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Alterations of haemato-biochemical and oxidative stress parameter in diarrhoeic buffalo calves

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Abstract
This study was envisaged to elucidate the clinico-pathological aspects viz haematological, serum biochemical and oxidative stress parameters in diarrhoeic buffalo calves. Investigation was carried out on 21 buffalo calves (6 healthy and 15 buffalo calves affected with diarrhoea). Haematological studies revealed significant decrease in value of haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC). There was no significant variation in values of erythrocyte indices i.e. mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV). Significant increase in total leukocytic count (TLC) was observed in calves affected with diarrhoea. Serum biochemical studies revealed that although there were increase in values of total protein, serum albumin, serum globulin in diarrhoeic buffalo calves but that was not significant. However, there was decrease in albumin: globulin ratio in diarrhoeic buffalo calves. Significant increase in activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was noticed in diarrhoeic buffalo calves whereas, non-significant increase in activity of lactate dehydrogenase (LDH) was observed. Studies of oxidative stress parameters showed that there was significant decrease in activity of superoxide dismutase (SOD), glutathione-s-transferase (GST) and glutathione reductase (GR) whereas significant increase in activity of catalase was noticed in diarrhoeic buffalo calves. The present study elucidated that there are alterations of haemato-biochemical and oxidative stress parameters in buffalo calves showing diarrhoeic symptoms.

Keywords: Alterations, haemato-biochemical, oxidative stress parameter, diarrhoeic buffalo calves

Introduction
Animal husbandry is one sector which has high potential for growth. The potential of sector needs to be exploited as this can play a key role in providing sustainable employment in their location itself and arrest migration of people to urban areas. As animal husbandry is an activity which can easily be taken up by rural communities as skill and resource requirements are minimal, inputs are locally available and marketing does not pose a major problem, it can act as an engine in poverty alleviation programmes by making asset less poor into income generating asset owing population. This will go a long way in not only augmenting food security, human security, empowerment of women and rural youths, but will also help in triggering and invigorating the rural economy ultimately contributing significantly to comprehensive socio-economic transformation of the State.

The new born calf has many challenges to face as it begins life on its own. The first of these challenges is a change in environment. However, some challenges won't manifest themselves until later in the calf's life. The first of these is enteric disease. Bacterial organisms and parasites play an important role in diseases causing heavy morbidity and mortality as they are important etiological agents causing gastroenteritis in calves (Singh *et al*., 2009; Singh *et al*., 2014) [16-17]. Some of the diseases are Johne's disease (paratuberclosis), colibacillosis, enterotoxemia, botulism, vibrio dysentery, salmonellosis, *Clostridium prefringens* type- B, C (enterotoxaemia) and type-D, bloat and diarrhoea. Gastrointestinal parasites are also considered as a major challenge for the health and the welfare of animals. Parasitism, especially by helminthic parasites, impairs health by causing inappetance, diarrhoea, anaemia and, in severe cases, death.

Haematological and biochemical variables are most widely used medical decision making tool. Haematological and biochemical analyses of blood are very useful to get an insight in metabolic and health status of animal. During diagnostic procedure it is very useful to compare the values obtained from ill animal with normal values in healthy animals. Therefore specific reference intervals are needed for each animal species for appropriate interpretation of results of haematological and biochemical analyses.

Materials and methods

Ethical approval

The study was conducted after the approval of the Institutional Animal Ethics Committee.

Collection of sample

Proposed study was conducted on 21 buffalo calves (6 healthy and 15 buffalo calves affected with diarrhoea) brought to Department of Veterinary Clinical Complex, LUVAS, Hisar and nearby organized/unorganized farms of Hisar region. Six apparently healthy buffalo calves which were showing normal physiological parameters like temperature, pulse rate, respiration rate, normal feeding and watering and showing normal behavior were kept as control. A complete clinical evaluation was performed before collection of samples for investigation of various parameters. About 5 ml of blood was collected through jugular vein aseptically in the clean sterilized glass vials containing anticoagulant. In addition to this 10 ml of blood was collected without anticoagulant in clean and sterilized glass vials and allowed to clot at room temperature to separate serum. Samples were shifted immediately to the laboratory, to avoid any deleterious effect. The serum samples were stored in deep freezer (-20^{0}C) in vials till further use.

Haematological study

Haematological studies viz. Hb, PCV, TEC, TLC, DLC, absolute leukocytic count and erythrocytic indices namely MCV, MCH and MCHC were undertaken following the method of Benjamin (2013) [3] within six hours of blood collection.

Serum biochemical study

Serum biochemical parameters were estimated by using Chem-5 (Erba Mannheim) chemistry analyzer using standard diagnostic kits of Erba (Transasia). The levels of the following serum constituents viz. total protein (Tietz, 1986b) [21], albumin (Doumas *et al*., 1972) [8], ALT (Wroblewski and La Due 1956) [23], AST (Tietz, 1986a) [20], ALP (Tietz, 1986b) [21] and LDH (Tietz, 1986b) [21] were measured. The gamma-globulin levels and albumin: globulin ratio were calculated.

Oxidative stress parameters

The plasma catalase activity was measured as per method described by the Aebi (1983). In brief 20 µl of 1% erythrocyte lysate was incubated in 1.0 ml of 30 m M H_2O_2 at 37^{0}C and decrease in absorbance was noted every 10 sec interval for one min. at 240 nm in a UV spectrophotometer (Yorco Digital Spectrometer). The catalase activity was expressed as µM of H_2O_2 decomposed/min/mg Hb using 36 as molar extinction coefficient of H_2O_2. The activity of SOD in 1% erythrocyte lysate was determined by the method of Marklund and Marklund (1974) [14]. The assay was based on the ability of SOD to exhibit the auto oxidation of pyrogallol in presence of ethylene diamine tetra acetic acid (EDTA). The values were expressed as units/mg Hb. Glutathione reductase (GR) activity was assayed according to the method of Calberg and Mannervik (1985) [7]. NADPH oxidation was monitored at pH 7.4 and 30^{0}C, and enzyme activity was expressed in terms of micromoles of NADPH oxidized per gram of Hb per min. Glutathione-s-transferase (GST) activity with 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate was measured according to Habig *et al*. (1974) [10]. Formation of the S-conjugate was followed by its absorbance at 340 nm. The activity was expressed in terms of micromoles of CDNB conjugated per gm of Hb/min.

Statistical analysis

Analyses of the data were undertaken by Student's t-test using SPSS 17.0 software (IBM Corporation, New York, USA) as per the Snedecor and Cochran (1994) [19].

Results and discussion

The results of haematological studies are presented in table 1 (a-c). It is evident that there was significant ($P \leq 0.05$) decrease in Hb (9.4 ± 0.75), PCV (32.0 ± 1.19), TEC (6.8 ± 0.36) and significant ($P \leq 0.05$) increase in TLC (16.0 ± 3.12) values diarrhoeic buffalo calves as compared to healthy buffalo calves. There was non-significant decrease in values of all the erythrocyte indices i.e. MCH (14.4 ± 0.85), MCHC (30.4 ± 1.48) and MCV (44.9 ± 2.69). Significant ($P \leq 0.05$) increase in absolute values of neutrophils (5.07 ± 0.71) and lymphocyte (9.8 ± 2.32) was observed in diarrhoeic buffalo calves whereas non-significant increase in value of monocytes (0.391 ± 0.15) and eosinophils (0.649 ± 0.15) was observed in diarrhoeic buffalo calves, respectively.

Decrease in value of Hb, PCV and TEC might be due to malabsorpation of principle nutrients which were required for haemopoiesis, presence of strongyle infection, which has been recognized as active blood sucker in stomach and infection of enterohaemorrhagic *E. coli* which is establish aetiology of haemorrhagic enteritis. Significant increase in absolute values of and neutrophils and lymphocytes in diarrhoeic buffalo calves due to response of cellular body defense mechanism to various aetiological agents like bacteria, bacterial toxin and virus (Jubb, 1993). These findings are in consent with finding of other workers (Alsaad *et al*., 2012; Malik *et al*., 2013; Botezatu *et al*., 2014; Singh *et al*., 2014; Brar *et al*., 2015) [2, 13, 5, 18, 6].

The results of serum biochemical studies of apparently healthy and diarrhoeic buffalo calves are depicted in table 2 (a–b). It is evident from serum biochemical studies that there was non-significant increase in values of serum total protein (7.8 ± 0.45), albumin (4.1 ± 0.27), globulin (3.7 ± 0.23) and albumin: globulin ratio (1.1 ± 0.06) in diarrhoeic buffalo calves. A significant ($P \leq 0.05$) increase in activities of ALT (26.0 ± 1.28), AST (67.1 ± 3.06) and alkaline phosphatase (68.3 ± 3.11) was noticed in comparison to non-significant increase activity of lactate dehydrogenase (244.2 ± 24.80) in diarrhoeic buffalo calves when compared with value of apparently healthy buffalo calves.

Increase in values of serum total protein, albumin and globulin might be due to haeomoconcentration however decrease in albumin: globulin ratio due to increase in fraction of relative globulin in diarrhoeic cow calves and buffalo calves (Duncan, 1994; Kaneko, 1997) [9, 12]. These findings are more or less similar to finding of Malik *et al*. (2013) [13], Singh *et al*. (2014) [18] and Brar *et al*. (2015) [6]. Immunoglobulins are effectively produced by body defense mechanisms in response to infection. Increase in activities of ALT, AST, alkaline phosphatase and lactate dehydrogenase might be due to pathological lesions like cell necrosis, damage to cell membrane and change in permeability of cell membrane of liver, intestine, damage to cells of lining epithelium of intestine (mucous membrane) and muscle fibers, increase in activity of osteoclasts and osteoblasts and cardiac muscle. Damage or increase in permeability of cell membrane leads to leakage of these intracellular enzymes and

comes to plasma (Benjamin, 2013; Kaneko, 1997) [3, 12].

In diarrhoeic buffalo calves, analysis of oxidative stress parameters revealed that there was significant ($P \leq 0.05$) decrease in SOD (6.02 ± 0.37), GST (33.9 ± 3.75) and GR (0.96 ± 0.08) whereas significant ($P \leq 0.05$) increase in activity of catalase (91.6 ± 2.53) in diarrhoeic buffalo calves (Table 3). These finding are in accordance with the findings of Sharma *et al*. (2011). Oxygen derived free radical are produced during normal metabolic processes in reduction-oxidation reaction in normal respiration and oxygen dependent effective killing of bacterial agent by (myeloperoxidase dependent/H_2O_2-MOP- Halide System and myeloperoxidase independent) in diarrhoeic/diseased animals. Superoxide dismutase, glutathione-s-transferase and glutathione reductase are utilized in effective neutralization of these free radicals so their concentration were found decreased in diarrhoeic/diseased animals (Vegad and Katiyar, 2008) whereas increase in activity of catalase might be due to marked reduction in activity of catalase enzyme with increase in temperature (Morgulis *et. al.*, 1926) [15]. In present study, there was significant increase in temperature in diarrhoeic calves therefore *in vivo* activity of catalase was found decreased and *in vitro* values were found increased.

Based on the findings of present study it may be concluded that there was significant decrease in values of Hb, PCV and TEC along with leucocytosis due to absolute neutrophilia and absolute lymphocytosis in diarrhoeic buffalo calves. Erythrocytic indices indicated normocytic and normochromic anemia in buffalo calves. Increase in values of serum total protein, albumin and globulin in diarrhoeic buffalo calves might be due to occurrence of haemoconcentration in diarrheoa. Significant increase in activities of serum ALT, AST, ALP and LDH in diarrhoeic buffalo calves are indicating that there might be some cellular level damage in tissues of major organs like liver, heart, kidney and musculature of buffalo calves. Significant decrease in values of SOD, GST and GR in diarrhoeic buffalo calves might be due to utilization of these enzymes to neutralize free produced during diarrhoeic state. Alterations of haemato-biochemical and oxidative stress parameter in diarrhoeic buffalo calves can be used by veterinarian as indicator for applying adequate preventive and therapeutic measures to prevent further losses.

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