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Clinical, Hematobiochemical and Oxidative Biomarkers Changes in Bacterial Diarrhea in Dairy Calves Before and After Weaning.

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Abstract

DIARRHEA is one of the most common gastrointestinal disorders affecting calves and represents a major cause of economic loss in the cattle industry. This study was conducted to assess the clinical fecal score, hematobiochemical alterations, and oxidative stress markers in pre and post weaned calves diagnosed with bacterial diarrhea. A total of 70 calves were included in the survey, comprising 10 healthy calves (control) and 60 diarrheic calves aged from 1-day old to 1-year-old. The diseased calves exhibited clinical signs including diarrhea, anorexia, weakness, dullness, unsteady gait, pale mucous membranes and varying degrees of dehydration. In this study, protein profiles, electrolyte and mineral alterations, liver function parameters, cortisol levels, CRP, malondialdehyde, and glutathione peroxidase activity were evaluated. The results revealed a higher prevalence of diarrhea among pre-weaned calves, with male calves being more frequently affected. Bacteriological analysis identified *Escherichia coli, Salmonella spp., Proteus mirabilis, Proteus vulgaris,* and *Shigella spp*. as the predominant pathogens. 20 diseased and 10 healthy calves were subjected to further analysis. Hematological findings in diarrheic calves showed a significant reduction ($p<0.05$) in total RBCs and Hb concentrations, accompanied by a significant increase in PCV%, WBCs with neutrophilia. Biochemical analysis of both pre and post weaned diarrheic calves revealed lower levels of protein profile parameters, Na, and Ca along with elevated K and Cl concentrations. P level was significantly decreased in post weaned calves only. Furthermore, diarrheic calves of both groups exhibited elevated levels of C-reactive protein (CRP), cortisol, ALT, AST and malondialdehyde (MDA), along with a marked decrease in glutathione peroxidase (GPX). In conclusion, diarrheic calves in pre and post weaning periods are susceptible to hematological, biochemical and oxidative status alterations. However, care must be given to calves in pre-weaning stage because of their greater susceptibility to diarrhea.

Keywords: Bacterial, calves, diarrhea, oxidative stress, weaning.

Introduction

Calf diarrhea imposes substantial economic losses on the cattle industry due to mortality, treatment and diagnostic costs, labor, veterinary care, and reduced replacement rates. Surviving calves often experience poor growth and chronic ill-thrift, further compromising herd productivity and profitability [1].

Calf diarrhea arises from both infectious and non-infectious factors. Non-infectious causes include poor hygiene and nutritional mismanagement, such as overfeeding of milk or milk replacers. Infectious agents include bacteria (e.g., *Escherichia coli, Salmonella spp.*), viruses (rotavirus, coronavirus), and parasites (*Toxocara vitulorum*, *Trichostrongylus* spp., *Eimeria* spp., *Cryptosporidium* spp.) [2].

Among bacterial pathogens, *enterotoxigenic Escherichia coli (ETEC) and Salmonella* species are considered the most economically significant causes of enteric disease in calves. However, several other bacterial species have also been implicated in the pathogenesis of neonatal diarrhea[3].

Infectious diarrhea represents one of the leading causes of morbidity and mortality among neonatal dairy calves worldwide. This condition can be attributed to a wide range of pathogens, including viruses, bacteria, and protozoa[4]. Infectious agents

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including bacteria can induce primary damage to the intestinal mucosa; however, mortality associated with scours is predominantly due to dehydration, metabolic acidosis, and electrolyte imbalance. Therefore, accurate identification of the causative pathogens is crucial for the development and application of effective preventive and therapeutic strategies[5].

Diagnosis of diarrhea relies on clinical data such as the animal’s age, vaccination status, and clinical signs, combined with relevant farm history to help veterinarians determine the underlying cause[6]. Diagnosis of diarrhea also involves assessing complex clinico-hematological and biochemical changes, including severe imbalances in fluid, electrolytes, and acid-base status, which can be life-threatening if untreated [7] .The clinical scoring system serves as a tool for assessing the viability of neonatal calves and for aiding in the diagnosis and monitoring of diarrhea and respiratory disorders. It enables clinicians to promptly identify calves that require medical attention[8].

It may also serve as a practical clinical assessment tool for evaluating the clinical consequences of diarrhea in calves. The system can be utilized by non-trained primary caregivers to determine the severity of dehydration and to assist in monitoring the recovery process in response to treatment for calf diarrhea[9].

The clinical manifestations observed in diarrheic calves included loose feces, a lack of appetite, and abdominal discomfort. Persistent diarrhea results in dehydration, marked weakness, and a progressive loss of the suckling reflex[5].

Oxidative stress arises from an imbalance between pro-oxidants and antioxidants, leading to cell and tissue damage through the generation of reactive oxygen species (ROS)[10]. In calves, weaning stress disrupts ROS regulation and reduces antioxidant enzymes like glutathione peroxidase (GPX), contributing to intestinal barrier dysfunction through villus atrophy and crypt hyperplasia. This results in inflammation, diarrhea, malabsorption, and systemic illness, negatively impacting health and growth [11].

Malondialdehyde (MDA) is a byproduct of lipid peroxidation and serves as a reliable biomarker of oxidative stress. It readily interacts with various biological macromolecules, including proteins, lipids, carbohydrates, and nucleic acids, leading to structural and functional damage at the cellular and tissue levels[12].

C-reactive protein (CRP) is a positive acute-phase protein and serves as a non-specific but reliable biomarker for detecting inflammation and infection. Acute-phase proteins are a group of plasma proteins whose hepatic synthesis is markedly upregulated in response to various pathological conditions such as inflammation, infection, tissue injury, cancer, and cardiovascular disorders[13].

Therefore, this survey study aimed to record the prevalence of diarrhea in calves. Moreover, evaluate the role of hematobiochemical changes, and oxidative stress markers as diagnostic and prognostic tools in bacterial diarrhea in calves before and after weaning.

Material and Methods

Animals

A total of 70 calves (45 pre-weaned and 25 post-weaned) of both sexes (43 males and 27females) and different ages ranged from 1 day to 1-year-old were admitted to the Veterinary Teaching Hospital in the Faculty of Veterinary Medicine (Benha University), as well as calves admitted to private veterinary animal clinics in Qalioubia Governorate (Egypt) during the period from August 2024 to June 2025. 60 calves had diarrhea and 10 (5 pre-weaned and 5 post-weaned) clinically healthy used as controls were included based on clinical and physical examination. The affected calves showed diarrhea, fever, weakness, depression and emaciation. Based on parasitological, virological and bacteriological examinations, 20 diarrheic calves (10 pre-weaned and 10 post-weaned) with fecal score 3, positive bacterial infection, negative viral and parasitic infection were subjected for further hematological and biochemical analysis.

Ethical approval

All studies were conducted following approval from the Animal Care and Use Committee (ACUC) and the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (Ethical Approval No. BUFVTM 06-11-24). Informed consent was obtained from all animal owners prior to sample collection.

Clinical examination of animals

All calves enrolled in the study, whether clinically healthy or exhibiting diarrhea, were subjected to clinical examination. physical examination, including rectal temperature, respiratory rate, and heart rate, were recorded depending on the data [14] .clinical score of calves with diarrhea was estimated also during examination as shown in Table 1.[5, 15,16,17,8].

For the purpose of analysis, clinical health assessment scores were dichotomized into "normal" and "abnormal" categories. Calves were classified as having a normal health status if they met all of the following criteria: dehydration score = 0, fecal consistency score = 0 or 1, demeanor score = 0, body temperature < 39.7°C, suckling reflex score = 0, and normal appetite. Conversely, calves were categorized as having an abnormal health status if they presented with any of the following: dehydration score of 1, 2, or 3; fecal consistency score of 2 or 3; demeanor score of 1, 2, or 3; body temperature ≥ 39.7°C; or suckling reflex and appetite scores of 1, 2, or 3.

Samples for parasitological, virological and bacteriological examinations

A fecal sample was collected in a clean, dry plastic container for parasitological examination aimed at detecting gastrointestinal parasites according to method mentioned by zajac [18] **;** in which results were negative of any fecal parasite. All samples were put in ice box and transmitted to the laboratory; those set for parasitological findings, were freshly examined. Another sample was obtained using sterile swabs for subsequent bacteriological and virological analysis. The viral RNA was extracted from a 20 % fecal suspension using a Blood/Liquid Sample Total RNA Rapid Extraction Kit (Bioteke Corpora- tion, China) according to the manufacturer's instructions as previously reported [19] . Samples that tested negative in parasitological and virological analysis were subsequently subjected to further bacteriological examination.

Isolation and identification of causative agent of diarrheic calves

fecal swab samples were inoculated into nutrient broth and incubated at 37 °C for 24 hours to promote microbial activation. Subsequently, a sterile loopful of the enriched broth was sub cultured onto blood agar, MacConkey agar, and paired Barker media. The culture plates were then incubated, and bacterial growth was monitored and recorded at 24 and 48 hours. Bacterial identification was carried out [20].The isolates were sub-cultured, and the resulting colonies were characterized based on colony morphology. Subsequently, standard biochemical tests and Gram staining were performed to identify the bacterial colonies [21] **.**

The biochemical characteristics of the bacterial isolates were determined by observing colony morphology under a microscope and conducting a series of biochemical tests such as catalase activity, motility, indole production, oxidase test, lactose fermentation, Simmons citrate utilization, Voges-Proskauer (VP) test, triple sugar iron (TSI) test, lysine iron agar (LIA) test, and urease activity. The biochemical characteristics of the bacterial isolates were determined using MacFaddin's methodology[22] .

Molecular identification of the viruses was done according to the procedures of previous studies [23,24,25] . Oligonucleotide Primers used for the molecular identification of viruses by Polymerase chain reaction (PCR) are listed in Table 2.

Blood sampling

Two blood samples were collected from both diarrheic and healthy (control) calves via jugular venipuncture. All samples were immediately placed on crushed ice and transported to the laboratory for subsequent hematological and biochemical analyses. The first sample was drawn into labeled tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA, potassium salt) and was used for hematological evaluation. The second sample was collected into plain tubes without anticoagulant. These tubes were centrifuged at 3000 revolutions per minute (r/min) for 15 minutes. Clear serum was carefully separated, aliquoted, and stored at -20 °C for biochemical analysis.

Hematological analysis

Hematological parameters were assessed using automated hematology analyzers (Mindray BC-3000 Plus and Edan H30), which perform complete blood count (CBC) analyses with high accuracy and efficiency. The procedures were in accordance with the methodology described by Feldman [26].

Biochemical analysis

Serum total protein and albumin concentrations were determined spectrophotometrically using commercial diagnostic kits (Bio-Diagnostics Ltd., Egypt). Serum globulin concentration was calculated by subtracting the albumin value from the total protein concentration. The albumin-to-globulin (A/G) ratio was calculated by dividing the serum albumin concentration by the globulin concentration. In addition, serum samples were used to determine AST and ALT using specific commercial kits [27] .

Serum concentrations of potassium (K), sodium (Na), phosphorus (P), calcium (Ca), and chloride (Cl) were measured using commercial diagnostic kits (Bio-Diagnostic Co., Egypt), following the methods specified by the manufacturer. All analyses were performed in accordance with the manufacturer's instructions. In addition, serum cortisol and C-reactive protein (CRP) concentrations were determined using ELISA kit (Eucardio Laboratory, Inc., Encinitas, and CA., U.S.A.). Serum malondialdehyde (MDA) levels were measured using an MDA assay kit, while glutathione peroxidase (GPx) activity was assessed using commercial kits, according to the standard protocols provided by the supplier (Bio-diagnostic, Egypt).

Statistical analysis

The statistical analysis was carried out using two-way ANOVA using SPSS, ver. 27 (IBM Corp. Released 2013). Data were treated according to steel [28] . Multiple comparisons were carried out. The significance level was set at < 0.05

Results

Clinical findings and Epidemiology

The predominant clinical signs observed in diarrheic calves, as summarized in Table 3, included anorexia, uncoordinated or staggering gait (80%) or unable to stand and recumbent (Fig.1), enophthalmos (Fig.2) (sunken eyes, 75%), and diarrhea (100%). Dehydration was also evident, classified as mild (25%), moderate (50%), or severe (25%). Additional clinical findings included general dullness (Fig.3) and changes in the mucous membranes (Fig.4), which appeared congested in the early stages of infection but became pale in severely dehydrated calves. The severity of dehydration was assessed based on skin tent duration and ocular appearance. Calves with mild dehydration showed a skin tent duration of 1–2 seconds and normal (non-sunken) eyes. Moderately dehydrated calves exhibited a skin tent lasting 2–5 seconds with slightly sunken eyes, while those with severe dehydration (Fig.5) had a skin tent duration exceeding 5 seconds and markedly sunken eyes. The consistency and color of diarrheic feces varied considerably, ranging from pale to dark yellow, greenish, and milky white, with frequent presence of mucus or streaks of blood (Fig.6). In contrast, calves in the control group (n=10) did not exhibit any clinical signs of diarrhea or other abnormalities upon examination. The frequency of clinical signs was demonstrated in Table 3. The clinical score of diarrhea was demonstrated in Table 4. (Fig.7)

Based on field observations in this study found that the incidence of diarrhea was significantly higher in calves younger than 4 months of age compared to older animals. Furthermore, sex-based analysis indicated a greater prevalence of diarrhea in male calves than in females. Seasonal variation was also observed, with a higher incidence reported during the wet season (winter) in contrast to the other seasons (Table 5).

The pre and post weaned diarrheic calves showed a significant increase ($P<0.05$) in body temperature, pulse rate and respiratory rate compared to the control groups and there were not significant differences between both pre and post weaned calves in diseased and control groups (Table 6)

The result of bacteriological and virological analysis

Bacteriological analysis of swab cultures obtained from diarrheic calves revealed the presence of several pathogenic bacteria, including *Proteus mirabilis, Proteus vulgaris, Escherichia coli, Salmonella spp.,* and *Shigella spp.*

PCR results for Coronavirus, Rotavirus, and Bovine Viral Diarrhea Virus (BVDV) revealed no amplification of target genes in the tested samples, indicating negative results for all three viruses.

Hematological findings

Hematological analysis of pre and post weaned diarrheic calves revealed a significant decrease ($P < 0.05$) in hemoglobin (Hb) concentration and red blood cell (RBC) count. Conversely, there was a significant increase ($P < 0.05$) in packed cell volume (PCV), total white blood cell (WBC) count, and neutrophil percentage compared to control groups. No significant differences were observed between pre and post weaned calves in both the control and diarrheic groups (Table 7).

Biochemical findings

Biochemical analysis of protein fraction in pre-weaned diarrheic calves showed significant decrease in total protein, albumin and globulin compared to control group while showed non-significant increase in A/G ratio. Also, in post-weaning diarrheic calves there were significant decrease in total protein, albumin and A/G ratio compared to control group while showed non-significant increase in globulin. There was significant decrease in albumin and A/G ratio and significant increase in globulin in post-weaning diseased group compared to pre-weaning diseased group **(**Table 8**).**

Biochemical analysis of electrolytes in pre and post weaned diseased group showed significant decrease in Na and Ca while significant increase in K and Cl compared to control group. While in post-weaned diseased group there was significant decrease in phosphorous compared to control group. Also, there was significant decrease in calcium and phosphorous in post-weaned diseased group compared to pre-weaned diseased group (Table 9).

In pre and post weaned diarrheic calves there was significant increase in C-reactive protein (CRP), cortisol, (AST) and (ALT) compared to control group**.** In pre-weaned diseased and control calves there was significant decrease in cortisol compared to post-weaned calves (Table 10). There was significant increase in MDA in both pre and post weaned diarrheic calves compared to control groups while there was significant decrease in GPX in diseased group compared to control group (Table 11).

Discussion

Calf diarrhea is a widespread health issue in the cattle industry and constitutes a major contributor to economic losses among producers. The condition arises from a complex interplay of infectious and non-infectious factors. Although in some instances a single pathogen may be the primary cause, mixed infections involving multiple pathogens are frequently observed in diarrheic calves [6] **.** Diarrhea is a major cause of neonatal mortality in dairy and beef calves during the first month of life. It also leads to significant economic losses due to the costs of medical treatment, impaired growth rates , and increased risk of death[29] **.**

The predominant clinical signs observed in diarrheic calves included diarrhea, staggering gait, anorexia, sunken eyes and dehydration that was present in varying degrees (mild, moderate, and severe). Additional findings included dullness and changes in mucous membrane coloration initially congested during the febrile phase of infection, progressing to pallor in cases of severe dehydration. The color of diarrheic feces ranged from pale to dark yellow, greenish, and milky white also the feces may contained visible flecks of mucus or blood as previously described [5,14,1] . The clinical signs included dullness, pale mucous membranes, and sunken eyes were typically occurred due to excessive loss of water and electrolytes[30,1,29].

The prevalence of diarrhea was notably higher in calves younger than three months compared to older calves. Most of diarrheic cases were observed in calves under three months of age. This increased susceptibility in neonatal calves may be attributed to their underdeveloped immune systems, making them more vulnerable to enteric pathogens. Moreover, the highest morbidity and mortality rates typically occur in pre-weaned calves **,**as previously described [31] . Male calves tended to be more sick than female calves. The reason for this may be that farmers may take care for female calves more closely due to their economic importance[32]. Female calves exhibited a lower risk of morbidity compared to male calves. This disparity may be attributed to preferential management practices and enhanced health care services typically provided to female calves, as they are regarded as future replacement stock and hold greater economic value for the farm. In contrast, male calves often receive comparatively less attention in areas such as nutrition, medical care, and other things[33] **.** These results did not agree with that previously reported[31].

In the present study, the highest prevalence of diarrhea was recorded in winter, corresponding to the wet season. The cooler temperatures characteristic of this period may create favorable conditions for the survival and transmission of enteric pathogens[34].

The significant increase in mean rectal temperature, respiratory rate, and heart rate was observed in both pre and post weaned calves affected by diarrhea. These alterations are consistent with findings reported in previous studies [35,36]. The observed increase in respiratory rate (compensatory polypnea) appears to be a physiological response to metabolic acidosis, aiming to eliminate excess carbon dioxide (CO_2) and thereby restore normal blood pH levels. Concurrently, tachycardia likely serves as a compensatory mechanism for hypovolemia resulting from dehydration associated with diarrhea [29] **.** Moreover, rectal temperature may be influenced by both the duration of diarrhea and the degree of dehydration. In the early stages of diarrhea, there is increase in rectal temperature typically observed (ranging from 39°C to 40.5°C). However, in more severe or prolonged cases, rectal temperature tends to decrease, sometimes reaching values as low as 36°C to 38°C, likely due to systemic dehydration in severe cases of diarrhea [37] **.**

Calves were categorized based on their clinical diarrheal scores. A calf was considered to have a normal health status if it met all of the following criteria: dehydration score of 0, fecal consistency score of 0 or 1, demeanor score of 0, body temperature below 39.7°C, suckling reflex score of 0, and normal appetite. In contrast, a calf was classified as having an abnormal health status if it displayed any of the following: dehydration score of 1, 2, or 3; fecal consistency score of 2 or 3; demeanor score of 1, 2, or 3; body temperature ≥ 39.7°C; or suckling reflex and appetite scores of 1, 2, or 3[17] .

Infectious diarrhea is a common condition among calves. In the present study, bacteriological isolation of fecal samples from diarrheic calves led to the isolation of several pathogenic bacteria, including *Escherichia coli*, *Salmonella spp.*, *Proteus mirabilis*, *Proteus vulgaris* and *Shigella spp*. Among these, *Salmonella spp. and E. coli* are recognized as the most prevalent pathogens associated with diarrhea in calves. These results were consistent with previous findings [3,38,1,39,40].

The mean hematological indices in diarrheic calves demonstrated a significant decrease in total erythrocyte count and hemoglobin concentration. The marked decreased in RBCs count could be attributed to persistent diarrhea, dehydration and hemoconcentration as previously reported [5]**.** In contrast, a significant elevation was observed in total leukocyte count, neutrophil percentage, and hematocrit (HCT) levels when compared to the control group. The observed leukocytosis and neutrophilia in diarrheic calves are likely attributable to systemic inflammatory responses triggered by pathogenic bacterial infections. Similar elevations in hematocrit values during of diarrhea have also been reported in previous studies[35,36,41])**.** Hematocrit (HCT) serves as an important indicator of overall blood volume and oxygen-carrying capacity. It is typically elevated at birth and gradually declines with age as part of the normal physiological adaptation. Additionally, hemoglobin concentration is directly associated with the efficiency of oxygen transport within the bloodstream, reflecting the blood's capacity to meet metabolic demands [29]. The increase in PCV% observed in diarrheic calves is likely attributable to hemoconcentration resulting from dehydration and reduced plasma volume (hypovolemia) [7].

Serum biochemical analysis in this study showed significant decrease in total protein and albumin in both diarrheic pre-weaning and post weaning calves than control calves. In diarrheic calves, the concentrations of albumin and total protein were reduced , that may be attributed to enteric protein loss through the intestinal lumen associated with diarrhea [14,1].

Albumin, the primary serum protein synthesized by the liver, plays a key role in maintaining colloid osmotic pressure and transporting water and metabolites. Its levels typically decline in conditions such as malnutrition, infection, liver dysfunction, and kidney disease. In this study, diarrheic calves showed reduced albumin levels compared to healthy ones, likely due to intestinal losses. This loss appears to be exacerbated by the presence of large volumes of fluids, ingesta, and hypertonic solutions in the intestinal lumen [42].

This study demonstrated a significant reduction in globulin levels in pre-weaned diseased calves compared to healthy controls. This decline may be attributed to decreased gamma-globulin concentrations, which are critical for immune defense against pathogens. The reduction in gamma-globulins may result from insufficient colostrum intake shortly after birth, immunosuppression induced by pathogenic agents, or a shift in protein synthesis favoring alpha-globulin production. As a result, the compromised gamma-globulin levels may weaken the immune response, increasing the calves' susceptibility to infections and aggravating the clinical manifestations of diarrhea as previously described [5,42] .

The results also showed a significant increase in globulin levels in post-weaned diarrheic calves compared to pre-weaned diseased calves. This elevation in globulin may be attributed to acute inflammation induced by various intestinal pathogens as previously reported[43]. The observed increase in globulin concentrations in post-weaned animals may also be attributed to a higher intake of total crude protein resulting from increased consumption of solid feed, as previously reported [44] .The progressive increase in globulin may attributed to increase γ-globulin concentrations in calves with increasing age has been shown to be closely related to the normal growth process and maturation of the immune system[45] .

In diarrheic calves, serum concentrations of sodium (Na^+), calcium (Ca^{2+}), and phosphorus (Ph) were significantly decreased, whereas levels of potassium (K^+) and chloride (Cl^-) were significantly elevated compared to healthy control calves. The observed hyponatremia is primarily attributed to the excessive loss of sodium ions through the intestinal tract, resulting from increased secretion by intestinal villus epithelial cells. This mechanism is particularly evident in cases of diarrhea caused by *enterotoxigenic Escherichia coli (ETEC)* infection[46,7].The reduction in serum calcium (Ca^{2+}) levels may be associated with persistent diarrhea and dehydration, leading to increased fecal loss of calcium. Similarly, the decrease in serum phosphorus (Ph) levels is likely due to greater electrolyte loss than water loss[5]. Hyperkalemia may result from impaired renal excretion of potassium, leading to its retention, and may also be attributed to cellular damage, which causes the release of intracellular potassium into the extracellular fluid [47,48].

The occurrence of hyperchloremia observed in this study may be explained by the physiological ion exchange processes that occur during diarrhea. Specifically, bicarbonate ions (HCO_3^-) are secreted into the intestinal lumen in exchange for chloride ions (Cl^-) at multiple sites along the gastrointestinal tract. This exchange leads to substantial bicarbonate loss and a relative retention of chloride in the bloodstream, resulting in decreased serum bicarbonate concentrations and compensatory elevation of serum chloride levels. Consequently, hyperchloremia is frequently associated with metabolic acidosis in animals with diarrhea[49,50]. Diarrheic calves in this study showed significant increase in CRP compared to control group. C-reactive protein (CRP) is an acute-phase protein and a well-established inflammatory biomarker that increases rapidly in response to inflammation or tissue injury. It plays a role in host defense by binding to metabolites released during cellular degeneration, thereby preventing their utilization by invading pathogens and facilitating their clearance. Elevated CRP levels in diarrheic calves suggest the presence of tissue damage and an underlying pathogenic infection [1,13] .

Cortisol is the main hormone released in case of stress to restoring homeostasis and physiological conditions. In the current study there was increase in cortisol in pre and post weaned diarrheic calves compared to control group to relieve stress resulted from the clinical and biochemical disturbances caused by diarrhea [1] . Cortisol levels were found to be lower in pre-weaning diseased calves compared to post-weaning diseased calves. This difference may be attributed to the dietary transition from milk to increased forage intake during the late pre-weaning stage. The elevated cortisol concentrations observed in post-weaning calves likely reflect the heightened stress associated with pre-weaning handling and the weaning process itself, which typically involves maternal separation, increased human interaction, and additional processing [51].Weaning stress encompasses both the psychological stress caused by the disruption of maternal and social bonds and the physical stress associated with dietary transitions from milk to solid food. Consequently, weaning stress in animals is recognized as a major contributing factor to various health problems in cattle, with significant economic implications**[**52] . Stressors activate both the sympatho-adrenal system and the hypothalamic–pituitary–adrenal (HPA) axis, leading to the release of stress hormones such as cortisol. Cortisol, in turn, can influence the proliferation and differentiation of immune-mediated cells [53] **.**while pre-weaned calves depend on passive immunity that obtained from consuming sufficient high-quality colostrum immediately after birth[54] so cortisol hormone is increased in post weaned calves than pre-weaned calves as it is stress related hormone.

Serum levels of ALT and AST were elevated in both pre- and post-weaning diarrheic calves compared to their respective control groups. This increase is likely attributed to gastrointestinal tract inflammation, which may have induced pathological changes affecting liver function[5,1,29]**.** Malondialdehyde (MDA) levels were significantly elevated in both pre- and post-weaning diseased calves compared to the control groups. As a well-established biomarker of oxidative stress, MDA may be associated with the enteric health status of neonatal calves. Measuring MDA is essential for evaluating lipid peroxidation and potential DNA damage, thereby providing a reliable indicator of oxidative stress severity[55] . The phospholipid membranes of enterocytes are particularly susceptible to oxidative damage induced by reactive oxygen species (ROS). As a result of this oxidative stress, enterocytes generate MDA as a byproduct of lipid peroxidation [56] . The increased serum MDA levels observed in diarrheic calves in the present study confirm that oxidative stress contributes to enterocyte damage during diarrhea [57] .

During stress such as weaning and diarrhea, the balance between the production of reactive oxygen species (ROS) and their scavengers by endogenous antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) becomes disrupted, leading to cell damage. Stress also induces villus atrophy and an increase in crypt depth, which together compromise the integrity of the intestinal barrier. This disruption can lead to inflammation, diarrhea, malabsorption, systemic diseases, and elevated oxidative stress [11] so serum level of (GPX) was decreased in diarrheic calves compared to control group.

In the current study, the bacterial diarrhea in calves had negative impacts on electrolyte imbalance, inflammatory markers and oxidative stress status. The condition was associated with a significant decrease in serum sodium, phosphorus and calcium levels, as previously reported [5], while potassium level was increased as previously discussed [47], along with elevated chloride concentrations as previously mentioned [50]. These electrolyte disturbances resulted from excessive fluid and ion loss due to intestinal damage. Additionally, bacterial infection induced inflammation and tissue injury, leading to a significant increase in C-reactive protein (CRP) levels, as previously reported [1]. Moreover, bacterial diarrhea was accompanied by increased oxidative stress, reflected by elevated level of malondialdehyde (MDA) [12] and a marked decrease in antioxidant enzymes such as glutathione peroxidase (GPx), as previously described [57].

Conclusion

Pre-weaned calves exhibited a higher susceptibility to bacterial diarrhea compared to older calves. The clinical diarrhea scoring system represented a rapid and non-invasive approach that could help the diagnosis and monitoring of diarrheic conditions in calves. Hematological and biochemical changes observed in diarrheic dairy calves indicated dehydration, electrolyte imbalances, and metabolic disturbances. These alterations can be used as important diagnostic and prognostic markers for the early detection of diarrhea and the evaluation of its severity. From a clinical perspective, monitoring these parameters may help veterinarians select appropriate treatment strategies. Additionally, from a zootechnical standpoint, applying these findings before weaning may improve nutritional management, promote growth performance, and reduce morbidity and mortality rates.

Conflict of interest

The authors declare that they have no conflict of interest.

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TABLE 1. Clinical scoring criteria of calves with diarrhea.

Parameter	Score 0	Score 1	Score 2	Score 3
Demeanor score	Normal (alert, active)	Slightly depressed or dull	Depressed, sternal recumbency	Lateral recumbency
Skin tent test	<1 sec	1-2 sec	2-5 sec	5-10 sec
dehydration status	No dehydration	Mild	Moderate	Sever
Fecal consistency	Normal	Pasty (soft), sits on bedding	Semi-liquid (runny) sits on and sifts through bedding,	Watery, liquid, sifts through bedding
Suckling reflex	Normal vigorous suckling	Calf suckles but not vigorously	Suckling is weak or absent	Suckling reflex is absent
Appetite	Normal	Mild anorexia	Moderate	Sever anorexia
Intestinal motility	Normal	Mild hyper motility	Moderate hyper motility	Sever hyper motility

TABLE 2. Primer sequences, target genes for SYBR green RT-PCR.

Target genes	Primer sequences	Reference
Bovine corona virus	TGGATCAAGATTAGAGTTGGC	[25]
	CCTTGTCCATTCTTCTGACC	
Bovine Rota virus	GATATTGGACCATCTGATTCTGCTTCAAA	[24]
	GAAATCCACTTGATCGCACCCAA	
BVDV (bovine viral diarrhea virus)	GGGNAGTCGTCARTGGTTCG	[23]
	GTGCCATGTACAGCAGAGWTTTT	

TABLE 3. Frequency of the clinical signs in diarrheic calves.

Clinical finding	Number of diseased cases (Total number=60)	Percentage
Inappetence	15	(25%)
Anorexia	45	(75%)
Staggering in gait	48	(80%)
Demeanor	Slightly dull (20)	(33.3%)
	Depressed (35)	(58.3%)
	Recumbent (5)	(8.3%)
Sunken eye	45	(75%)
Skin fold test:		
Mild (1-2 sec)	15	(25%)
Moderate (2-5 sec)	30	(50%)
Severe(5-10 sec)	15	(25%)
Feces consistency		
Pasty	12	(20%)
Semi-liquid	20	(33%)
Watery	28	(47%)

TABLE 4. Clinical score of calves with diarrhea.

Score of diarrhea	Total Number of animals (60)	Percentage
1	12	20%
2	20	33%
3	28	47%

N.B. The scoring system, ranging from 1 to 3, was used to indicate the presence and severity of clinical signs of diarrhea.

TABLE 5. Epidemiological data of animals.

Points of comparison	Sex		Age		Season			
Total number	Male	Female	1day:3months (pre-weaning)	>3months (post-weaning)	Autumn	Winter	spring	Summer
(6o calves)	40	20	39	21	15	25	10	10
Percentage	66.6%	33.3%	65%	35%	25%	41.7%	16.7%	16.7%

TABLE 6. Physical examination of control and diarrheic pre and post weaned calves (mean±SE)

Parameter	Pre-weaning		Post-weaning	
	Control N=5	**Diseased N=10**	**Control N=5**	**Diseased N=10**
Temperature (°C)	38.07 ± 0.12^{bA}	40.00 ± 0.12^{aA}	38.37 ± 0.15^{bA}	40.00 ± 0.17^{aA}
Resp. rate (cycle/min)	25.67 ± 1.45^{bA}	35.33 ± 1.45^{aA}	25.33 ± 1.20^{bA}	35.00 ± 1.15^{aA}
Pulse rate(pulse wave/min)	98.67 ± 1.76^{bA}	123.33 ± 1.76^{aA}	98.00 ± 1.15^{bA}	123.00 ± 0.58^{aA}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences ($P < 0.05$).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences ($P < 0.05$).

TABLE 7. Hematological parameters of pre and post weaned control and diarrheic calves (mean±SE).

Parameter	Pre-weaning		Post-weaning	
	Control N=5	Diseased N=10	Control N=5	Diseased N=10
RBCs(x10^{12}/l)	5.33±0.09^{aA}	3.57±0.17^{bA}	5.96±0.18^{aA}	3.57±0.19^{bA}
Hemoglobin(g/dl)	9.50±0.18^{aA}	7.96±0.65^{bA}	10.00±0.15^{aA}	8.03±0.58^{bA}
PCV (%)	25.08±2.02^{bA}	30.60±1.08^{aA}	24.67±1.24^{bA}	28.29±1.18^{aA}
WBCs(x10^{9}/l)	9.13±2.36^{bA}	14.00±1.45^{aA}	10.53±2.23^{aA}	14.30±1.37^{aA}
Neutrophil (%)	15.33±1.76^{bA}	27.33±4.91^{aA}	16.67±0.67^{bA}	24.67±1.33^{aA}
Lymphocyte (%)	74.33±2.91^{aA}	65.33±8.01^{aA}	72.00±2.08^{aA}	65.33±2.73^{aA}
Monocyte (%)	5.33±0.88^{aA}	5.00±0.58^{aA}	6.00±0.00^{aA}	5.67±0.33^{aA}
Esinophil (%)	0.33±0.33^{aA}	1.00±0.58^{aA}	1.00±0.58^{aA}	1.33±0.33^{aA}
Basophil (%)	1.33±0.67^{aA}	2.00±0.58^{aA}	2.00±0.58^{aA}	2.33±1.33^{aA}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences (P < 0.05).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences (P < 0.05).

TABLE 8. Protein fraction of pre and post weaned control and diarrheic calves (mean±SE).

Parameter	Pre-weaning		Post-weaning	
	Control N=5	Diseased N=10	Control N=5	Diseased N=10
Total protein (g/dL)	7.89±0.04^{aA}	6.83±0.13^{bA}	8.08±0.05^{aA}	6.82±0.35^{bA}
Albumin (g/dL)	3.50±0.14^{aA}	3.14±0.04^{bA}	3.61±0.12^{aA}	2.80±0.19^{bB}
Globulin (g/dL)	4.40±0.17^{aA}	3.59±0.05^{bB}	4.13±0.30^{aA}	4.35±0.16^{aA}
A/G ratio	0.80±0.06^{aA}	0.88±0.02^{aA}	0.88±0.06^{aA}	0.65±0.07^{bB}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences (P < 0.05).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences (P < 0.05).

TABLE 9. Alterations in serum electrolytes and minerals in control and diarrheic calves before and after weaning (mean±SE).

Parameter	Pre-weaning		Post-weaning	
	Control N=5	Diseased N=10	Control N=5	Diseased N=10
Sodium (Na) (mg/dL)	146.23±2.15^{aA}	132.02±1.08^{bA}	143.95±4.00^{aA}	130.42±2.32^{bA}
Calcium (Ca) (mg/dL)	11.16±0.06^{aA}	10.37±0.05^{bA}	10.27±0.06^{aB}	10.00±0.01^{bB}
Phosphorus (P) (mg/dL)	7.92±0.05^{aA}	7.79±0.12^{aA}	7.82±0.07^{aA}	6.37±0.05^{bB}
Potassium (K) (mEq/L)	4.76±0.06^{bA}	5.55±0.23^{aA}	4.44±0.13^{bA}	5.69±0.18^{aA}
Chloride (mEq/L)	91.48±1.98^{bA}	114.63±2.8^{aA}	92.62±7.63^{bA}	117.13±1.36^{aA}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences (P < 0.05).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences (P < 0.05).

TABLE 10. Biochemical changes of CRP, cortisol and liver function tests of pre and post weaned control and diarrheic calves (mean±SE).

Parameter	Pre-weaning		Post-weaning	
	Control N=5	Diseased N=10	Control N=5	Diseased N=10
CRP (mg/dL)	2.21±0.26^{bA}	4.97±0.10^{aA}	2.62±0.34^{bA}	4.98±0.17^{aA}
Cortisol (µg/dl)	0.47±0.05^{bB}	1.72±0.07^{aB}	0.78±0.07^{bA}	1.92±0.04^{aA}
AST (U/L)	85.33±1.70^{bA}	120.99±4.99^{aA}	91.19±1.71^{bA}	126.78±3.77^{aA}
ALT (U/L)	31.35±1.25^{bA}	45.22±1.14^{aA}	32.54±1.29^{bA}	45.76±0.94^{aA}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences (P < 0.05).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences (P < 0.05).

TABLE 11. Oxidative biomarkers changes of pre and post weaned control and diarrheic calves (mean±SE).

Parameter	Pre-weaning		Post-weaning	
	Control N=5	Diseased N=10	Control N=5	Diseased N=10
MDA (µmol/l)	26.78±1.54^{Ba}	53.3±7.86^{aA}	26.3±3.98^{bA}	65.12±5.33^{aA}
GPX (U/mg Hb)	8.01±0.52^{Aa}	3.19±0.11^{bA}	8.71±1.27^{aA}	4.99±1.00^{bA}

a, b: Different lowercase superscripts within the same row within the group indicate significant differences (P < 0.05).
A, B: Different uppercase superscripts within the same row between groups indicate significant differences (P < 0.05).

Fig.1. Sternal (A) and lateral (B) recumbent severely diarrheic calves

Fig.2.Sunken eye in severely diarrheic calf

Fig.3.Dull appearance of diarrheic calf

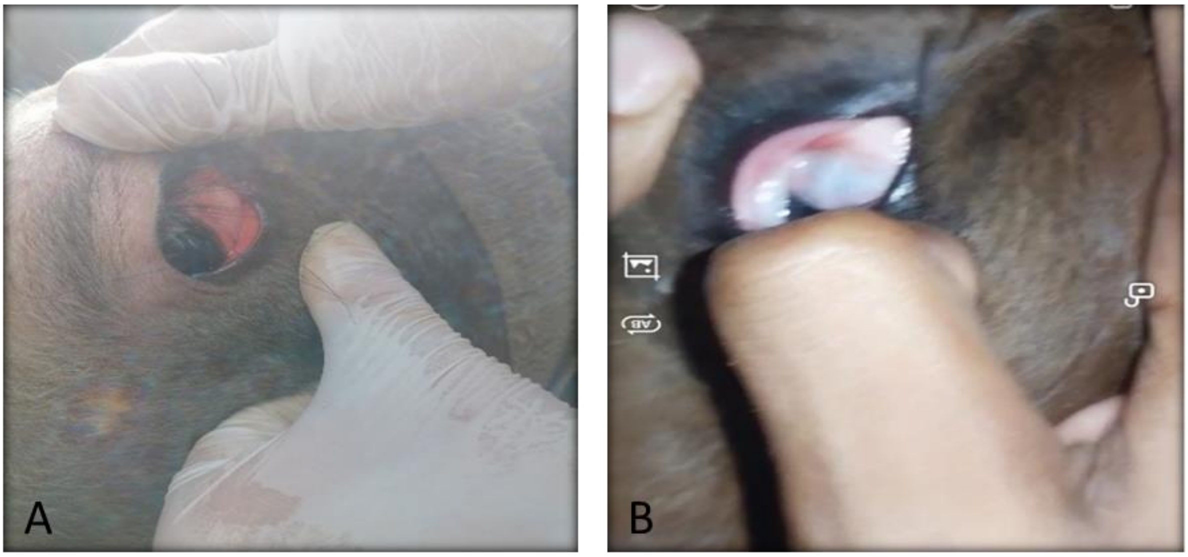

Fig.4. Congested(A) and pale (B) conjunctival mucous membrane in diarrheic calves

Fig.5. Severely dehydrated calf showing visible ribs and deep hunger fossa

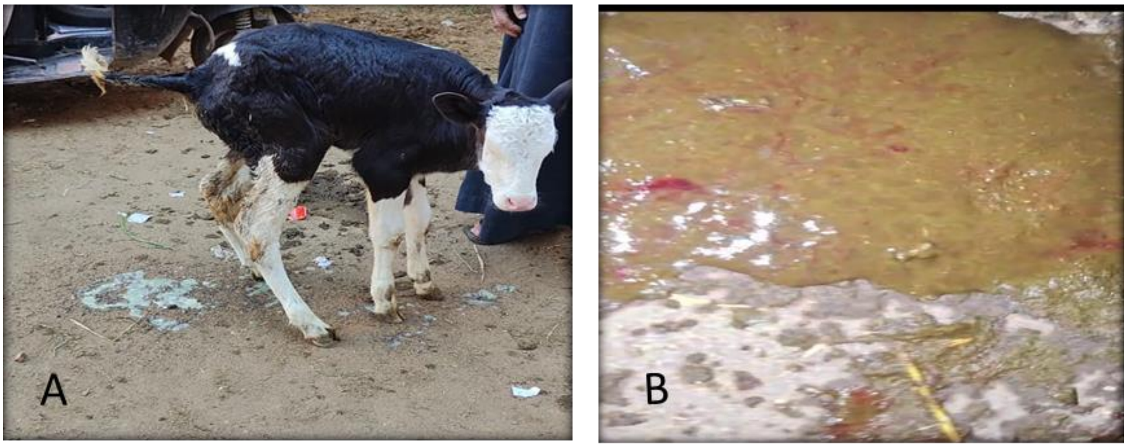

Fig.6. Different colors and consistency of diarrheic fecal matter (A): milky white watery diarrhea, (B): yellowish brown diarrhea with presence of blood streaks.

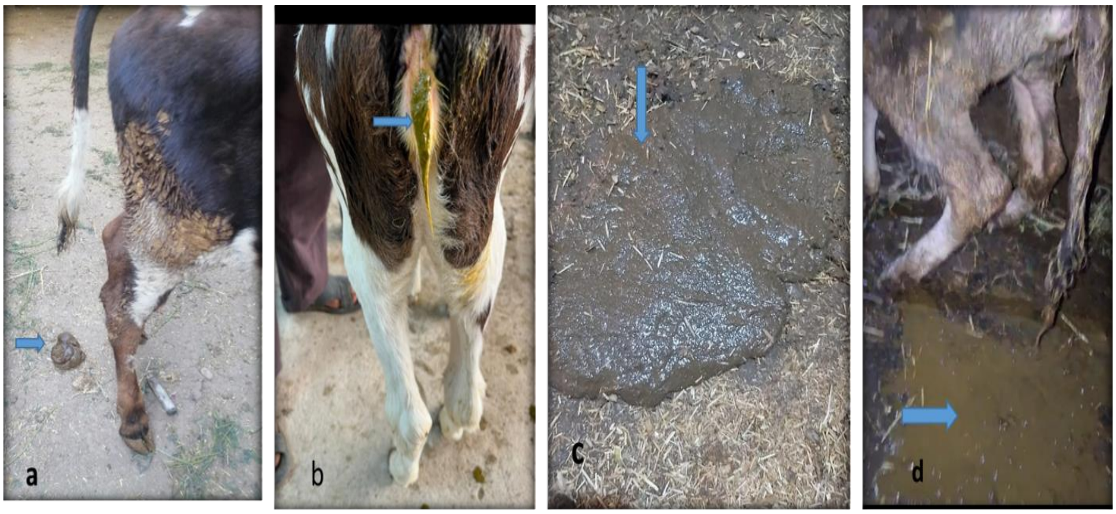

Fig.7. Different score for calf diarrhea (A): score zero (normal fecal matter), (B): score 1 (pasty diarrhea), (C): score 2 (semi-liquid), (D): score 3(watery diarrhea)

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التغيّرات السريرية والدموية-الكيميائية ومؤشرات الإجهاد التأكسدي في حالات الإسهال البكتيري لدى عجول الحليب قبل وبعد الفطام

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قسم طب الحيوان، كلية الطب البيطرى، جامعة بنها، مصر.

الملخص

يُعد الإسهال أحد أكثر اضطرابات الجهاز الهضمي شيوعًا بين العجول، ويمثل سببًا رئيسيًا للخسائر الاقتصادية في مجال تربية الأبقار. أُجريت هذه الدراسة بهدف تقييم الدرجة السريرية للبراز والتغيرات الدموية-الكيميائية ومؤشرات الإجهاد التأكسدي في العجول قبل وبعد الفطام المصابة بالإسهال البكتيري. شملت الدراسة 70 عجلًا، تضم 10 عجول سليمة (مجموعة ضابطة) و60 عجلًا مصابًا بالإسهال تتراوح أعمارهم من يوم واحد حتى عام واحد. أظهرت العجول المريضة علامات سريرية شملت الإسهال، فقدان الشهية، الضعف، الخمول، مشية غير ثابتة، شحوب الأغشية المخاطية، جحوظ العينين بدرجات متفاوتة، إضافة إلى الجفاف. أظهرت النتائج انتشارًا أعلى للإسهال في العجول قبل الفطام مقارنة بالعجول بعد الفطام، وكانت الذكور أكثر عرضة للإصابة. وأظهر الفحص البدني ارتفاعًا ملحوظًا في درجة حرارة الجسم ومعدل ضربات القلب ومعدل التنفس في العجول المصابة. أظهرت التحاليل البكتريولوجية أن الاي كولاي ، والسالمونيلا، وبروتيوس ميرابيليس، وبروتيوس فولغاريس، والشيغيلا كانت المسببات السائدة. وقد خضع عشرون عجلًا مريضًا وعشرة عجول سليمة لمزيد من التحليل. وأظهرت النتائج الدموية في العجول المصابة انخفاضًا معنويًا في العدد الكلي لكريات الدم الحمراء وفي تركيز الهيموغلوبين لعجول المصابة انخفاضًا معنويًا (في عدد كريات الدم الحمراء الكلي وتركيز الهيموغلوبين، بالتزامن مع ارتفاع معنوي في الباكد سيل فوليم وعدد كريات الدم البيضاء وخلايا النتروفيل. وأظهر التحليل الكيميائي الحيوي لكل من العجول قبل وبعد الفطام المصابة بالإسهال انخفاضًا في مستويات البروتين الكلي والصوديوم والكالسيوم، مع ارتفاع مستويات البوتاسيوم والكلور. كما انخفض مستوى الفوسفور بشكل معنوي في العجول بعد الفطام فقط. بالإضافة إلى ذلك، أظهرت العجول المصابة قبل وبعد الفطام ارتفاعًا في مستويات البروتين المتفاعل ، والكورتيزول، وإنزيمي الالت و الاست، وكذلك المالونداي ألدهيد ا، مع انخفاض واضح في إنزيم غلوتاثيون بيروكسيداز. وفي الختام، فإن العجول المصابة بالإسهال في مرحلتي ما قبل وما بعد الفطام تكون عرضة لتغيّرات دموية وكيميائية وإجهاد تأكسدي. ومع ذلك، يجب إيلاء عناية خاصة للعجول قبل الفطام نظرًا لارتفاع قابليتها للإصابة بالإسهال.

الكلمات الدالة: بكتيري، عجول، إسهال، إجهاد تأكسدي، قبل وبعد الفطام.