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Investigation of the Relationship Between Blood Gas and Oxidative Stress Parameters in Calves with Diarrhea *, **

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The aim of this study was to determine the etiology of diarrhea in neonatal calves with diarrhea and to determine the changes in blood gases and oxidative stress parameters. 20 diarrheal calves and 20 healthy calves brought to Fırat University Faculty of Veterinary Medicine Animal Hospital were used in the study. After identifying the etiological agents in diarrheic calves using immunochromatographic test kits, blood samples were collected from the jugular vein for further analysis. Blood gas parameters were measured using a blood gas analyzer. Additionally, serum malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) levels, as indicators of oxidative stress, were analyzed using a spectrophotometer. Blood gas analysis revealed that pH, bicarbonate (HCO_3^-), and partial oxygen pressure (pO_2) levels were decreased, whereas partial carbon dioxide pressure (pCO_2) and serum potassium (K) levels were elevated. When compared to the serum MDA levels of healthy calves, it was determined that the serum MDA levels of calves with diarrhea increased significantly, while the serum GSH, GSH-Px, CAT and SOD enzyme activities decreased. As a result of this study, it was concluded that, independent of the etiological agents causing diarrhea, metabolic acidosis will develop in addition to oxidative stress in the neonatal period, and that serum HCO_3^- levels may decrease and electrolytes, especially K, may increase.

Key Words: *Diarrhea, calf, oxidative stress, blood gases*

İshalli Buzağılarda Kan Gazı ve Oksidatif Stres Parametreleri Arasındaki İlişkinin Araştırılması

Bu çalışmanın amacı, ishalli neonatal buzağılarda ishalin etiyolojisini ortaya koyarak, kan gazları ve oksidatif stres parametrelerindeki değişimlerin belirlenmesidir. Çalışmada Fırat Üniversitesi Veteriner Fakültesi Hayvan Hastanesi'ne getirilen 20 adet ishalli buzağı ile 20 adet sağlıklı buzağı kullanılmıştır. İshalli buzağıların immunokromatografik test kitleri kullanılarak etiyolojik tanıları yapıldıktan sonra, vena jugularislerinden kan örnekleri alınmıştır. Alınan kan örneklerinden kan gazı analizatörü ile kan gazı analizleri yapılmıştır. Ayrıca kan örneklerinden elde edilen serumlardan spektrofotometre ile oksidatif stres parametrelerinden malondialdehit (MDA), indirgenmiş glutatyon (GSH), glutatyon peroksidaz (GSH-Px), katalaz (CAT) ve süperoksit dismutaz (SOD) düzeyleri belirlenmiştir. Kan gazı analizleri neticesinde pH, bikarbonat (HCO_3^-), kısmı oksijen basıncı (pO_2) gibi değerlerin düştüğü, kısmi karbondioksit basıncı (pCO_2) ve serum potasyum (K) düzeylerinin ise arttığı belirlenmiştir. Sağlıklı buzağıların serum MDA düzeyleriyle kıyaslandığında, ishalli buzağıların serum MDA düzeylerinin önemli derecede arttığı, serum GSH, GSH-Px, CAT ve SOD enzim aktivitelerinin ise azaldığı belirlenmiştir. Yapılan bu çalışma neticesinde ishale neden olan etiyolojik ajanlardan bağımsız şekilde neonatal dönemde oksidatif stres yanında metabolik asidozun gelişeceği, serumda HCO_3^- düzeylerinin azalıp özellikle K gibi elektrolitlerde artışların olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: *İshal, buzağı, oksidatif stres, kan gazları*

Introduction

Calf diarrhea is a disease that causes serious economic losses in livestock farms due to its effect on treatment costs and mortality rates. Viral, bacterial, and protozoal pathogens are major causes of this disease, often occurring as mixed infections (1-5). Neonatal calf diarrhea, frequently caused by *Escherichia coli, Cryptosporidium spp.*, rotavirus, and coronavirus, is highly prevalent during the first four weeks following birth (6). In neonatal calves with diarrhea, electrolyte losses such as bicarbonate (HCO_3^-), chloride (Cl), sodium (Na), potassium (K) and hydrogen (H) are observed along with fluid loss (7, 8). In addition to fluid electrolyte loss, decreased milk intake, changes in intestinal flora, metabolic acidosis, hypothermia, septicemia and azotemia are also observed (2, 3, 9-12). In healthy calves, venous pH ranges between 7.35 and 7.45. A venous pH below 7.35 indicates acidosis. Diarrhea is particularly recognized as the primary factor leading to metabolic acidosis observed in calves (13, 14). Metabolic acidosis typically arises as a result of hyponatremia, the accumulation of D-lactate and volatile fatty acids, or intestinal HCO_3^- loss, all of which contribute to the development of

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symptoms (15). In dehydrated calves, inadequate perfusion leads to reduced acid secretion in the kidneys, while tissue hypoxia results in the formation of lactic acid, further contributing to the development of metabolic acidosis (4, 16). In the case of metabolic acidosis, respiratory compensation results in a decrease in partial arterial pCO_2. Metabolic acidosis, characterized by HCO_3^- depletion, excessive acid production, and impaired renal acid excretion, is a critical and commonly observed condition in diarrheic calves (17). The acidosis observed in diarrhea is associated with intestinal HCO_3^- loss and the accumulation of organic acids such as L-lactate and D-lactate (18, 19). Additionally, diarrhea is frequently accompanied by significant electrolyte imbalances. Although there is a reduction in the total body K levels, an increase in blood K concentration is observed in diarrheic calves (18, 20). Moreover, moderate L-lactic acidosis may develop in both healthy and diarrheic calves due to dehydration (21, 22). In metabolic acidosis, particularly in cases of elevated plasma D-lactate concentrations, the central nervous system becomes progressively depressed over time. This condition is further characterized by a reduction in the suckling reflex, ataxia, recumbency, coma, and ultimately, death (3). In calves with severe diarrhea, it is reported that mortality is more often attributable to metabolic acidosis rather than dehydration (8).

The severity of acidosis and base deficit can be accurately determined using a blood gas analyzer (23). The assessment of acid-base imbalances and the degree of dehydration is crucial for determining the treatment protocol and ensuring effective therapeutic management (7). A blood gas analyzer measures parameters such as blood pH, pCO_2 (mmHg), and HCO_3^- (mmol/L). These parameters allow the determination of whether the acid-base imbalance is acidemia or alkalemia and whether the underlying issue is respiratory or metabolic in origin (24).

Oxidative stress refers to an imbalance between oxidants and antioxidants, skewed towards an excess of oxidants. This condition is primarily characterized by lipid peroxidation and the generation of reactive oxygen species (ROS) or free radicals, resulting in cellular damage within the organism. When the body's defense mechanisms against oxidative stress fail, oxidative damage occurs in cells, resulting in significant disruptions in bodily functions. Oxidative stress plays a critical role in the pathogenesis of numerous diseases, including aging, cardiovascular diseases, cancer, sepsis, degenerative neurological disorders, renal failure, infertility, and various muscle and liver diseases (25, 26). Under normal conditions, there is a balance between oxidant and antioxidant systems within the organism. In the case of oxidative stress, this balance is disrupted, leading to an increase in the number of oxidants (27, 28). Free oxygen radicals function in two significant ways. First, they act on the fatty acids present in the cell membrane, leading to the generation of new radicals. Second, they incorporate released hydrogen atoms, converting them into lipid peroxides (29). Malondialdehyde (MDA), a lipid peroxide product, causes alterations such as disruption of ion transport, enzyme activities, and the integrity of the cell membrane (30). Therefore, MDA measurement is utilized to assess the severity of cellular damage (31). Catalase (CAT) and glutathione peroxidase (GSH-Px) reduce hydrogen peroxide (H_2O_2) to water and atomic oxygen. Glutathione (GSH), as a substrate in the redox cycle, plays a crucial role in scavenging hydroxyl radicals and singlet oxygen. In addition to directly neutralizing free radicals, GSH also exerts enzymatic effects in collaboration with GSH-Px. The primary function of GSH is to maintain enzymes and other cellular components in their reduced state within cells (32). Superoxide dismutase (SOD) is the primary detoxification enzyme within cells and is known to play a central role in defense against ROS (33, 34).

The aim of this study was to investigate the etiology of diarrhea in neonatal calves and to determine the relationship in blood gas and oxidative stress parameters.

Materials and Methods

Research and Publication Ethics: Ethical approval for this study was obtained from Fırat University Animal Experiments Local Ethics Committee as stated in the decision numbered 2022/19-10.

In this study, calves aged 1-30 days, brought to the Clinics of Fırat University Faculty of Veterinary Medicine Animal Hospital with complaints of diarrhea, were used. Etiological diagnoses were performed on diarrheic calves that had not received any prior treatment, were born normally, and showed no anomalies, using immunochromatographic test kits. Subsequently, blood samples were collected from the jugular veins of the calves in accordance with standard techniques, and these samples were used to determine blood gas and oxidative stress parameters. Blood gas analyses were conducted through a service obtained from the Central Laboratory of Fırat University Faculty of Veterinary Medicine Animal Hospital. In the samples, MDA levels were determined using the spectrophotometric method described by Placer et al. (35) GSH levels were measured according to the method defined by Sedlak and Lindsay (36) CAT activity was determined using the method outlined by Goth (37) and GSH-Px activity was measured following the methods described by Lawrence et al. (38).

Statistical Analysis: In this study, 20 calves with diarrhea and 20 healthy calves were used. All comparisons were performed using the SPSS software (Version 22.0). Initially, the Kolmogorov-Smirnov normality test was applied to the data, and it was determined that all values, except for pH and $pHCO_3^-$, followed a normal distribution. Therefore, the differences in pH and $pHCO_3^-$ levels between the control and diseased groups were assessed using the non-parametric Mann-Whitney U test. For other parameters, comparisons between the groups were conducted using the independent t-test. Parametric values are presented as mean ± standard deviation (S.D.), while non-

parametric values are expressed with both mean ± S.D. and median values. A p-value of <0.05 was considered statistically significant (39).

Power Analysis: The sample size in the study was calculated using G*Power (version 3.1.9.7) package program as 40 cows (N=40) in total, with 10 animals in each group (n=20) with an effect size of 0.88, type 1 error of 0.05 and 85% power.

Results

Analysis performed using immunochromatographic test kits on the diarrheic calves included in the study revealed *Cryptosporidium spp.* infection in 1 calf, rotavirus infection in 6 calves, coronavirus infection in 3 calves, *E. coli* infection in 6 calves, and rotavirus-*Cryptosporidium* co-infection in 1 calf. No etiological agent was identified in 3 of the diarrheic calves.

Table 1 provides the mean values, standard deviations, and statistical significance levels of oxidative stress parameters and blood gas results for both the control group and neonatal diarrheic calves. Analysis of the table indicates that MDA levels, representing lipid peroxidation, were markedly elevated in neonatal diarrheic calves compared to the control group (p<0.001). Conversely, the activities of antioxidant enzymes such as GSH (p<0.001), GSH-Px (p=0.010), and SOD (p<0.001) were significantly diminished in diarrheic calves, reflecting the impact of oxidative stress. While CAT levels were found to be lower in the diarrheic group relative to the control group, this reduction did not reach statistical significance (p=0.164).

Table 1. Mean values, standard deviations, and statistical significance levels of oxidative stress parameters and blood gas results in the control group and neonatal diarrheic calves

Parameters	Control Groups	Neonatal Diarrhea Groups	p value
MDA (µmol/L)	15.30±3.82	24.84±5.72	***
GSH (mmol/L)	0.52±0.09	0.42±0.05	***
GSH-Px (U/g protein)	266.79±59.85	222.29± 53.09	0.010*
Katalaz (kU/L)	109.99±26.76	91.08±53.21	-
SOD (ng/mL)	72.03±4.01	8.69±0.52	***
pH	7.39±0.03	7.20±0.17	***
$pHCO_3^-$ (mmol/L)	27.51±1.97	16.69±6.32	***
pO_2 (mmHg)	46.02±4.51	41.20±9.60	0.041*
pCO_2 (mmHg)	29.85±0.43	41.75±7.93	***
K (mmol/L)	4.16±0.49	5.56±1.28	***

*: p<0.05, ***: p<0.001

In neonatal diarrheic calves, blood pH levels, indicative of metabolic acidosis, were significantly lower (p<0.001) compared to the control group. Correspondingly, plasma HCO_3^-levels in neonatal diarrheic calves were also significantly reduced (p<0.001). Plasma pO_2 levels were significantly decreased (p=0.041), whereas pCO_2 (p<0.001) and K levels (p=0.001) were significantly elevated in neonatal diarrheic calves.

Discussion

Diarrhea is a common problem in newborn calves. Clinically, it can present in various forms, ranging from mild diarrhea to severe forms that rapidly lead to dehydration and electrolyte imbalances. Numerous enteropathogens are responsible for the etiology of diarrhea encountered during this period, and *Escherichia coli*, rotavirus, coronavirus, and *Cryptosporidium parvum* play important roles in its development (1, 3, 40). When the etiological findings of the present study are examined, it is clear that the identified etiological agents are consistent with reports in the current literature. Oxidative stress, which develops during the course of many diseases and acts as a secondary factor that worsens the condition, occurs when the body's defense systems are inadequate to counteract ROS (26, 41). Lipid peroxidation, which is among the most commonly used methods to determine oxidative stress, is determined by measuring the increase in MDA concentration in plasma (42). Increased serum MDA levels and decreased serum antioxidant enzyme activities have been reported in calves affected by diarrhea (43-45). When the results of the current study were examined, it was seen that MDA levels were significantly higher (p<0.001), GSH (p<0.001), GSH-Px (p=0.010) and SOD (p<0.001) levels were significantly lower and CAT levels were lower compared to the control group, but not statistically significant (p=0.164). The increase in MDA levels indicates increased cellular damage caused by increased free radical production during the diarrhea process. The decrease in antioxidant enzyme levels indicates that oxidative stress-related destruction may have occurred in the cell. Metabolic acidosis and various metabolic abnormalities occur primarily due to fluid and electrolyte loss (12). In a study examining the venous blood results of neonatal diarrheal calves, it was reported that pre-treatment pH, HCO_3^- and pO_2 levels were lower compared to the control group, and that developing metabolic acidosis caused a decrease in pH and HCO_3^- levels (21, 47). Hyperkalemia may occur in calves as a result of diarrhea. In such cases, an increase in blood K concentration is detected in calves with diarrhea; however, total body K content actually decreases (47, 48). During this process, intracellular K levels decrease while extracellular K levels increase (49). Increases in blood serum K levels have been reported (50, 51). When Table 1, which presents the results of the present study, is examined, it is seen that blood pH (p<0.001), HCO_3^- levels (p=0.001) and partial pO_2 (p=0.041) were significantly lower, while partial pCO_2 (p<0.001) and serum K levels (p=0.001) were significantly higher compared to the control group. These findings are indicative of metabolic acidosis and indicate that systemic acid-base balance is disturbed. Interestingly, the changes observed in oxidative stress markers seem to coincide with these blood gas changes, suggesting a possible link. Increases in pCO_2 and K may also be associated with impaired cellular ion transport

and mitochondrial dysfunction under oxidative conditions, while decreases in pO_2 may imply increased tissue oxygen demand or impaired oxygen utilization (52). This impairment may negatively affect aerobic metabolism and energy production, thus worsening the clinical condition of affected calves.

Taken together, these results suggest that oxidative stress and acid-base disorders may develop simultaneously in neonatal diarrhea and may possibly reinforce each other's effects. This interaction may play an important role in the progression of clinical symptoms and highlights the importance of addressing oxidative imbalance as part of a supportive treatment strategy in diarrheic calves.

We were only able to evaluate 20 healthy and 20 diarrheic calves in our study. Although this sample size was considered sufficient for the present analyses, it is unknown how applicable the results are to different calf populations. We believe that studies that include calves of various ages, breeds and regions and examine clinical and environmental factors in more depth can overcome these limitations and the results obtained can be more reliable and widely applicable. Our findings and literature data indicate that, regardless of the etiological agents causing diarrhea, newborn calves are likely to develop oxidative stress together with metabolic acidosis, and in addition, a decrease in serum HCO_3^- levels and an increase in K, can be expected.

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