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## RESEARCH ARTICLE

## CLINICAL, HEMATOLOGICAL AND SOME BIOCHEMICAL ALTERATIONS IN CALVES DURING DIARRHEA

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### Abstract

From different farms in menofia governorate, Blood and serum samples were collected from 25 diseased crossbreed Egyptian calves (3-6 months old) and 10 apparent healthy calves to be used as control. The diseased calves were suffering from diarrhea, anorexia, depression, depraved appetite, hyperthermia, rough hair coat, weakness and loss of body weight. Careful clinical examinations as respiration, pulse, rumen function, reflexes were manually recorded in all animals. Fecal samples were screened for parasitic load using standard techniques (Coles, 1986). The estimated values of total RBCs, Hb, and MCH showed significant decrease in diarrheic calves compared with that in healthy ones. Estimation of serum minerals revealed decrease in calcium, magnesium, sodium and potassium levels, with normal physiological serum phosphorus level. Serum, glucose, total protein and albumin were significantly decreased, while, serum globulin were not changed in diarrheic animals compared to healthy ones. Also, significant increase was found in the activities of AST, ALT, in diarrheic calves compared to normal ones.

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## INTRODUCTION

Diarrhea defined as increased fecal water content or to increased volume of feces excreted or a combination of both and characterized clinically by excessive water, electrolyte discharge in the intestine (Goljan, 2010). Diarrhea is one of the major problems facing animal production especially those are bred under intensive or semi-intensive system of breeding; it also causes economic loss due to significant mortalities of young (s), cost of treatment, weight loss or even retarded growth (Abubakar et al., 2007).

Etiology of diarrhea is multiple, including infectious agents, poor management, reproductive factors, nutritional factors and immune status (Diaz-Lee et al., 2011). The diarrheic animals loosed fluid, rapidly dehydrated and suffered from electrolyte loss, acidosis and infectious agents may cause initial damage to the intestine but death from scours usually result from dehydration, acidosis and loss of electrolytes (Radostits et al., 2007). Dehydration in calves that have diarrhea is accompanied by large decreases in the extracellular fluid volume along with small increases in intracellular fluid volume (Naylor, 1987; Constable et al., 1998 and Smith, 2009). Neonatal calf diarrhea is one of the most devastating diseases of the dairy industry worldwide (Elhassan et al., 2011; Pourjafar et al., 2011). Clinical symptoms observed in calves with diarrhea are manifested by lack of appetite, loose stools and abdominal pain. Longer diarrhea results in dehydration, weakness and loss of sucking reflex. Loss of fluids leads to hypovolaemia and circulation disorders, whereas disturbances in the acid-base balance and electrolytic balance are likely to induce neural symptoms with convulsion leading to death (Grove-White, 2004). Different studies have shown that administration of water and electrolytes to diarrheic calves improves survival (Naylor, 1989). Electrolyte imbalance in diarrheic calves may cause death in some cases and estimation of the severity of electrolyte

imbalance is necessary before administration of electrolytes to diarrheic calves. Although it is believed that serum concentrations of sodium (Na) and potassium (K) are more important in relation to the composition of the fluids used for therapy (**Groutides and Michell, 1990**). There is little information about the changes of some other electrolytes such as calcium (Ca) and magnesium (Mg) and trace elements, and their relationships with clinical signs in diarrheic calves. Untreated changes in serum concentrations of Ca and Mg and some trace elements may be the cause of some death or post diarrhea complications such as growth retardation. Nutritional surveys carried out in different areas of Egypt have revealed pathological symptoms existing in many livestock due to deficiency of minerals and trace elements in the soil and consequently, in the fodder grazing on them, thus, deficiency symptoms due to subnormal intake of phosphorus copper, zinc, cobalt, selenium have been observed in certain areas (**Naser, 1995**). Diarrhea associated with hematological changes such as marked increase in the values of Hb, PCV%, RBCs, WBCs count (**Ghanem, et al., 2012**), also diarrhea associated with biochemical changes such as decreased total protein, hypoglycemia, hyponatremia, hyperkalemia, hypocalcaemia, increase AST, ALT activity (**Ghanem and Abd El-Raof, 2006**), increase urea nitrogen and creatinine level (**Ghanem et al., 2012**). The present study was planned to study diarrhea in farm animals to secure the following information: Diagnostic procedures to determine the probable cause of dietary diarrhea in farm animals, Detection of the mean levels of some biochemical constituents and hematological profile of the blood during diarrhea and detection of the mean levels of some minerals in diseased animals.

## MATERIALS AND METHODS

### Animals and study design.

This study was conducted on a total of 35 calves, 3-6 months of the ages (diarrheic calves; n=25, apparently healthy; n=10) in Menofia Governorate, All animals were subjected to detailed clinical examination including recording of temperature, pulse and respiratory rates, mucous membranes and ruminal movement investigation (**Radostits, et al., 2007**). The degree of dehydration had been estimated by capillary refill time and other clinical parameters (**Smith, 1996**). Blood and/or fecal samples were collected from control and diarrheic calves at the onset of diarrhea.

### Blood and serum analysis:

Five ml of blood samples were collected from the jugular vein of each animal at the onset of diarrhea each blood sample divided into two portions. The 1<sup>st</sup> portion (2ml) with anticoagulant (EDTA) was used for hemogram investigation (**Feldman et al., 2000**). The 2<sup>nd</sup> portion (3ml) were allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation and used for biochemical studies. The following serum biochemical parameters were determined: Serum total protein (**Henary et al., 1974**), Serum albumin (**Doumas et al., 1971**), Serum globulin was determined by subtracting the value of serum albumin from the value of serum total proteins, A/G ratio, Serum glucose (**Trinder, 1961**). Activities of AST, ALT (**Reitman and Frankel., 1957**), ALP (**Tietz, 1986**), urea (**Patton and Crouch, 1977**) and creatinine (**Oliver and Biochem, 1955**). The reagents were supplied by biomérieux-France and randox-Co.

### Statistical analyses

Statistical differences were calculated according to the Student t-test with significance level at  $P < 0.05$ . All results were analyzed using the procedure of (**SAS, 2004**).

## RESULTS

Clinical findings of a total of 25 young calves 3-6 month old were suffering from diarrhea, inappetence, decreased feed intake and diarrhea. Internal body temperature, arterial pulse rate, lung auscultation and respiratory rate were within normal range while conjunctiva, oral, nasal and vaginal mucous membranes were slightly pale in colour with no lesions.

The obtained hematological pictures of apparent healthy and diarrheic calves are described in (**Table 1**). When the mean values of total red blood corpuscles (RBCs), hemoglobin (Hb), mean corpuscular hemoglobin (MCV) showed significant decrease in (diarrheic) calves compared with corresponding values in healthy (control) ones while significant increase were seen in Packed cell volume (PCV). The mean values of total white blood cells (WBCs) and differential leucocytic count in (apparent) healthy and (diarrheic) calves revealed significant leucopenia in diseased calves.

**Table 1: Hemogram and Leukogram Healthy and Diarrheic Calves (Mean  $\pm$  S.E)**

	<b>Healthy (n=10)</b>	<b>Diarrheic (n=25)</b>
RBCs( $10^6/\text{mm}^3$ )	8.22 $\pm$ 1.32	8.76 $\pm$ 2.06
Hb (g/dl)	13.36 $\pm$ 1.12	12.29 $\pm$ 1.25
PCV %	35.21 $\pm$ 2.42	46.50 $\pm$ 3.12*
MCV (FL)	44.50 $\pm$ 2.60	53.71 $\pm$ 1.98*
Platelets ( $10^3/\text{ul}$ )	4.87 $\pm$ 0.60	3.50 $\pm$ 0.42 *
TLC( $10^3/\text{ul}$ )	14.52 $\pm$ 2.33	11.15 $\pm$ 3.06
Neutrophile ( $10^3/\text{ul}$ )	6.14 $\pm$ 1.86	5.57 $\pm$ 1.90
lymphocytes ( $10^3/\text{ul}$ )	7.60 $\pm$ 2.51	4.86 $\pm$ 2.83*
Monocytes ( $10^3/\text{ul}$ )	0.68 $\pm$ 0.03	0.72 $\pm$ 0.11

**Biochemical Examination:**

The mean values of activities of serum enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) in apparent healthy and diarrheic calves before and post treatment are presented in (Table 2). The tabulated results showed significant increase of serum AST, ALT, activities in diarrheic calves compared to healthy ones. On the other hand, the mean values of blood serum total protein, albumin and glucose, showed significant decrease in diarrheic calves occurred in (Table 2). Regarding the estimated levels of blood serum calcium, chloride, sodium and potassium shown in (Table 2), a highly significant decrease was noticed in serum, calcium, Sodium concentrations in diarrheic calves than healthy ones while there is significant increase in Potassium level in diarrheic calves than in healthy ones.

**Table 2: Biochemical Parameters of Normal and Diarrheic Calves (Mean  $\pm$  S.E)**

	<b>Healthy (n=10)</b>	<b>Diarrheic (n=25)</b>
Sodium (mmol/L)	152.30 $\pm$ 6.75	128.60 $\pm$ 5.43*
Chloride (mmol/L)	82.05 $\pm$ 3.67	67.81 $\pm$ 4.15 *
Potassium (mmol/L)	4.80 $\pm$ 0.71	6.05 $\pm$ 0.24 *
Calcium (mg/dl)	12.93 $\pm$ 1.06	9.36 $\pm$ 1.15 *
Glucose (mg/dl)	75.04 $\pm$ 5.50	59.83 $\pm$ 6.80 *
Total protein (gm/dl)	5.95 $\pm$ 0.82	5.20 $\pm$ 1.04
Albumen (gm/dl)	3.12 $\pm$ 0.50	2.97 $\pm$ 0.28
Globulin (gm/dl)	2.68 $\pm$ 0.03	2.30 $\pm$ 0.86
A/G ratio (%)	1.05 $\pm$ 0.11	1.12 $\pm$ 0.02
AST (Iu/l)	63.60 $\pm$ 5.08	66.18 $\pm$ 7.15
ALT (Iu/l)	28.50 $\pm$ 1.40	30.08 $\pm$ 2.30
Creatinine	1.08 $\pm$ 0.30	1.83 $\pm$ 0.44*
Urea	21.90 $\pm$ 2.20	24.66 $\pm$ 1.98*

**Discussion**

Diarrhea constitutes one of the most serious problems among farm animals which lead to considerable economic loss. The diarrheic calves showed depression, dullness, loss of appetite and moderate degree of dehydration. The faeces was semi fluid to watery in consistency and grayish to yellowish green in color, contained mucous and sometimes blood. The perineum and tail were soiled with feces. Mucous membranes were pale in colour. These results come in agreement with those previously recorded by **Martin and Aitken, (1991)**. The recorded anorexia, depression and dullness may be attributed to muscular weakness due to escape of intracellular potassium,

hyperkalemia and hypoglycemia (**Radostits, et al., 2007**). In our results hematological results observed are) increase in the total RBCs counts, Hb values, PCV than those in healthy ones. No significant differences were detected with other selected hemogramic parameters (**Table 1**). Those Results, reveled generally Presence of clinical marginal anemia, the increase in hematological parameters may be attributed to haemoconcentration, excessive loss of body fluid and dehydration which lead to decrease plasma volume. These results were in agreement with that reported by **Radostits et al. (2007)**, and **Tawfik, (2000)**. Leukogram in diarrheic calves found to be significantly depressed for total WBCs than the corresponding values in healthy ones. The decrease of total leucocytic count in diarrheic calves may be attributed to the stress of malnutrition. This suggestion was supported by the result obtained by **Mogongo et al. (1985)**. The degree of leucocytosis within different individuals was influenced by the severity of the infectious agent and the susceptibility of the animal to the infection (**Coles, 1986**). Serum analysis of diarrheic calves showed significant decrease in serum Na, Cl and Ca, while there was a significant increase in serum K in compared with control, this result were in agreement with that reported by [**Ghanem and Abd El-Raof (2006)**; **Radostits et al., (2007)**]. The significant decrease in the serum calcium may be attributed to malabsorption and its loss in the gastrointestinal tract (**Ghanem and Abd El-Raof, 2006**). Hyperkalemia in diarrheic calves could be attributed to increase renal tubular reabsorption of potassium in response to acidosis. Also it could be attributed to oligouria or anuria in which kidney failed to eliminate excess potassium (**Wakwe and Okon, 1995**). Hyponatremia, hypochloriemia in diarrheic sheep were attributed to direct loss of sodium and chloride ions via feces as well as failure of intestinal absorption **Michell (1989)**. The significant decrease in serum total protein and albumin levels shown in (**Table 2**) in diarrheic calves was in agreement with **Coles (1986)**. Also it could be attributed to stress of dietary diarrhea which may affect worsely the hepatic parenchyma resulting in the failure of protein synthesis. Data presented in (**Table 2**) showed significant increase in AST, and ALT, activities in the diarrheic calves when compared with control ones. Similar results were previously reported by **Fell, 1981**. This increase in AST and ALT might be) attributed to inflammation of gastrointestinal tract of diarrheic calves and cellular destruction of the liver and intestinal mucosa (**Ghanem and Abd El-Raof (2006)**). Serum kidney function tests of diarrheic calves showed significant increased in the mean values of creatinine and highly significant increase in serum level of urea nitrogen. The increased values of serum urea nitrogen and creatinine may be due to decrease renal function and reduction in glomerular filtration rate and decrease urine production resulting from hypovolemia, systemic arterial hypotension and vasopressin release (**El-Sangary, 1999**). It could be also due to excessive production of urea by catabolism of body proteins in toxic conditions (**Coles, 1986**). Serum analysis of diarrheic calves showed significant decrease in serum glucose level and in the level of total serum protein, while there was non significant decrease in levels of albumin, globulin and A/G ratio compared with control ones. These findings are in agreement with those reported by (**Ghanem, et al., 2012 and Jimenez et al., 1993**). The decrease in serum glucose level in the diseased animal as reported in (**Table 2**) may be due to copper deficiency (**Duncan et al., 1994**).The occurrence of hypoglycemia in diarrheic calves may be due to bacterial infection and may be attributed to lack of glucose absorption from damaged intestine, while reduction in the levels of serum total protein and albumin in diarrheic sheep could be attributed to the destructive effect of bacteria or bacterial toxin on the liver cells resulting in impaired synthesis of albumin or malabsorption from the intestinal tract as recorded by **Coles (1986)**.

## Conclusions

In severely dehydrated calves with diarrhea the most impotent action must be done firstly is to correct electrolyte imbalance due to diarrhea. One of the most important factors for decreasing mortality rates associated with diarrhea in calves is parenteral and oral administration of appropriately formulated electrolyte solutions, which can correct the dehydration, acidemia, strong ion (metabolic) acidosis, and electrolyte imbalances, particularly hyperkalemia and hyponatremia. Inclusion of an alkalinizing agent is required to correct systemic metabolic acidosis. Oral electrolyte solutions containing acetate or propionate are preferred over solutions containing bicarbonate. Oral electrolyte solutions are indicated in any diarrheic calf that has at least a partially functional gastrointestinal tract (especially suckle reflex). Sodium bicarbonate solutions are indicated for use in the treatment of severe acidosis.

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