Effect of catecholamines and thermal exposure on lymphocyte proliferation, IL-1α & β in buffaloes

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ABSTRACT: In order to study the effect of catecholamines (epinephrine/norepinephrine) and thermal exposure on in vitro buffalo Lymphocyte Proliferation (LP) apparently healthy 2-2 1/2 years old Murrah buffalo heifers maintained as per the standard feeding and management practices were selected from Institute herd. Jugular blood was collected in the forenoon on the day of experiment and processed for Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC). Lymphocyte proliferation assays were performed using whole blood and cells were incubated with epinephrine and norepinephrine (1, 1.5,2 ng/ml) at 37°C with 5% CO2. Cells were counted after 72 hrs of incubation and Lymphocyte Proliferation Index (LPI) was calculated. Thermal stress effect on the cultures was observed after exposure at 45°C for 4 hr after 72hrs of incubation. The cells were separated from media and media was used for analysis of IL-1α & 1β by ELISA kit. Lymphocyte proliferation Index decreased in responses to Epinephrine and Norepinephrine (P<0.01). Concentration of epinephrine and norepinephrine (1, 1.5,2 ng/ml) had no distinguishable effect on LPI. IL-1α & IL-1β levels when compared with control in supernatant (exposed to 45°C) were low (P<0.01 and P<0.05, respectively). There was a significant positive correlation between LPI and IL-1α (r=0.80; P<0.01) and between LPI and IL-1β (r=0.78; P<0.05). The study indicated that lymphocyte proliferation in vitro and IL-1α & β levels were affected by catecholamines and thermal exposure. Further the levels of catecholamines had significant (P<0.01) negative effect on LPI indicating that catecholamines levels modulate immunity through IL-1α and IL-1β in buffaloes.

Key words: Buffalo, Catecholamines, Lymphocyte proliferation index (LPI), IL-1α & 1β.

INTRODUCTION - Stress is the state manifested by a specific syndrome, which consists of all the non-specifically induced changes within a biological system (Selye, 1956). Both external and internal stressors cause pronounced behavioral and physiological alterations in tropical livestock and homeostatic mechanisms ensure recovery or adaptation to these stressors irrespective of type or level of stress. The hypothalamic-pituitary-adrenal axis and the sympathetic nervous system are markedly activated during thermal and other stress conditions leading to the release of glucocorticoids and catecholamines. Interleukin-1 (IL-1) is a pleiotropic pro-inflammatory cytokine that is produced by lymphoid and non-lymphoid cells. IL-1 plays an important role in immuno-modulation particularly during stress and...
helps in initiation of lymphocyte proliferation and maturation. IL-1 also induces several other factors related to hematopoiesis. The catecholamines modulate the lymphocyte proliferative activity indirectly by decreasing the IL-1 levels in the blood.

MATERIAL AND METHODS - Six Murrah buffalo heifers of 2-2.5 years were selected from the Institute herd and were maintained as per the standard practices followed at the Institute for growing buffaloes. The feed and water was available ad lib.

Whole Blood Lymphocyte Proliferation Assay (LPA). Blood samples were collected from jugular vein in sterile tubes containing EDTA (1mg/ml of blood) and heparin (20 IU/ml). The tubes were immediately transported to the laboratory for further processing for hematology and lymphocyte culture assays. Blood with EDTA anticoagulant was used for Total Leukocyte Counts (TLC) and Differential Analysis as per standard methods used in hematological measurements. Whole blood lymphocyte culture was performed as described by Fletcher et al., (1996) using Heparinized blood.

Measurement of IL-1α and 1β. Five milliliters of diluted blood was dispensed to the flasks containing mitogen and different concentrations (1.0, 1.5 and 2ng/ml) of epinephrine and norepinephrine. All flasks were incubated at 37°C with 5% CO2 for 72 hrs. After 72 hrs, all the flasks were exposed at 45°C for 4 hrs and cells were separated from media by centrifugation at 1500 rpm for 10-min. IL-1α & 1β were assayed by ELISA in separated media following manufacturer’s (Cayman) instructions supplied with kits.

Statistical Analysis. Two ways ANOVA with interaction was performed for finding out effect of temperature and concentrations of epinephrine and nor-epinephrine using Least Square Analysis. Means were compared using Duncan’s Multiple Range Test (DMRT) and correlation Coefficients were analyzed as per Snedecor and Cochran 1967.

RESULTS AND CONCLUSIONS - The effect of catecholamines (epinephrine and norepinephrine) on lymphocyte proliferation was assessed in buffalo heifers, by performing in vitro lymphocyte proliferation assays using whole blood (Fletcher et al., 1996) and Tetanus toxoid as a mitogen. The mean total leucocyte count in blood was 8216 ± 467-cells/mm³. Lymphocytes on an average were 65% and Neutrophils, Eosinophils and Monocytes were 26, 3, and 5%, respectively. The N: L ratio was 0.41, which is indicative of no stresses on animals either due to physiological production or diseases (Stull and McDonough, 1994).

A study conducted on young calves under different housing conditions also concluded that neutrophil levels increased and number of lymphocytes lowered in stalls than those compared to animals kept in loose house system (Friend and Dellmeier, 1986).

Lymphocyte blastogenic activity using tetanus toxoid was observed 8 folds in control, 7, 7, 6 respectively in 1.0, 1.5, 2.0 ng/ ml of epinephrine treated cultures and 7 in all three concentration of norepinephrine treated cultures. The mean lymphocyte proliferation decreased by 17.9 % (7.6 to 6.5) in epinephrine treated cultures and 12.0 % (7.6 to 6.7) in norepinephrine treated cultures (Figure 1). This indicated that epinephrine and norepinephrine significantly decreased LPI (P<0.01). Lymphocyte proliferation response varied at different concentration and due to catecholamines type. Different concentrations of epinephrine and norepinephrine were observed to have no significant differences indicating that physiological levels of epinephrine and norepinephrine had no significant effect on LPI. However, epinephrine or norepinephrine presence in culture significantly (P<0.01) declined the LPI.
Exposure of cultures at 45° C also affects LPI. Earlier studies of Kiranbai et al., (2004) on young and adult buffaloes have indicated that heat exposure at 40°C caused a decline in lymphocyte proliferation index (LPI) in young buffaloes but in adult buffaloes LPI was markedly increased.

IL-1α level in supernatant of thermal exposed lymphocyte culture in control was 6.7 pg/ ml. The levels were 2-pg/ ml and 5.7- pg/ ml in epinephrine and norepinephrine respectively. These results indicated that the IL-1α level decreased significantly (P<0.01) in supernatants after thermal exposure. IL-1β level in supernatant of lymphocyte culture after thermal exposure in control was 9 pg/ ml, in epinephrine treated 5 pg/ ml, and in norepinephrine treated culture was 6-7 pg/ ml (Figure 2). These results indicated that the IL-1β levels in supernatants of lymphocyte culture decreased significantly (P<0.05) after thermal exposure and epinephrine and norepinephrine had different effect on IL-1β.

Interrelationship between LPI and IL-1α and IL-1β were analyzed and Correlation coefficient (r) between LPI and IL-1α was r=0.80 and between LPI and IL-1β was r=0.78. This indicated a significant (P<0.01) positive correlation and dependence of LPI on IL-1. Lymphocyte proliferation has been found to be positively associated with interleukin levels during exercise (Cannon et al., 1986) due to IL-1 secretion and stress hormones production. IL-1 secreted by peripheral blood mononuclear cell (PBMC) has been observed to decrease (P<0.01) due to addition of physiological concentration of epinephrine in vitro. Catecholamine induced suppression of IL-1 production might be mediated by elevated intracellular cAMP levels (Koff, 1986). Different aspects of immune modulation during thermal stress in buffaloes require in depth studies.

The study indicated that lymphocyte proliferation of buffaloes in vitro and IL-1α & β levels were affected by thermal exposure. The levels of catecholamines had significant (P<0.01) negative effect on LPI which indicated that the higher concentration of catecholamines exhibit negatively impact immunity of heat exposed cells through IL-1α and IL-1β.
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