rpm; fan diameter-24 inches; water output-1.25 litre/nozzle /hour) and ceiling fans (speed- about 360 rpm) were also fitted to modify microclimate for animals' comfort. On the contrary, windows of T_0 group buffalo shed were opened. Only few ceiling fans, but no mist fan, were used. All buffaloes of T_1 and T_0 group were tied individually within the shed throughout 24 hours except at the time of wallowing. As a routine practice of the farm, during summer months, all experimental buffaloes were allowed to the wallowing tank for 10-15 minutes twice daily i.e. around 7 AM and 2.30 PM.

All experimental buffaloes were individually fed. The farm prepared balance concentrate mixture was offered. The T_1 group was supplemented with feed grade niacin (≤ 95% niacin) @6 gm/buffalo/day, feed grade yeast (*Saccharomyces cervisiae* @100 billion C.F.U. per 25 gm) @10 gm/buffalo/day to reduce heat arising from fermentation, and edible oil i.e. mustard oil @150gms/buffalo/day to increase energy density to meet out the nutrient requirements. The feeding regimens of two experimental groups have been given in Table **1**.

The minimum and maximum temperature within shed of T_0 and T_1 group were 17°C and 42°C, and 15°C and 38°C, respectively during hot dry (HD) period. The respective values during hot humid (HH) period were 20°C and 40°C, and 19°C and 36°C.

Blood Collection and Sampling

Blood was collected *via* jugular veinipuncture using 10 ml BD Vacutainer tube containing sodium heparin as anticoagulant. Four samples during HD period particularly when ambient temperature exceeded 40°C and four samples during HH period when ambient temperature exceeded 30°C were collected for analysis of haematological parameters. Samples were collected between 6 to 7 AM. Sampling days were spread about equally (divided by 12 days on average) between the start and the end of the HD and the HH period.

Haematological parameters, viz. total leucocytes count (TLC), differential leucocyte counts (DLC), per cent differential leucocyte counts (%DLC), total erythrocyte count (TEC), haemoglobin (Hb), haematocrit (HCT)/packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), total platelet counts (TPC), platelet crit (PCT),

Table 1: Feeding Regime of Two Experimental Groups

Parameters	**Control group (T_0)**		**Treatment group (T_1)**		
Nutrient supplementation	**Nil**		**Niacin, Yeast and Mustard oil**		
Concentrate feed	½ part provided during morning milking around 4 AM	½ part provided during evening milking around 4 PM	½ part mixed with nutrient supplements		½ part (without nutrient supplements) mixed with green fodder and straw & provided around 12 noon
			½ part provided during morning milking around 4 AM	½ part provided during evening milking around 4 PM	
Green fodder	½ part provided at 10 AM	½ part provided at 3 PM	½ part provided around 6 AM	Remaining part provided around 7 PM	Little amount provided around 12 noon
Water	Clean, fresh and *ad lib*.		Clean, fresh and *ad lib*.		

mean platelet volume (MPV) and platelet distribution width (PDW) were estimated by automatic blood cell counter (Vet Scan HM5; Abaxis).

Statistical Analysis

All data of control (T_0) and treatment (T_1) group during HD and HH season are expressed as means ± S.E.M. Data were analysed by analysis of contrast variables using GLM (generalized linear model) procedures on analysis of variance for repeated measures using the Greenhouse-Geisser adjusted univariate significance tests as described earlier [8] using SPSS [9]. The differences between treatment means were considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

The results of haematological parameters of control (T_0) and treatment (T_1) group lactating Nili-Ravi buffaloes under hot dry (HD) and hot humid (HH) seasons are presented in Table **2**. During HD season, total leucocyte count (TLC) of T_1 group buffaloes was significantly ($P<0.02$) lower than T_0 buffaloes. The HH season also had similar trend being the values differed significantly ($P<0.05$) between the two experimental groups. As per DLC were concerned, under both seasons lymphocyte counts were much higher ($P<0.013$, $P<0.003$) in T_0 group as compared to T_1 group. Earlier [10] report depicted that leucocyte numbers decreased significantly (from 8.32 to 5.02 G/l, $P<0.05$) during hot period until a period of lower ambient temperatures. During cool period of experiment, a significant increase of leucocyte upto 8.22 G/l was recorded. And the use of sprinkler during hot period resulted no change of leucocyte number in animals. In their experiment, the reason behind decreasing white blood cell count after hot period was probably due to significant fall of lymphocyte number. Their relative distribution significantly decreased from 64 to 46% ($P<0.05$) in the middle of cool period after hot period. A decrease in leucocute number in the alarm phase and leucocytosis in the resistance phase of heat stress was also pointed out in earlier report [11].

The results revealed that lactating buffaloes had either slightly higher or almost similar monocyte, eosinophil and basophil counts in T_0 group compared to T_1 group under HD season. On the contrary, monocyte, neutrophil, eosinophil and basophil counts were slightly lower in T_1 than T_0 group under HH season. However, there were no statistically significant differences in the values between the experimental groups. As per available report [10] in the literature, there was a rise in relative neutrophil number and a fall in relative lymphocyte number in cool period, but, in hot period with cooling, the neutrophil number fell again. Considering the proportional distribution of white blood cells changes at high temperature, a decrease in neutrophils and eosinophils, and an increase in lymphocytes and monocytes were found in other reports [12].

In T_1 group, lymphocyte % was significantly lower than T_0 in HD season ($P<0.002$) and HH season ($P<0.004$). The average rectal temperature was also significantly ($P<0.0001$) lower in T_1 group than T_0 group. The lesser change of body temperature indicated the lesser variability in homeostatic mechanism, lesser stress and better regulation of body functions in T_1 group buffaloes compared to T_0 group. Earlier report [12] in rat depicted that during hibernation period when body temperature was low there was depletion of lymphocytes from general circulation and storage in secondary lymphoid organs. This activity was mediated by temperature dependant drop in plasma sphingosine-1-phosphate. A similar mechanism might be responsible in buffaloes with low body temperature of treatment group which needs to be ascertained.

The neutrophil per cent was significantly ($P<0.0001$) higher in T_1 than T_0 group under HD season. Similar result was also found in hot humid season. Two experimental groups had comparatively higher monocyte, eosinophil and basophil per cent in T_1 than T_0 group. Increase of neutrophil per cent in T_1 group compared to T_0 group under both seasons might be due to the compensatory adjustment resulting from decreased leucocyte per cent. A relative increase in neutrophil number occurred from 32 to 51% ($P<0.05$) and their number decreased again to 27% in hot period with cooling as per reports of earlier researcher [10].

There was slight increase in TEC in T_1 group as compared to T_0 group during HD period. The observation of the present investigation was just opposite in HH period. The concentration of Hb and HCT per cent values were found to be little bit higher in T_0 than T_1 group under both HH and HH season. However, values between two experimental groups were not statistically significant. The earlier experiment [10] showed significant increase in haematocrit value and erythrocyte number at the beginning of hot period. The rise in those parameters was not significant in hot period with cooling. Same literature envisaged the

Table 2: Haematological Profile of Lactating Nili-Ravi Buffaloes Under Heat Stress Alleviated Conditions in Hot-Dry and Hot-Humid Seasons

Parameters	Time	Control (T_0) group	Treatment (T_1) group	SEM	P Value
TLC(x10^9/l)	HD	17.20	12.32	0.56	0.02
	HH	16.97	13.05	0.66	0.05
Lymphocyte (x10^9/l)	HD	12.52	7.32	0.58	0.003
	HH	11.40	7.36	0.42	0.013
Monocyte (x10^9/l)	HD	0.24	0.16	0.08	0.15
	HH	0.23	0.22	0.05	0.69
Neutrophil (x10^9/l)	HD	4.30	4.72	0.44	0.43
	HH	5.08	4.99	0.37	0.83
Eosinophil (x10^9/l)	HD	0.15	0.12	0.02	0.10
	HH	0.25	0.22	0.02	0.48
Basophil (x10^9/l)	HD	0.004	0.004	0.37	0.87
	HH	0.008	0.007	0.52	0.45
Lymphocyte%	HD	69.46	59.68	3.86	0.002
	HH	66.54	55.59	1.96	0.004
Monocyte%	HD	1.41	1.42	0.44	0.99
	HH	1.49	2.19	0.58	0.16
Neutrophil%	HD	25.36	42.01	7.64	<0.0001
	HH	31.02	40.42	1.99	0.006
Eosinophil%	HD	0.86	1.03	0.21	0.21
	HH	1.47	1.70	0.10	0.40
Basophil%	HD	0.02	0.03	0.01	0.21
	HH	0.04	0.06	0.01	0.18
TEC (x10^{12}/l)	HD	7.60	7.73	0.14	0.73
	HH	6.61	6.58	0.13	0.91
Haemoglobin (g/dl)	HD	11.20	11.08	0.26	0.80
	HH	9.85	9.36	0.33	0.28
HCT/PCV (%)	HD	38.58	38.41	0.94	0.92
	HH	34.69	33.19	0.73	0.42
MCV (fl)	HD	50.58	49.85	0.47	0.62
	HH	52.85	50.73	0.33	0.27
MCH (pg)	HD	14.75	14.32	0.17	0.35
	HH	14.96	14.20	0.10	0.14
MCHC (g/dl)	HD	29.26	28.80	0.27	0.09
	HH	28.35	27.98	0.23	0.17

(Table 2). Continued.

Parameters	Time	Control (T_0) group	Treatment (T_1) group	SEM	P Value
RDW (%)	HD	20.60	20.79	0.15	0.61
	HH	21.11	20.64	0.19	0.35
TPC ($x10^9$/l)	HD	196.07	210.83	9.62	0.46
	HH	162.88	179.38	13.80	0.40
PCT (%)	HD	0.21	0.23	0.01	0.44
	HH	0.17	0.19	0.01	0.48
MPV(fl)	HD	11.09	10.88	0.29	0.57
	HH	10.66	10.40	0.21	0.45
PDW (%)	HD	39.72	39.97	0.86	0.81
	HH	38.41	37.36	0.96	0.42

HD= Hot dry and HH= Hot humid.

significant difference between haematocrit values and erythrocyte numbers between hot period and hot period with cooling. Our study indicated that there was similar but non-significant trend. Earlier [14] research envisaged that at the beginning of hot period, there was an evidence to increase blood concentration. That haemoconcentration was initially induced by an increase in erythrocyte number, later by plasma dehydration. However, the haemoglobin concentration did not show significant differences in their studies. Similar finding of non-significant difference between haematocrit value and haemoglobin concentration in hot and cool weather were also put forth by other searchers [15]. The result of another [16] experiment showed the increase in haematocrit values and haemoglobin concentration during summer months. It was perhaps caused by a rise in erythrocyte destruction and haemo-dilution effect resulted from more water transportation in circulatory system for evaporative cooling. The report [17] also indicated that the cooling of heat stressed dairy cows caused increase in blood haemoglobin concentration.

Table **2** envisaged that under HD period, control group buffaloes had comparatively higher MCV, MCH and MCHC values than treatment group. Hot humid period also depicted similar results. However, no statistically significant differences between two groups were found. The RDW per cent was slightly lower in T_0 group than T_1 group under both seasons. But, values did not differ significantly between two groups.

As per platelets were concerned, TPC values were comparatively higher in T_1 group than T_0 group under both seasons. Similar observation was noted for PCT per cent values. The PDW per cent was slightly higher in treatment group than control under HD period. Contrary to this result, control group showed higher values in HH season. In no case, values differed significantly between two groups.

CONCLUSION

It was concluded that heat stress during sub-tropical summer months affected the haematology of buffaloes and the stress could be reduced through the change (use of fans and curtains, nutritional supplementations, and feeding alterations together in the form of one package) of microclimate.

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