IMPACT ON HEMATOLOGICAL PARAMETERS IN YOUNG AND ADULT MURRAH BUFFALOES EXPOSED TO ACUTE HEAT STRESS

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ABSTRACT

The present study was designed to investigate the effect of acute heat stress on some hematological parameters in which young and adult Murrah buffaloes (n=6) were exposed to 40°C, 42°C, 45°C for 4 h duration in climatic chamber and thermoneutral temperature (22°C). Blood samples were collected by jugular vein puncture in sterile vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) from animals after 4 h exposure to different temperatures. In packed cell volume (PCV) and hemoglobin, there was an increasing trend with increase of temperature, but there was no effect of age on these parameters. In case of total erythrocytic count (TEC), there was no effect of temperature but adult animals had higher TEC. Total leukocytic count (TLC) was significantly increased at 40°C and 42°C in young, but there was no effect of temperature on TLC in adult animals. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values were similar in all the four temperatures groups in both young and adult animals. There was no effect of temperature and age on mean corpuscular hemoglobin concentration (MCHC) values. The intensity of changes in all parameters were more pronounced in young animals compared to adult, indicating that young animals were more susceptible to heat stress compared to their adult counterparts. This study concludes that acute heat stress evokes a series of drastic changes in the animal's hematological functions.

Keywords: young and adult Murrah buffalo, heat stress, hemoglobin, packed cell volume, total erythrocytic and leukocytic count

INTRTODUCTION

Stress is the state manifested by a specific syndrome, which consists of all the non-specifically induced changes within a biological system. Both external and internal stressors cause pronounced behavioral, physiological and hematological alterations in tropical livestock. Swamp buffaloes (*Bubalus bubalis*) are found in hot-humid climates and are important livestock in east and southeast Asia. Hafez *et al.* (1955) reported that in Egyptian buffaloes, glandular surface of sweat gland per cm^2 of skin surface was 1.07 in buffaloes and 3.08 in cattle and that the skin thickness of buffaloes was about twice that of cattle. The sweat glands in buffaloes are underdeveloped (Koga, 1999). This indicates less efficiency in sweating in buffaloes than cattle, placeing buffaloes at a disadvantage

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under solar radiation as enhanced reabsorption of solar radiation interferes with heat loss resulting in higher heat storage. So, buffaloes have poor capacity to withstand high temperatures and so need greater attention to protection against adverse climatic conditions. The physiological responses of these animals to environmental stress during winter and summer and their energy balance showed that seasonal heat and cold stress have a profound effect on some biochemical, hematological parameters (Nazifi *et al.,* 1999). Therefore, the present study was undertaken to measure the comparative influence of heat stress on hematological parameters at different temperatures in young and adult buffaloes. This information is likely to further help to minimize stress in Murrah buffaloes.

MATERIALS AND METHODS

Two groups of healthy Murrah buffaloes, young (1-2 yrs) and adult (3-4 yrs), were selected for the experiment. Each group contained six animals. An insulated climatic chamber (22'26"x 10'10" x 8') fitted with thermostatically controlled heat convector was used for exposing animals to heat. The temperature of of the chamber was maintained at 40.0 ± 1.0°C, 42.0 ± 1.0°C and 45.0 ± 1.0°C prior to the experiments. After exposure of animals for 4 h at the above mentioned temperatures, blood was collected. Blood samples were also collected from the animals in the month of March, when average environmental temperature was 22°C, which is considered to be the temperature of thermoneutral environment for tropical animals and used as control. Blood samples were drawn in sterile vacutainer tubes containing EDTA (1mg/ml) anticoagulant by jugular vein puncture posing minimum disturbances. Immediately the tubes were brought to the laboratory for evaluation of hematological parameters.

PCV estimation was done by the Wintrobe, or macrohematocrit, method . Hemoglobin concentration was estimated with Drabkins solution, and the optical density was measured at 540 nm on a spectrophotometer. TEC and TLC were done in a Neubauer chamber under a compound microscope at 40X. Differential leukocyte count (DLC) was done using Leishman stain of 0.15% in methyl alcohol. The cells were counted using a high power oil emersion lens in a strip running the whole length of the film. The erythrocyte indices such as MCV, MCH and MCHC were calculated based on TEC, hemoglobin and PCV.

All the data generated were statistically analyzed by two-way ANOVA using SigmaStat (3.1 software package) Statistical Analysis System (Jandel Scientifiic, San Rafael, CA, USA), according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The data on hematological parameters of young and adult Murrah buffaloes exposed to different temperatures are presented in Table 1. The PCV increased with the increase of temperature in both young and adult buffaloes. In young animals, PCV (%) was increased from 33.83 at 22°C to 38.17 at 45°C whereas the values were 33.17 and 37.33, respectively, in adult animals. The results also showed a significant increase ($P<0.05$) of PCV at 40°, 42° and 45°C as compared to 22°C in young animals whereas in adults, significant differences were only observed at 22°C and 42°C; and 22°C and 45°C interactions only. At other temperature interactions, the values were statistically the same. There was no effect of age on PCV since the values

Table 1. Comparision of hematological parameters in young and adult Murrah buffaloes exposed to different temperatures.

Parameters	Young				Adult				Significance of effects (p)			SEM
	Temperature (°C)								Age	Temp.	Age x Temp.	
	22°C	40°C	42°C	45°C	22°C	40°C	42°C	45°C				
PCV (%)	33.83^{ax}	36.67^{bx}	36.83^{bx}	38.17^{bx}	33.17^{ax}	35.08^{abx}	36.00^{bx}	37.33^{bx}	0.062	<0.001	0.922	0.722
Hb conc. (g%)	12.52^{ax}	13.54^{bx}	13.84^{bcx}	14.50^{cx}	11.94^{ax}	13.41^{bx}	13.70^{bx}	14.23^{bx}	0.118	<0.001	0.776	0.246
TEC (10^6/µl)	7.65^{ax}	7.94^{ax}	8.19^{ax}	8.89^{ax}	9.59^{ay}	9.89^{ay}	10.01^{ay}	9.68^{ay}	<0.001	0.034	0.546	0.408
TLC (10^3/µl)	7.93^{ax}	10.75^{bx}	11.54^{by}	8.92^{ax}	8.08^{ax}	9.73^{ax}	9.17^{ax}	8.40^{ax}	0.005	<0.001	0.045	0.442
Lymphocyte	72.33	75.00	77.17	74.00	72.67	75.00	74.67	72.83	0.377	0.261	0.162	1.319
Neutrophil	24.00	22.17	20.17	24.00	22.50	20.67	21.67	22.33	0.246	0.593	0.202	1.351
Monocyte	2.83	2.33	1.83^{x}	1.33^{x}	3.67	3.33	2.83^{y}	3.67^{y}	0.002	0.385	0.429	0.537
Eosinophil	0.83	0.50	0.83	0.67	1.17	0.83	0.83	1.00	0.260	0.696	0.986	0.310
Basophil	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.17	0.165	0.577	0.577	0.0833
MCV (fl/cell)	44.36^{y}	46.73^{y}	45.26^{y}	43.01^{x}	35.05^{x}	35.69^{x}	34.76^{x}	39.14^{x}	<0.001	0.857	0.358	2.056
MCH (pg/cell)	16.41^{y}	17.26^{y}	17.00^{y}	16.36^{x}	12.57^{x}	13.64^{x}	14.16^{x}	14.89^{x}	<0.001	0.409	0.414	0.764
MCHC (g/dl)	37.06^{x}	36.94^{x}	37.60^{x}	38.06^{x}	36.08^{x}	38.46^{x}	38.12^{x}	38.15^{x}	0.692	0.445	0.668	1.011

Means with different superscripts in rows for a parameter differ significantly (P<0.05).

$^{a, b, c}$ indicate significant difference between temperatures; ^{x,y} indicate significant difference between age groups.

were statistically the same in both the age groups. The result is consistent with the observation of Fagiolo *et al.* (2004) who reported that the PCV was higher at the higher environmental temperature (summer - 40.75% vs winter - 32.63%.). However, in comparision of age groups, a higher value for young was also reported by Ciaramella *et al.* (2005) who found that PCV was higher in heifers than in adult buffaloes. The increase in PCV may be due to the hemoconcentration and dehydration of plasma because when any animal faces severe heat stress, it tries to maintain its body temperature through evaporative water losses, which ultimately lead to the hemoconcentration.

The average hemoglobin concentrations (g %) in young Murrah buffaloes were 12.52, 13.54, 13.84 and 14.50 and those of adults were 11.94, 13.41, 13.70 and 14.23 at 22°, 40°, 42° and 45°C, respectively, and showed an increasing trend with increase of temperature. There was a significant increase ($P<0.05$) in hemoglobin concentration at 40°, 42° and 45° C as compared to 22°C in both young and adult animals. The age had no effect on the concentration of hemoglobin. The observations are in agreement with Fagiolo *et al.* (2004) who reported higher Hb in lactating buffaloes during the summer season (13.62 g/dl) than in the winter season (11.37 g/dl). Like PCV, the increase in hemoglobin also may be due to hemoconcentration. The increase may also be attributed to the fact thatan animal requires more oxygen in any stressful condition and as a consequent, hemoglobin concentration may rise.

Our findings demonstrated that the average TEC (10^6/µl) showed an increasing trend with elevation of temperature (7.65 vs. 8.89 in young and 9.59 vs. 9.68 in adult at 22°C and 45°C, respectively) but statistically, temperature had no effect on TEC in either age group. In comparision of age groups, it was found that the adult animals had significantly higher levels ($P<0.05$) as compared to young at all the temperatures. These observations are similar to the findings of El-Nouty *et al.* (1990), Mayengbam (2008) and Broucek *et al.* (2009).

TLC was found to be increased 35.56% when temperature was increased from 22°C to 40°C and 45.22% when increased from 22°C to 42°C in young buffaloes whereas the values were 20.42 and 13.49 in adults. The TLC had significantly ($P<0.05$) increased at 40° and 42°C temperature as compared to 22°C, which is considered as the thermo-neutral temperature. In case of effect of age on TLC, it was significantly higher ($P<0.05$) in young as compared to adult animals only at 42°C but no difference ($P<0.05$) was found at other temperatures. The increasing trend in TLC with increase of temperature may be attributed to the fact that leukocytes are generally engaged in the immune system. So, when an animal is exposed to thermal stress, the immune system becomes activated, and as a result, TLC may increase. Abdel-Samee (1987) also reported that the white blood cell (leucocytes) count values increased by 21–26% in Friesian cattle under heat stress conditions. Lallawmkimi (2009) reported the same trend in buffalo heifers and lactating buffaloes. This may be due to thyromolymphatic involution under heat stress. After 42°C, there was again a decline in TLC. This may be due to destruction of blood cells at higher temperature. In adult animals, there was no effect of temperature on TLC. These results are in consistent with the findings of El-Nouty *et al.* (1990), Mayengbam (2008) and Broucek *et al.* (2009) who did not observe any effect of temperature on total leukocytic count. The higher value in younger animals might be due to the fact that they are easily affected by thermal stress as compared to adults.

All DLCs were statistically similar (P<0.05) in all temperature interactions and in both age groups except for monocytes, which were found to be increased in adult buffaloes compared to young at 42° and 45° C. There was no significant effect of temperature or age group on the count, which was found consistent with the result of Broucek *et al.* (2009), who also did not find any significant differences in the percentage of basophils, monocytes, and neutrophils when Holstein calves were allotted to different temperature groups. Fagiolo *et al.* (2004) found seasonal changes in early lactating buffaloes in terms of neutrophil percentage (from 64% in summer to 7% in winter). Early lactating buffaloes showed a decrease in lymphocytes during summer (41%) with respect to winter values (77%). Ciaramella *et al.* (2005) also reported significant reduction in the absolute values of lymphocytes in buffaloes above eight years of age. Canfield *et al.* (1984) found eosinophils were significantly higher compared to those in immature females, and Ciaramella *et al.* (2005) reported that buffaloes over ten years of age show higher absolute values of eosinophil levels. Lallawmkimi (2009) observed that lymphocytes, monocytes, eosinophils were decreased after buffalo heifers were exposed to 42°C for 3 h whereas neutrophil values showed the opposite trend. In contrast, Mayengbam (2008) showed that changes in eosinophil and basophil counts due to thermal exposure were not significant in Sahiwal and Karan-Fries after exposure to 40°C and 45°C. The lymphocyte counts decreased whereas neutrophil counts increased after thermal exposure. May *et al.* (1977) also reported a significant increase of neutrophils and decrease of eosinophils when environmental temperature rose from 32°C to 52°C by direct solar radiation for 6 h.

The MCH and MCV values were statistically similar (P<0.05) at all the four temperatures in both young and adult. But, in case of age group, the values were higher in young animals compared to adult at all temperatures except 45°C, where the MCH and MCV were statistically similar. But, in case of MCHC, there was no effect of temperature and age. These results are in agreement with the observations of Fagiolo *et al.* (2004) who reported nonsignificantly different mean values of MCV (53 vs. 56 femtolitres), MCH (17.8 and 19.5 picograms), MCHC as (33.5 vs. 34.8 g/dl) during different seasons in lactating buffaloes. In adults, the low MCH could be due to smaller than normal cells with normal Hb concentration or normal sized cells with lower than normal Hb concentrations. But in contrast, Ciaramella *et al.* (2005) found that it was normally lower in heifers. El-Nouty *et al.* (1990) reported that hot summer weather resulted in significant reductions in mean cell volume and mean cell hemoglobin but had no significant effect on mean cell hemoglobin concentration of calves.

Hence, it is very clear that when buffaloes are exposed to heat stress even for a very short duration (acute heat stress), the total impact on hematological functions may be severe. Young animals, in this study, showing higher intensity of changes of hematological parameters indicates that they are more susceptible to heat stress. Therefore, these animals need greater attention towards protection against adverse climatic conditions.

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