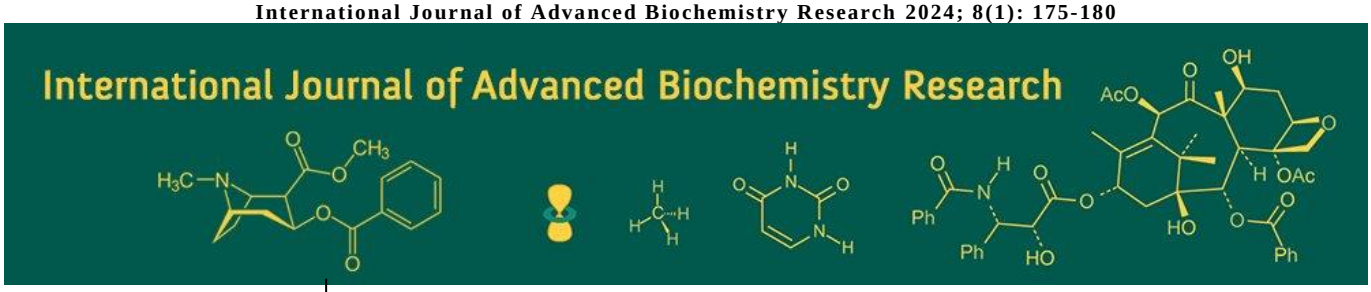

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Comprehensive haemato-biochemical profiling in sheep affected by diarrhea: Insights into physiological impact and veterinary management

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Abstract
The present study was undertaken to investigate the Haematological analysis and Serum biochemical profiles of diarrhoeic sheep/lambs. Haematological analysis of blood collected from diarrhoeic sheep showed a significant increase in values of haemoglobin, packed cell volume, total erythrocyte count and total leucocyte count. Absolute leucocytic count in diarrheic sheep revealed significant leukocytosis due to neutrophilia, lymphocytosis, monocytosis and eosinophilia and no change was observed on erythrocytic sedimentation rate (ESR). Serum biochemical profiles of diarrhoeic sheep/lambs in present study were significant decrease in values of total protein, serum globulin and serum albumin where as significant increase in the albumin to globulin ratio. The activity of functional enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase and Gamma glutamyl transferase were significantly increased in diarrhoeic sheep.

Keywords: Diarrhea sheep, haematological analysis, albumin and globulin, ESR

Introduction
In the dynamic landscape of India's advancing agricultural economy, the indispensable contribution of animal husbandry to economic prosperity is undeniable. The livestock sector holds a key position in driving the nation's economy, particularly considering that more than 70 percent of the population is actively involved in agricultural and animal husbandry pursuits. Livestock resources are vast in India, contributing 4.9 percent to the GVA (Gross Value Added) and 30.13 percent to the total Agriculture and allied sector GVA. Selecting these specific animals within households facing land scarcity and economic challenges is a deliberate and strategic decision. Opting for smaller animals like sheep is driven by their relatively low initial investment requirements and minimal ongoing operational costs. This choice makes them particularly accessible for families with constrained resources and limited land holdings. Additionally, these animals serve as a consistent source of income through the sale of meat and wool, offering these households a sustainable means to improve their economic well-being. Despite advancements in management practices and strategies for prevention and treatment, diarrhea remains the predominant and economically burdensome disease affecting neonatal small ruminants. According to a study on sheep, it was found that diarrhea contributed to a significant 46% of lamb mortality. The present study was formulated on haemato-biochemical investigations in sheep with diarrhea.

Materials and Methods
Hematolo-biochemical studies
Blood collection
The 52 blood samples were collected from sheep/lambs (10 healthy and 42diarroheic sheep/lambs from sheep farm and sheep from clinical cases affected with diarrohea. Ten blood sample were collected from healthy sheep/lambs, as control. 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 freeze (-20 °C) in vials till further use.

Haematological parameters

Haemoglobin (Hb): Haemoglobin was estimated by Sahli's haemoglobinometer and values were expressed in gram percentage (gm%). The Haemoglobinometer tube was filled up to 2 gm% with N/10 HCl and 0.02ml fresh blood was added, then after mixing the content the solution was left to stand for about 10 minutes. The acid haematin was diluted by adding distilled water drop by drop and mixing with the stirrer thoroughly. It was diluted till its colour matches with the standard and the reading were noted down.

Packed Cell Volume (PCV): This was obtained by centrifuging blood (containing 5mg/ml EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a percentage of the original volume of blood with the aid of capillary pipette a Wintrobe's haematocrit tube was filled to the 100 mark with the anticoagulants blood and centrifuged for 30 min at approximately 3000 rpm. As the original column of blood in the tube is 100 mm long, the volume of packed cell was read directly as percentage (%).

Erythrocyte Sedimentation Rate (ESR): The estimation of ESR was done by Wintrobe's method. The Wintrobe's tube is about 11 cm long with a bore diameter 2.5mm and the 10cm of the tube is graduated. The graduations are from zero (top) to hundred (bottom) for ESR and Zero (bottom) to hundred (top) for PCV. Blood containing EDTA was used for the estimation of ESR. Wintrobe's tube was filled upto zero mark on top and the tube was kept vertically in ESR stand for one hour. This is the rate at which erythrocytes sediment by their own weight when blood containing anticoagulant is held in a vertical column. It is expressed as the fall of RBC's in mm at the end of first hour. After an hour reading was taken from the tube directly.

Total Leukocyte Count (TLC): Total leukocyte count was done as per standard procedure using Neubaur's counting chamber and values were expressed in thousands/cu.mm (1000/cu.mm.). WBC diluting fluid was taken in watch glass. The blood was sucked up in the WBC Pipettes upto the 0.5 mark and then WBC dilution fluid was drawn upto the 11 mark of pipette. Solution was mixed gently and few drops of WBC fluid was discarded and the counting chamber was charged by the resulting mixture. The cells were counted under low power objective lens. TLC/cu.mm. = Cells counted x 50

Differential Leukocyte Count (DLC): DLC was done by preparing blood smears of naturally infected animals and staining them with Geimsa stain. A total of 100 leucocytes were counted and number of lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophils were expressed in percentage. A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. The air dried blood smear were fixed in absolute Methyl alcohol for 30 second and the smear was dried. Stain was added in 1:20 dilution, and it was allowed to stand for 30 minutes, then washed and dried finally examined under oil immersion (100x) high power of microscope and number of cells were noted.

Biochemical parameters

1. Serum Total Protein
2. Serum Albumin
3. Alanine amino transferase (ALT)
4. Aspartate amino transferase (AST)
5. Gamma glutamyl transferase (GGT)
6. Alkaline Phosphatase (AP)

Biochemical examination of serum samples separated from above sheep was done. Total protein, albumin, globulin, ALT, AST, GGT & AP were estimated using single step ERBA kits from Transasia Biomedicals Ltd., Mumbai.

Results

Haemato-biochemical studies

Haemotological studies

The results of haematological studies are presented in Table (1, 2 & 3). The present study revealed significant increase ($p \leq .05$) in value of haemoglobin (13.384±.184), packed cell volume (PCV) (41.52±1.164), total erythrocyte count (12.34±.257) and significant increase in leukocytic value (19.146±1.443) in sheep affected with diarrhoea, in comparison with the corresponding values of healthy animals. It was very much evident from these finding that in sheep affected with diarrhoea; there was significant increase ($p \leq .05$) in absolute values of eosinophils (1462.0±117.4), lymphocyte 9687.04±765.15), monocytes (523.28±57.085) and neutrophil (7245.44±697.48) was observed when compared with the value of healthy animals. In present study there was no significant change observed with respect to erythrocytic sedimentation rate (ESR).

Table 1: Haematological observation of healthy and diarrhea sheep

Groups	Hb(g/dl)	PCV (%)	TEC (10^6/µl)	TLC (10^3/µl)	ESR (mm/hr)
Healthy sheep	11.433±.462[a]	39.86±.899[a]	10.88±.514[a]	5.612±.184[a]	.13±.008
Diarrhoeic sheep	13.384±.184[b]	41.52±1.164[b]	12.34±.257[b]	19.146±1.443[b]	.13±.006

Mean±S.E., Different superscripts $p \leq 0.05$.

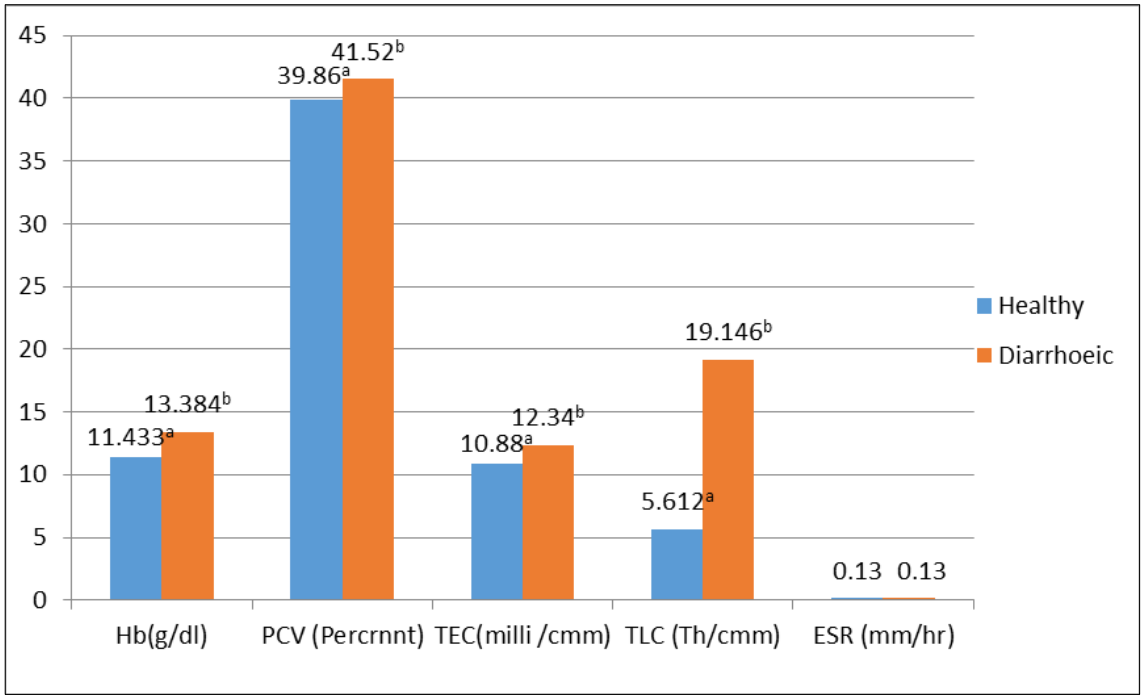

Fig 1: Haematological observation of healthy and diarrhea sheep

Table 2: Haematological observation of healthy and diarrhoeic sheep: Differential Leucocytic Count

Groups	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)
Healthy sheep	37.86±.864[a]	54.96±1.584[a]	5.01±.665[a]	3.42±.281[a]
Diarrhoeic sheep	39.19±1.564[b]	52.12±1.760[b]	6.74±.334[b]	2.82±.194[b]

Mean±S.E., Different superscripts $p \leq 0.05$

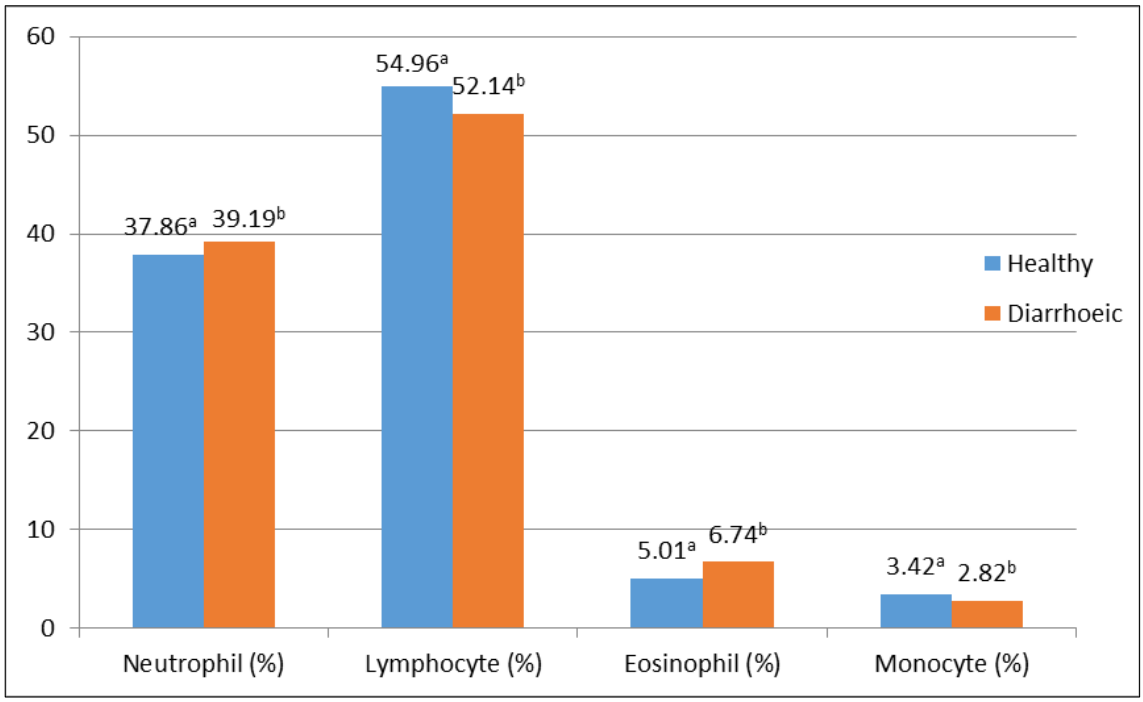

Fig 2: Haematological observation of healthy and diarrhoeic sheep: Differential Leucocytic Count

Table 3: Haematological observation of healthy sheep and diarrhoeic sheep: Absolute Count of Leucocytes

Groups	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
Healthy sheep	2223.67±105.37[a]	3125.±150.96[a]	188.00±23.491[a]	267.34±37.265[a]
Dirroheic sheep	7245.44±697.48[b]	9687.04±765.15[b]	523.28±57.085[b]	1462.0±117.42[b]

Mean±S.E., Different superscripts $p \leq 0.05$.

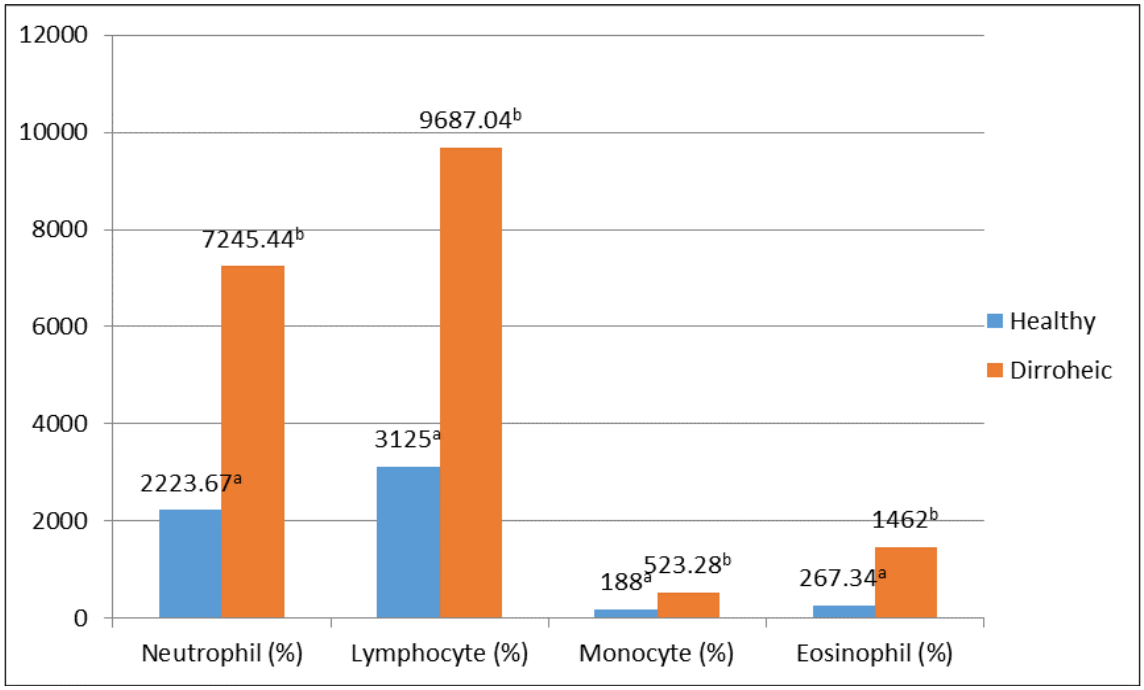

Fig 3: Haematological observation of healthy sheep and diarrhoeic sheep: Absolute Count of Leucocytes

Serum biochemical studies

In the present study results of serum biochemical studies are depicted in Table 4 & 5 and column diagram 4 & 5. It was evident from serum biochemical studies that in sheep affected with diarrhea there was significant decrease ($p \leq .05$) in values of total protein (4.86±.118), serum globulin (1.69±.120), how ever there was significant increase in albumin: globulin ratio. (Table 7).

A significant ($p \leq .05$) increase in activities of aspartate aminotransferase (207.6±12.96), alanine aminotransferase (63.34±1.54), alkaline phosphatase (154.28±19.662) and Gamma glutamyl Transferase (56.64±1.61) were observed in the sheep showing diarrhea (Table 8).

Table 4: Serum bio-chemical observation of healthy and diarrhoeic sheep: Total Serum Protein, Serum Albumin, Serum Globulin and Albumin: Globulin ratio (A:G ratio)

Groups	Total Serum Protein (g/dl)	Serum Albumin (g/dl)	Serum Globulin (g/dl)	A:G ratio
Healthy sheep	5.80±.367^a	3.33±.223^a	2.47±.18844^a	1.33±.09040^a
Diarrhoeic sheep	4.86±.118^b	3.17±.116^b	1.69±.12030^b	2.46±.29345^b

Mean±S.E., Different superscripts $p \leq 0.05$.

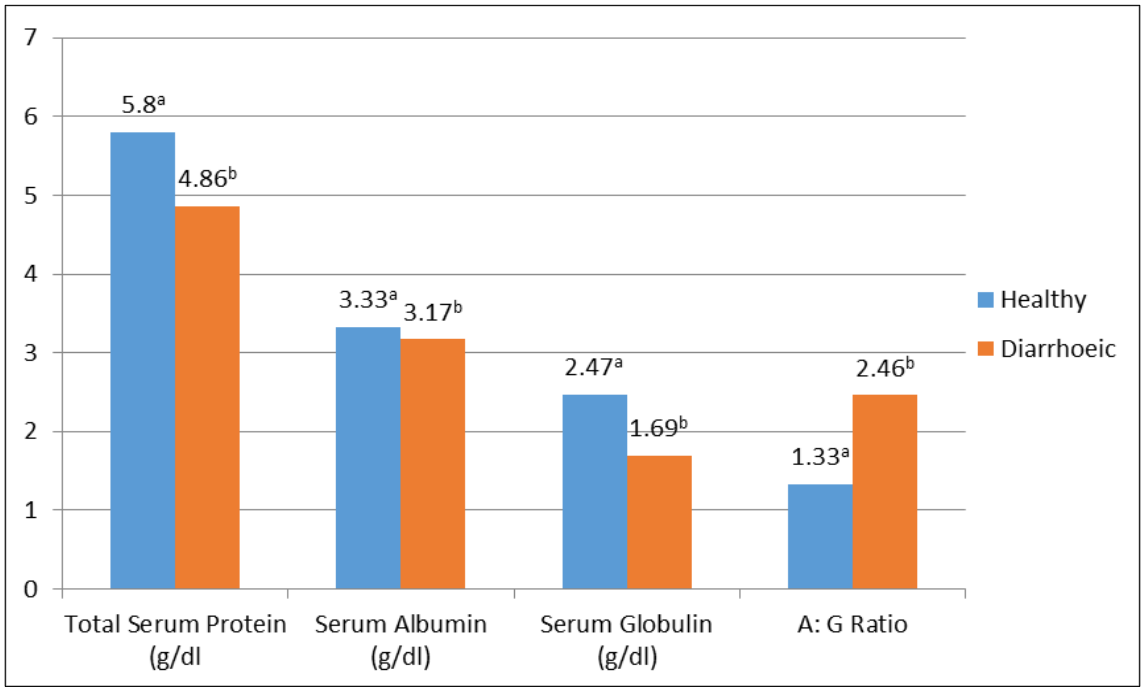

Fig 4: Serum biochemical observation of healthy and diarrhoeic sheep: Total Serum Protein, Serum Albumin, Serum Globulin and Albumin: Globulin ratio (A: G ratio)

Table 5: Serum biochemical observation of healthy and diarrhoeic sheep: Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase &GGT

Groups	Aspartate aminotransferase (IU/L)	Alanine Aminotransferase (IU/L)	Alkaline phosphatase (IU/L)	GGT (Gamma glutamyl Transferase) (IU/L)
Healthy sheep	97.23±5.34[a]	55.54±1.46[a]	77.67±6.5563[a]	42.44±1.54[1]
Diarrhoeic sheep	207.6±12.96[b]	65.34±1.49[b]	149.28±18.662[b]	56.64±1.61[b]

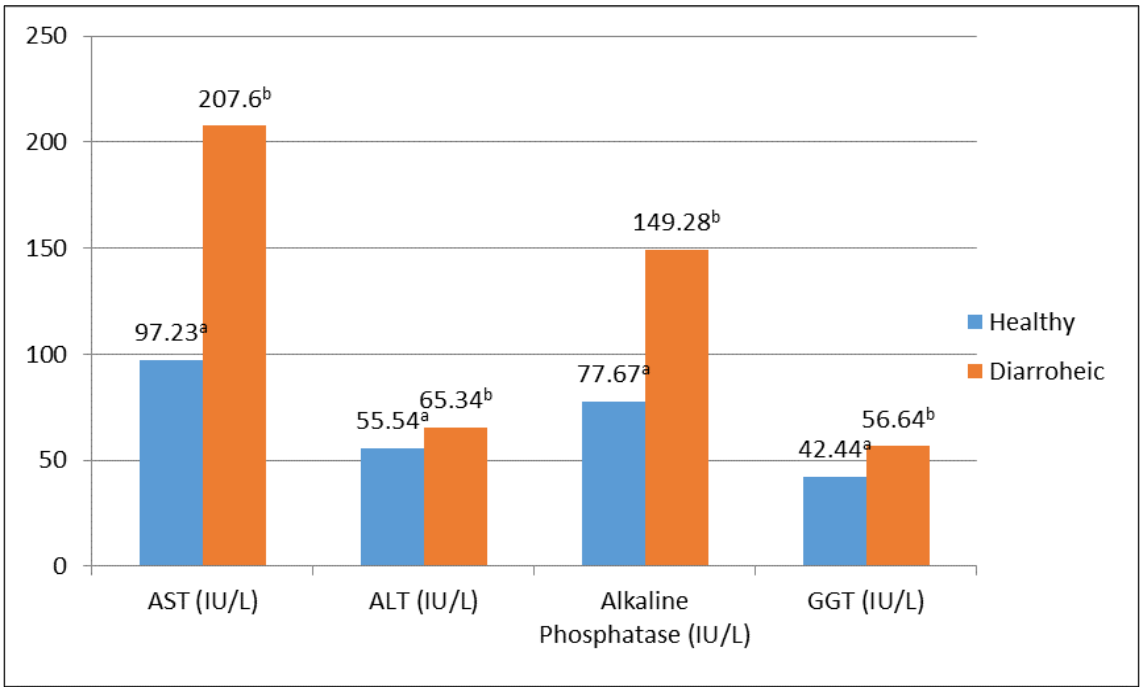

Fig 5: Serum biochemical observation of healthy and diseased sheep: Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase & GGT

Discussion

Haematological analysis of blood collected from diarrhoeic sheep showed a significant increase in values of haemoglobin, packed cell volume, total erythrocyte count and total leucocyte count. Absolute leucocytic count in diarrheic sheep revealed significant leukocytosis due to neutrophilia, lymphocytosis, monocytosis and eosinophilia and no change was observed on erythrocytic sedimentation rate (ESR). The haematological studies in present investigations were in unison with the observation of Bordoloi, (2012) [3], Ramesh and Chaudhary (2015) [11]; Kumar *et al.* (2015) [6]; Ahmed, *et al.* (2015) [1] and Tarunpreet *et al.* (2019) [13]. Increase in Hb, PCV and TEC is due to dehydration and hemoconcentration which occur invariably in cases of diarrhea as fluid volume is lost from body so relative count increases. Absolute leucocytic count in diarrhoeic sheep revealed significant leucocytosis due to neutrophilia, lymphocytosis, monocytosis and eosinophilia were in unison with the previous observation of Hassen *et al.* (2013) [5]; Ahmed (2020) [2] and Tarunpreet *et al.* (2019) [13]. Increased TLC might be due to reaction of body defence mechanism against infection and also due to dehydration and hemoconcentration as reported by Hassen *et al.* (2013) [5]. Serum biochemical profiles of diarrhoeic sheep/lambs in present study were significant decrease in values of total protein, serum globulin and serum albumin where as significant increase in the albumin: globulin ratio. These results are in agreement with the previous finding of Hassen *et al.* (2013) [5]; Singh *et al.* (2014) [12], Kumar *et al.* (2015) [6]; Ebrahim (2018) [4]. In diarrhoeic sheep inappetance with resultant reduction in dietry protein and plasma losses from demaged intestinal mucosa might be the main cause for hypoproteinemia. Decreased globulin concentration may be due to the lack of colostrum feeding in lambs and faecal losses of globulin. The inflammation of intestine by development stages of parasites and bacterial infection might also because of poor absorption of protein metabolites resulting hyperproteinemia. The decrease in the albumin value is likely due to liver damages and increased catabolism of albumin through the gastrointestinal leakage (Kumar S. *et al.* 2015) [6]. The activity of functional enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase and Gamma glutamyl transferase were significantly increased in diarrhoeic sheep. Similar finding were also reported by Kumar *et al.* (2015) [6]; Ahmed *et al.* (2015) [1]; Raghavendra *et al.* (2018) [10]. Increase in enzymatic activities might be due to pathological lesions of Gastrointestinal tract and liver (Radostits *et al.* 2000) [14] which were seen in present study.

Conclusion

Haematological analysis of blood collected from diarrhoeic sheep showed a significant increase in values of haemoglobin, packed cell volume, total erythrocyte counts and total leucocyte count. Absolute leucocytic count in diarrheic sheep revealed significant leukocytosis due to neutrophilia, lymphocytosis, monocytosis and eosinophilia and no change was observed on erythrocytic sedimentation rate (ESR). Serum biochemical profiles of diarrhoeic sheep/lambs in present study were significant decrease in values of total protein, serum globulin and serum albumin where as significant increase in the albumin: globulin ratio. The activity of functional enzymes aspartate aminotransferase, alanine aminotransferase and alkaline

phosphatase and Gamma glutamyl transferase were significantly increased in diarrhoeic sheep.

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