

Hematological, Serum Biochemical and Parasitological investigation of calf diarrhea

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ABSTRACT

This study aimed to isolate the most common pathogens causing diarrhea in calf in addition to studying the hematological, biochemical, and fecal changes associated with the disease. The study was conducted on 44 calves, 31 diarrhea-diseased cases and 13 animals were apparently healthy and were served as control. Hematological, biochemical, and fecal changes were investigated through examining whole blood, serum and fecal samples collected from both healthy and diseased groups. The results implicated that *Eimeria* and *Toxocara* were the most common parasitic pathogens isolated from both healthy and diseased animals. Hematological parameters showed a significant increase in red cell parameters including red cell count, hemoglobin, and packed cell volume as well as total leukocytic counts in the diseased group compared to control. Significant increases were observed in the serum enzymatic activities of ALT, AST, ALP GGT, amylase and lipase. Serum concentrations of glucose, urea, BUN, creatinine were significantly increased, whereas serum levels of total protein, albumin, triglyceride, Na, K and Cl showed a significant decrease. No significant changes were observed in blood pH and bicarbonate levels in the diarrheic animals when compared to control group. We can conclude that *Eimeria* and *Toxocara* appeared to be the main parasitic causes of diarrhea in calf. The disease is associated with significant hematological, and biochemical alterations which upon understanding can provide a good knowledge of the pathogenesis and thus controlling the condition in calf.

Keywords: Diarrhea, Calf, Clinical Pathology, *Eimeria*, *Toxocara*

INTRODUCTION

Diarrhea is a disease of the digestive system characterized by watery feces and increased frequency of bowel movements. In livestock, the commonest cause of calf-hood disease is diarrhea (Wudu *et al.*, 2008). Diarrhea commonly affects newborn calves because of their liquid diet (milk), the higher water content in their bodies compared to adult cattle, and their susceptibility to certain age-specific infectious diseases of the intestinal tract (Villarroel, 2009).

Calf diarrhea remains one of most the leading causes of mortality in dairy calves and an

important cause of morbidity and mortality in beef calves (Peter, 2004). The 2007 National Animal Health Monitoring System (NAHMS) for U.S. dairy reported that 57% of weaning calf mortality was due to diarrhea and most cases occurred in calves less than 1 month old (USDA, 2007). A similar mortality rate (53.4%) for dairy calves due to calf diarrhea was recently reported in Korea (Hur *et al.*, 2013).

The economic losses due to diarrhea occur not only from mortality but also from other costs including treatment, diagnostics, labor, veterinary intervention and decreased number

of herd replacements as well as subsequent chronic ill thrift and impaired growth performance (Bazeley, 2003).

Diarrhea in calves is a multi-factorial disease associated with predisposing and determinant factors (environment, management, nutrition, immunology, and microorganisms) (Stipp *et al.*, 2009). Many of enteropathogens producing diarrhea result in severe intestinal lesions, alterations in enzyme activity and nutrient transport mechanism, or a combination of these effects (Wudu, 2008).

Due to the importance of clinical pathology in supporting the diagnosis of the disease, to provide a greater understanding of the clinical picture of the disease and thus a rationale for subsequent management, as well as a tool to assess the benefits of therapy, the present investigation aimed to study some clinicopathological changes which involve comparing hematological, biochemical changes and fecal analysis between the healthy and diseased animals with special reference to the most common parasitic causes of the disease in calf.

MATERIALS AND METHODS

Animals:

The present study was conducted on up to 6 months old (7 days till 6 months) 44 bovine calves in different localities in Menufiya governorate, Egypt. 31 calves were suffering from calf diarrhea. The rest 13 calves were apparently healthy and considered as control group.

Samples:

Blood samples were collected from all calves from jugular vein under aseptic techniques using clean tubes and syringes and were divided into 3 portions. The first portion was collected on disodium ethylene diamine tetracetic acid (EDTA) for the hematological assays. The second portion was collected on heparin (20 IU/ ml) for blood pH and bicarbonate measurement. Finally, the third portion was placed in plain centrifuge tubes for separation of serum. Serum samples were stored at -20°C until assayed for other biochemical tests. Fecal samples were collected directly from the rectum of diarrheic calves in sterile plastic bags. Fecal samples were subjected to both macro and microscopical examination for detecting various etiological agents of diarrhea.

Analytical methods:

Fecal examination

Fecal samples were subjected to the following techniques:

Direct fecal smear:

A pinhead piece of feces was spread on a microscopic slide and mixed with 1-2 drops of saline solution by the aid of glass stick, then covered by cover slip and examined under light microscope (Soulsby, 1988). Direct fecal smears are most useful for the diagnosis of motile protozoa trophozoites or cysts.

Floatation method:

About 1ml of sieved mixed sediment fecal specimen with water was diluted by 10-20 ml of saturated salt solution in a test tube and filled to the top. A cover glass was put over the top of the tube so that it was in contact with the liquid. After about 10-20 minutes, the cover glass was gently removed and examined under a low power (Soulsby, 1988). Floatation method is useful for the detection of nematode and cestode eggs.

Detection of Cryptosporidium oocyst:

Fine fecal smear was done, dried in air fixed with methanol, stained with modified Ziehl-Neelson stain, and was examined with light microscope under oil immersion lens $\times 1000$ for detection of *Cryptosporidium* oocyst according to Henriksen and Pohlenz., (1981).

Hematological studies:

The hematological parameters evaluated in this study included red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count (PLT) and total (TLC) and differential leukocytic counts. Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using RBCs, Hb and PCV values. All hematological parameters were measured using 3 Part Differential Veterinary Hematology Analyzer H32 (Avantor/ J.T. Baker, BeneSphera, USA). These hematological parameters were performed according to the procedures adopted by Feldman *et al.*, (2000)

Biochemical studies:

All serum biochemical parameters were measured by spectrophotometric method (Unico spectrophotometer UV 480, USA) using commercial kits and following the manufacturer's instructions.

Assay of serum total proteins (TP) was carried out by the Biuret method as described by

Gornal et al., (1949). Serum albumin (Alb) level was determined by the method of **Doumas (1971)**. Serum globulin (Glob) was calculated by subtracting serum albumin from serum total protein and then A/G ratio was calculated by dividing albumin on globulin. Blood urea concentration was measured colorimetrically based on the method of **Fawcett and Soctt (1960)**. Serum concentration of creatinine was determined according to **Schirmeister et al., (1994)**.

Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) activities were determined according to the method described by **Reitman and Frankel (1957)**. Serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities were detected colorimetrically according to the method described by **Belfield and Golderg (1971)** and **Saw et al., (1983)** respectively.

Serum cholesterol level was measured according to the method of **Richmond (1973)**. Serum concentrations of triglycerides was assayed according to **Fossati and Prencipe (1982)**. HDL level was measured by using the method of **Burstein et al., (1970)**. Serum LDL was measured based on the method of **Wieland and Seidel (1983)**. VLDL was calculated by dividing triglycerides by 5 according to **Wieland and Seidel (1983)**.

Serum level of sodium (Na) was measured according to **Trinder (1951)** while, serum chloride (Cl) concentration was estimated following method of **Schales and Schales (1941)**. Determination of serum potassium level (K) was done as described by **Sunderman and Sunderman (1958)**.

Serum calcium (Ca) and inorganic phosphorus (ip) concentrations were estimated according to methods described by **Gindler and King (1972)** and **El-Merzabani et al., (1977)** respectively. Blood pH was directly measured at 37°C by Rapid point 340® Blood Gas Analyzer (England) using kits supplied by Symbiotic Corporation (11011 via Frontera, San Diego). Plasma bicarbonate levels (HCO₃) was calculated automatically by the blood gas analyzer.

Statistical analysis procedures:

All the values were presented as mean ± standard deviation (SD). Mean values of diseased group and control group were compared by student's t test at 0.05 level of probability (**Snedecor and Cochran 1980**).

RESULTS

Clinical signs:

Affected calves showed watery stools that may be brown, grey, green, yellow in color. Occasionally blood and mucus may be evident in the stools. Calves were weak, depressed and developed a sunken-eyed appearance because of dehydration. Fever, anorexia, depression, tenesmus, colic and emaciation were also reported.

Dehydration degree did not affect survival rate significantly, but the severity of depression significantly affected the survival of diarrhetic calves. Three calves died in this study. The dead calves were severely depressed and were in lateral recumbency during physical examination.

Fecal examination:

Macroscopic examination:

Diseased calves showed diarrhea in the form of semisolid to watery feces of whitish or yellowish color. Some samples contained blood and mucous.

Microscopic examination:

As shown in Table 1, 44 fecal samples (31 diseased and 13 control) were examined for isolation of parasitic causative agents and the results clarified that 7 coccidia isolates were recovered, (2 from control samples and 5 from diarrhetic samples) while 5 Toxocara isolates were recovered, (2 from control samples and 3 from diarrhetic samples) at percentages of 15.9% and 11.3% respectively. One diarrhetic sample showed mixed infection between Coccidia and Toxocara at a percentage of 2.2% (Table 1). On the other hand, *Cryptosporidium sp.* was not demonstrated in diarrhetic calves on fecal smear stained with modified Ziehl-Neelson stain.

Fecal examination of control and diseased samples by ordinary direct smear method showed that there was increasing numbers of RBCs and pus cells in fecal smear of diarrhetic calves compared to control healthy calves. Fat globules, vegetable cells, starch granules and muscle fibers were clearly evident in diarrhetic samples as compared to control healthy ones (Table 2).

Hematology:

Data shown in Table 3, indicated that there was a significant ($p \leq 0.05$) increase in RBCs count, Hb and PCV values in the diarrhetic calves compared to the control healthy animals while, MCV, MCH and MCHC values did not show significant differences between the two

groups. Platelet count was significantly ($p \leq 0.05$) higher in affected calves compared to the control.

Diarrheic calves demonstrated significant ($p \leq 0.05$) increase in the TLC as well as neutrophilic and monocytic percentages as compared to healthy calves (Table 3). On the other hand, affected group showed a significant ($p \leq 0.05$) decrease in lymphocytic percentage but no significant changes were observed in the percentages of eosinophils and basophils.

Serum biochemistry:

The effect of diarrhea on the basic metabolic profile as shown in (Table 4) cleared that there was a significant ($p \leq 0.05$) decrease in serum concentrations of total protein and albumin in the diarrheic calves compared to the control healthy animals while, serum globulin and A/G ratio showed no significant changes in the affected calves. The mean values of glucose, urea, BUN, and creatinine were significantly ($p \leq 0.05$) higher in affected calves compared to control.

Regarding serum enzymatic activities, the data presented in Table 5, showed that there was a

significant ($p \leq 0.05$) increase in the serum enzymatic activities of ALT, AST, ALP, GGT, amylase and lipase in the diarrheic calves compared to the control healthy animals.

Evaluation of serum levels of lipid profile parameters as shown in Table 6, demonstrated a significant decrease in the triglycerides level of the affected animals compared to the control group. On the other hand, diarrheic calves did not show significant differences in the values of total cholesterol, HDL, LDL and VLDL as compared to the control group.

Changes in serum levels of minerals and electrolytes as shown in Table 7, indicated that there were no significant changes in both total and ionized calcium in the diarrheic group. The results of serum phosphorus implicated a significant ($p \leq 0.05$) increase in diarrheic calves compared to healthy group. Comparison the mean values of serum of Na, K and Cl in both groups showed significant decrease ($p \leq 0.05$) in the diseased animals (Table 7). Diarrheic calves did not show any significant changes in Mg, pH or bicarbonate values as compared to the control.

Table 1: Prevalence rate of parasitic isolates from apparently healthy and diarrheic calves.

Fecal isolate	Method of detection	No of +ve samples		Total	(%)
		Control	Diarrhetic		
Coccidia	Direct smear and flotation	2	5	7	15.9
Toxocara	Direct smear and sedimentation	2	3	5	11.3
Mixed infection		0	1	1	2.2
Number of samples =44(13 control, 31 diseased)					

Table 2: Macroscopic and Microscopic findings of fecal smears.

Finding	Control	Diarrhetic
Macroscopic findings		
Color	Brown	Yellow, greenish, white
Consistency	Formed	Soft to watery
Mucus	Nil	Nil to +
Gross blood	Nil	Nil to +
Microscopic findings		
Pus cells/HPF	2-6	30-60
RBC/HPF	0-3	10-55
Fat globules	+	++
Vegetable cells	+	+++
Starch granules	+	++
Muscle fibers	+	++
Total number of samples= 44		

HPF: High power field; RBC: Red blood cell.

Table 3 : Hematological parameters of diarrhetic calves compared to the control healthy group. Values are mean \pm SD.

Parameters	Groups	
	Control (N =13)	Diseased (N =31)
RBCs ($\times 10^6$ / μ l)	6.46 \pm 0.64	10.73 \pm 0.32*
Hb (g/dl)	11.12 \pm 0.41	16.02 \pm 0.48*
PCV (%)	33.08 \pm 1.88	45.49 \pm 1.01*
MCV (fl)	46.74 \pm 1.35	46.14 \pm 0.34
MCH (pg)	15.37 \pm 0.50	15.26 \pm 0.34
MCHC (%)	32.45 \pm 0.11	32.75 \pm 0.32
Platelets($\times 10^3$ / μ l)	357.20 \pm 13.01	535.83 \pm 24.62*
TLC($\times 10^3$ / μ l)	12.77 \pm 0.90	17.93 \pm 0.60*
Neutrophils (%)	48.60 \pm 2.30	63.10 \pm 2.30*
Lymphocytes (%)	46.00 \pm 2.00	31.17 \pm 1.17*
Monocytes (%)	3.10 \pm 0.07	4.20 \pm 0.45*
Eosinophils (%)	1.82 \pm 0.30	1.76 \pm 0.19
Basophils (%)	0.20 \pm 0.15	0.20 \pm 0.17

RBCs: Red Blood Cells; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; TLC: Total leucocyte count.

Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

Table 4: Basic metabolic profile of calves suffering from diarrhea compared to the control healthy group. Values are mean \pm SD.

Parameters	Groups	
	Control (N =13)	Diseased (N =31)
Total protein (g/dl)	6.69 \pm 0.40	5.53 \pm 0.37*
Albumin (g/dl)	3.13 \pm 0.01	2.37 \pm 0.41*
Globulin(g/dl)	3.56 \pm 0.41	3.16 \pm 0.56
Albumin/Globulin ratio	0.89 \pm 0.11	0.78 \pm 0.23
Glucose (mg/dl)	59.096 \pm 13.23	101.59 \pm 4.32*
Urea (mg/dl)	25.87 \pm 1.91	54.81 \pm 7.64*
BUN (mg/dl)	12.09 \pm 0.89	25.61 \pm 3.57*
Creatinine (mg/dl)	1.067 \pm 0.0058	2.18 \pm 0.80*

Bun: Blood urea nitrogen.

Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

Table 5 : Serum enzymatic activities of diarrhetic calves compared to the control healthy group. Values are mean \pm SD.

Parameters	Groups	
	Control (N =13)	Diseased (N =31)
ALT (U/L)	16.97 \pm 1.26	23.27 \pm 0.55*
AST (U/L)	8.76 \pm 3.52	71.59 \pm 10.51*
ALP (U/L)	111.63 \pm 1.52	202.30 \pm 24.62*
GGT (U/L)	5.50 \pm 0.50	20.00 \pm 2.00*
Amylase (U/L)	7.63 \pm 1.08	23.19 \pm 1.08*
Lipase (U/L)	6.50 \pm 1.50	16.00 \pm 2.0*

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyltransferase.

Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

Table 6: Lipid profile of calves suffering from diarrhea compared to the control healthy group. Values are mean \pm SD.

Parameters	Groups	
	Control (N =13)	Diseased (N =31)
Total cholesterol (mg/dl)	47.50 \pm 7.50	48.00 \pm 2.66
Triglycerides (mg/dl)	31.66 \pm 1.53	23.74 \pm 4.43*
HDL (mg/dl)	27.45 \pm 2.03	22.16 \pm 3.90
LDL (mg/dl)	21.13 \pm 1.27	25.31 \pm 3.99
VLDL (mg/dl)	6.33 \pm 0.31	5.00 \pm 0.81

HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein. Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

Table 7: Serum minerals and electrolytes and pH of diarrhetic calves compared to the control healthy group. Values are mean \pm SD.

Parameters	Groups	
	Control (N =13)	Diseased (N =31)
Ca-Total (mg/dl)	6.42 \pm 0.36	5.72 \pm 0.58
Ca-Ionized (mg/dl)	2.40 \pm 0.21	2.55 \pm 0.42
Phosphorus (mg/dl)	6.63 \pm 0.86	10.43 \pm 1.50*
Na (mmol/L)	136.85 \pm 1.98	125.83 \pm 0.39*
K (mmol/L)	6.15 \pm 0.20	4.94 \pm 0.66*
Cl (mmol/L)	113.18 \pm 1.34	99.29 \pm 4.42*
Mg (mg/dl)	1.70 \pm 0.08	2.47 \pm 0.517
pH	8.08 \pm 0.061	7.94 \pm 0.14
Bicarbonate(mmol/L)	24.70 \pm 0.26	25.37 \pm 0.55

Ca: Calcium; Na: Sodium; K: Potassium; Cl: Chloride; Mg: Magnesium.

Significant differences in the values between the diseased and the control groups were indicated by (*) at $P \leq 0.05$.

DISCUSSION

In young calves, diarrhea is considered as one of the most important diseases, because the resulting economic loss following mortality, treatment costs, and decrease of growth rate would be detrimental (Maes *et al.*, 2003).

Diarrhea is a well-known clinical sign in neonatal animals. Its etiology is complex involving management, environmental, nutritional, physiological variations. Variety of pathogens including bacteria, viruses, protozoa and intestinal parasites are described as important agents causing diarrhea (either separately or in combination) in buffalo calves (Prescott *et al.*, 2008). The incidence of calf diarrhea occurs all over the year with some increase in calving seasons.

The clinical signs observed in affected calves in this study matched well with those reported in previous studies which included watery stools that may be brown, grey, green, yellow in color. Occasionally blood and mucus may be evident in the stools. Calves are often weak to stand, depressed and developed a sunken-eyed appearance as a result of dehydration. Left untreated, death typically occurs within 24 hours. Depending on the cause(s) and the severity of the infection, a case of scours in a calf can last 1-2 days or as long as 2 weeks (Gould, 2014).

Routine fecal examination in the present study revealed that coccidia and *Toxocara* species were the most common parasites recovered from both control and diarrhetic samples at percentages of 15.9% and 11.3% respectively

while, *Cryptosporidium* sp. was not demonstrated in fecal smears stained with modified Ziehl-Neelson stain.

In this respect, previous studies indicated that 58.5% of diarrhetic cases in suckling buffalo calves were due to internal parasitic infection with *Toxocara vitulorum* and *Eimeria* sp. were the most common protozoal agents recorded in these studies (Reberio, *et al.*, 2000). Ramadan *et al.*, (2015) added that *Eimeria* sp. and *Toxocara vitulorum* were the most parasitic pathogens isolated from diarrhetic suckling buffalo calves at percentages of 30.4% and 17.4% respectively.

Regarding the hematological parameters, the results presented in this work implicated a significant increase in RBCs count, Hb and PCV values in the diarrhetic calves compared to the control healthy animals. These findings are in accordance with those as obtained by (Fernandes *et al.*, 2009b, Bipin *et al.*, 2010, Malik *et al.*, 2013 and Shekhar *et al.*, 2017). Increased red cell count and PCV% could be attributed to hemoconcentration of blood due to excessive loss of body fluid and inadequate intake of milk and fluids during diarrhea (Eddy and Pinsent, 2004). The resultant hemoconcentration can be further aggravated by the losses of plasma and interstitial water.

On contrary to the present results, Kumar *et al.*, (2018) revealed a significant decrease in values of Hb, PCV and RBC count in diarrhetic buffalo calves. Erythrocytic indices indicated normocytic and normochromic anemia. Platelet count was significantly ($p \leq 0.05$) higher in affected calves compared to control. Consistently, thrombocytosis was observed in many cases of calf diarrhea along with presence of immature platelets, indicating septicemia (Apminder, 2009).

Gurbuz *et al.*, (2006) reported no significant difference in the number of thrombocytes between diarrhetic and healthy calves, while a visible drop in platelets counts were observed in diarrhetic group of calves (Sobiech *et al.*, 2013). The splenic pool contains 20%-30% of platelets and they can be released into the blood circulating during stress, pain, anxiety, or concurrent disease (Barton *et al.*, 1998).

Concerning the white blood cell parameters, results of this study showed a significant ($p \leq 0.05$) increases in TLC of diarrhetic calves as well as neutrophilic and monocytic percentages when compared to healthy calves. Affected group showed a significant ($p \leq 0.05$)

decrease in lymphocytic percentage but no significant changes were observed in the percentages of eosinophils and basophils.

Similar results of leukogram (neutrophilia and lymphopenia) observed by Malik *et al.*, (2013) and Brar *et al.*, (2015). Leukocytosis might have occurred due to normal reaction of body defense mechanism against infection whereas marked neutrophilic response with lymphopenia is characteristic of acute bacterial enteritis (Brar *et al.*, 2015). Dehydration and hemoconcentration could be possible causes.

Hayat *et al.*, (1999) added that the decrease in lymphocytes could be attributed to its supply of globulins, which is under the control of adrenocortical hormones upon lymphoid tissue and lymphocytes, resulting in increased rate of cytoplasmic budding and dissolution of cells during the disease. Moreover, the toxins produced by bacteria during diarrhea may produce stress to the infected animals with significant decrease in relative and absolute numbers of lymphocytes (Coles, 1974).

No significant changes were seen in both eosinophilic and basophilic count in the affected calves which agrees with the results of Malik *et al.*, (2013). On contrary, eosinophil percentage was significantly increased in calves naturally infected with cryptosporidiosis compared to healthy calves (Sahu and Maiti, 2011). In addition, *Eimeria*-infected calves demonstrated eosinophilia and basophilia in the study of Ahmed and Soad (2007).

The results of serum biochemical parameters showed a significant ($p \leq 0.05$) decrease in serum levels of total protein and albumin of the diarrhetic calves compared to the control group. These results are in consistency with the findings of Rasha *et al.*, (2015) who found that the mean values of total serum proteins and albumin were significantly lower in diarrhetic calves than control group. The possible explanation for the decreased levels of total protein and albumin is the excretion of those parameters in the intestinal lumen with diarrhea (Constable *et al.*, 2016). Others found significantly higher serum concentrations of total protein and albumin in diarrhetic calves due to colibacillosis (Asati *et al.*, 2008). The differences between studies could be due to type of causative agent, degree of dehydration and other environmental conditions.

The result of increased serum glucose level reported in this study comes into line with the finding of Guzelbektes *et al.*, (2007) who reported increased plasma glucose concentration along with the increased dehydration degree in older calves with diarrhea. Bovine tends to produce marked stress hyperglycemia therefore, stress associated with general systemic illness and infections may lead to increased endogenous glucocorticoids production that inhibits the action of insulin on glucose metabolism and thus hyperglycemia results (Kaneko *et al.*, 1997)

In this work, investigation of renal function revealed a marked increase in serum concentration of urea, BUN and creatinine in diarrheic calves compared to control. Also, Seifi *et al.*, (2006) found that diarrheic calves had significantly higher serum concentrations urea nitrogen and creatinine than the control calves. Elevation of serum urea and creatinine concentrations in diarrheic calves might be due to deficit in renal blood perfusion thus reducing urine formation as previously reported by Singh *et al.*, (2014). This explanation was further supported by Guzelbektes *et al.*, (2007) who reported an increased blood urea along with the increase of degree of dehydration in calves with diarrhea.

Significant ($p \leq 0.05$) increases in serum activities of ALT, AST, ALP, GGT, amylase and lipase were seen in the diarrheic calves in the present study as reported in previous studies (Kumar *et al.*, 2018 and Sherif *et al.*, 2020). Chronic inflammation of GIT and pathological affection of liver might be the cause of recorded elevation of serum AST, ALT and GGT diarrheic calves (Chernecky, 2013). Kleczkowski *et al.*, (2008) confirmed that the significant increase in ALP level in diarrhea might be due to damage of intestinal mucosa, progressive inflammatory process, and release of the intestinal fraction of the enzyme into circulation. These findings disagree with some reports which recorded no significant alterations in the activities of ALT and AST between the diarrheic and healthy calves (Singh *et al.*, 2014 and Shekhar *et al.*, 2017).

Concerning the lipid profile parameters, results of this study showed a significant decrease in the serum levels of triglycerides in the affected animals compared to control

group. This could be attributed to disturbance in lipid absorption resulting from deficiency of digestive enzymes as supported in this study by elevated serum lipase activity.

No significant changes were observed in other lipid parameters in the present work. On contrary, significant increases in triglycerides with significant decreases in serum levels of total cholesterol, HDL, LDL and VLDL were determined in neonatal calves with diarrhea (Bozukluhan *et al.*, 2017).

With respect to minerals and electrolytes evaluated in this study, the present data did not show significant changes in both total or ionized Ca. Consistently, Guzelbektes *et al.*, (2007) demonstrated that serum calcium level of calves during diarrhea was within the normal range. Santos *et al.*, (2002) and Ghanem *et al.*, (2012) however, found that calcium levels were significantly higher ($p < 0.05$) in diarrheic calves of 1-7- and 22-30-days age groups as compared to corresponding reference range.

Hyperphosphatemia was documented in diarrheic calves which agree with the observation of Guzelbektes *et al.*, (2007) who stated that serum phosphate concentration increased along with the increase of calves' degree of dehydration during diarrhea.

Concerning the electrolytes parameters, results of this study showed a significant decrease in serum values of Na, K and Cl in diarrheic group which might relate to their loss with feces (Kamal, 2008). Additionally, decrease in blood plasma chloride concentration in diarrheic calves was observed only when the animals were highly dehydrated (Guzelbektes *et al.*, 2007). Dratwa *et al.*, (2012) suggested hyponatremia, hyperkalemia and hypochloremia are usually observed together with an increasing degree of dehydration in diarrheic calves. Hyperkalemia was also seen in diarrhetic calves in some studies, and it had a direct relationship with disturbances in the acid-base balance (Bellino *et al.*, 2012). In this work diarrheic calves did not demonstrate any significant changes in the values of PH and bicarbonate. In conclusion, the results of the present study demonstrated that parasitic agents appeared to be important causes of diarrhea in buffalo calves with the *Toxocara vitulorum* and *Eimeria sp.* were the most common leading protozoal agents. Calf diarrhea is associate with significant hematological, biochemical and fecal changes

which may provide a good knowledge about the clinical and laboratory picture of the disease that may lead to better management and proper treatment protocols.

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