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Assessment of Serum Malondialdehyde (MDA), Reduced Glutathione (GSH) and Thiol–Disulfide Homeostasis in Neonatal Calves Diagnosed with Cryptosporidiosis

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ABSTRACT

Cryptosporidiosis is an important cause of neonatal calf diarrhea and may contribute to systemic oxidative and redox imbalance through intestinal epithelial injury, inflammation, and antioxidant consumption. Although malondialdehyde (MDA) and reduced glutathione (GSH) are commonly used indicators of lipid peroxidation and antioxidant defense, respectively, dynamic thiol–disulfide homeostasis (TDH) may provide complementary information on the circulating thiol pool and protein-based redox buffering capacity. However, data evaluating conventional oxidative stress biomarkers together with TDH parameters in neonatal calves with naturally occurring cryptosporidiosis remain limited. Therefore, this study aimed to assess serum MDA, GSH, and TDH parameters in neonatal calves diagnosed with cryptosporidiosis and to determine whether TDH components provide additional insight into cryptosporidiosis-associated redox alterations. The study was conducted on 40 calves aged 0–28 days, including 30 calves with cryptosporidiosis and 10 healthy controls. Diagnosis was established using an immunochromatographic test on fresh fecal samples, and only calves that tested negative for other enteric agents in the same panel were included. Serum MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) method, GSH levels using the Beutler method, and total thiol (TT) and native thiol (NT) levels using the Erel–Neşelioğlu method; disulfide (Ds) levels and related ratios were calculated. In calves with cryptosporidiosis, serum GSH, TT, and NT levels were significantly lower than those in healthy controls (p=0.010, p=0.010, and p=0.030, respectively). Although MDA levels were numerically higher in the diseased group, the difference was not statistically significant (p=0.150). No significant differences were detected between groups for Ds levels or TDH-derived ratios (Ds/TT, Ds/NT, and NT/TT). These findings suggest that neonatal calf cryptosporidiosis may be associated mainly with depletion of antioxidant and thiol pools rather than a marked systemic lipid peroxidation response. In this context, TT and NT may represent supportive redox indicators for the evaluation of cryptosporidiosis-associated neonatal diarrhea.

Keywords: *Cryptosporidiosis, Malondialdehyde, Oxidative stress, Reduced Glutathione.*

ÖZ

Kriptosporidiyozlu Neonatal Buzağılarda Serum Malondialdehit, Redükte Glutatyon ve Tiyol–Disülfit Homeostazının Değerlendirilmesi

Kriptosporidiyoz, neonatal buzağı ishallerinin önemli nedenlerinden biri olup intestinal epitel hasarı, inflamasyon ve antioksidan tüketimi aracılığıyla sistemik oksidatif/redoks dengenin bozulmasına katkıda bulunabilir. Malondialdehit (MDA) lipid peroksidasyonunun, redükte glutatyon (GSH) ise antioksidan savunmanın yaygın kullanılan göstergeleri olmakla birlikte, dinamik tiyol–disülfit homeostazı (TDH) dolaşımdaki tiyol havuzu ve protein temelli redoks tamponlama kapasitesi hakkında tamamlayıcı bilgi sağlayabilir. Bununla birlikte, doğal olarak gelişen kriptosporidiyozlu neonatal buzağılarda klasik oksidatif stres belirteçleri ile TDH parametrelerini birlikte değerlendiren veriler sınırlıdır. Bu nedenle bu çalışmada, kriptosporidiyoz tanılı neonatal buzağılarda serum MDA, GSH ve TDH parametrelerinin değerlendirilmesi ve TDH bileşenlerinin kriptosporidiyozla ilişkili redoks değişiklikleri hakkında ek bilgi sağlayıp sağlamadığının ortaya konulması amaçlandı. Çalışma, 0–28 günlük toplam 40 buzağıda yürütüldü; 30 buzağı kriptosporidiyozlu, 10 buzağı ise sağlıklı kontrol grubunu oluşturdu. Tanı, taze dışkı örneklerinde immünokromatografik test ile konuldu ve aynı test panelinde diğer enterik etkenler yönünden negatif olan buzağılar çalışmaya dâhil edildi. Serum MDA düzeyleri tiyobarbitürik asit reaktif maddeler (TBARS) yöntemiyle, GSH düzeyleri Beutler yöntemiyle, toplam tiyol (TT) ve natif tiyol (NT) düzeyleri ise Erel–Neşelioğlu yöntemiyle ölçüldü; disülfit (Ds) düzeyi ve ilişkili oranlar hesaplandı. Kriptosporidiyozlu buzağılarda serum GSH, TT ve NT düzeyleri sağlıklı kontrollere göre anlamlı olarak daha düşük bulundu (sırasıyla p=0,010; p=0,010; p=0,030). MDA düzeyi hastalıklı grupta sayısal olarak daha yüksek olmasına rağmen fark istatistiksel olarak anlamlı değildi (p=0,150). Ds düzeyi ve TDH'den türetilen oranlar (Ds/TT, Ds/NT ve NT/TT) açısından gruplar arasında anlamlı fark saptanmadı. Bu bulgular, neonatal buzağı kriptosporidiyozunda sistemik redoks yanıtın belirgin bir lipid peroksidasyon artışından çok antioksidan ve tiyol havuzunun tükenmesiyle ilişkili olabileceğini düşündürmektedir. Bu bağlamda, TT ve NT kriptosporidiyozla ilişkili neonatal ishalin değerlendirilmesinde destekleyici redoks göstergeleri olarak değerlendirilebilir.

Anahtar Kelimeler: *İndirgenmiş glutatyon, Kriptosporidyoz, Malondialdehid, Oksidatif stres.*

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INTRODUCTION

Cryptosporidiosis is a major enteric disease of neonatal calves and represents an important component of neonatal calf diarrhea during the first weeks of life. Among *Cryptosporidium* species affecting ruminants, C. parvum is most commonly associated with diarrhea in young calves (Gong et al. 2017; Díaz et al. 2018; Kaduková et al. 2024). The infection primarily targets the intestinal epithelium, where epithelial damage, villus atrophy, and impaired absorptive function contribute to malabsorption, diarrhea, dehydration, weakness, and systemic deterioration (Naylor 2009; Thomson et al. 2017). Transmission occurs mainly through the fecal–oral route, and the environmental persistence of oocysts makes disease control difficult under farm conditions (Nugraha et al. 2021; Ryan et al. 2021; Cappellaro et al. 2023). In this context, cryptosporidiosis-associated diarrhea may disturb systemic oxidant–antioxidant balance, making redox-related biomarkers relevant for evaluating the host response in neonatal calves (Yüksek et al. 2021).

Oxidative stress occurs when oxidant production exceeds antioxidant defense capacity, leading to reactive oxygen species accumulation and oxidative damage to cellular components (Celi 2011; Ercan and Fidancı 2012). In neonatal calves with diarrhea, intestinal inflammation, impaired absorption, dehydration, and metabolic stress may increase oxidant burden and accelerate antioxidant consumption (Celi 2011; Yüksek et al. 2021). Malondialdehyde (MDA) and reduced glutathione (GSH) are frequently used to evaluate oxidative status. MDA is a secondary product of polyunsaturated fatty acid peroxidation and reflects oxidative membrane damage (Ayala et al. 2014; Hajimohammadi et al. 2023; Tesena et al. 2024), whereas GSH is a major endogenous antioxidant involved in the detoxification of hydrogen peroxide, lipid peroxides, and free radicals (Lu 2013; Uzun 2019). However, oxidative stress is a multifactorial process and may not be fully explained by a single lipid peroxidation marker or antioxidant parameter.

Thiols are redox-active compounds that contribute to antioxidant defense by reacting with oxidants and undergoing reversible conversion to disulfide bonds (Cremers and Jakob 2013; Erdoğan et al. 2019). Dynamic thiol–disulfide homeostasis (TDH), defined by native thiol, total thiol, disulfide levels, and their related ratios, is involved in detoxification, enzymatic regulation, apoptosis, and intracellular signaling pathways (Circu and Aw 2010; Erel and Neşelioğlu 2014). Compared with single oxidative stress markers, TDH provides broader information on the circulating thiol pool and protein-based redox buffering capacity. Therefore, alterations in TDH parameters under inflammatory or oxidative conditions may reflect consumption of thiol-dependent antioxidant reserves and disruption of systemic redox balance (Erdoğan et al. 2019; Erel and Erdoğan 2020).

Although oxidative stress has been investigated in neonatal calf diarrhea and in calves with cryptosporidiosis, studies evaluating MDA, GSH, and TDH parameters together in naturally occurring neonatal calf cryptosporidiosis remain limited. This represents an important knowledge gap, because combined assessment of conventional oxidative stress markers and TDH components may provide a broader biochemical perspective on cryptosporidiosis-associated redox alterations. Therefore, this study aimed to determine serum MDA, GSH, and TDH parameters in neonatal calves with cryptosporidiosis and to compare them with those of healthy controls. We hypothesized that neonatal calves with cryptosporidiosis would exhibit oxidative/redox imbalance characterized particularly by depletion of GSH, total thiol, and native thiol levels.

MATERIAL AND METHODS

Ethical Approval

The study protocol was approved by the Kafkas University Local Ethics Committee for Animal Experiments (Decision date: 03.04.2024; Approval No. KAÜ-HADYEK/2024-064), ensuring compliance with relevant ethical standards. Additionally, blood samples were collected by following the recommended "standard sample collection procedure," ensuring that the animals were not exposed to stress in any way or harmed. All procedures related to the animals were carried out in accordance with institutional guidelines and national regulations on animal welfare.

Animal Material

This single-center clinical study was conducted at the Animal Hospital of the Faculty of Veterinary Medicine, Kafkas University, between 15 April 2024 and 15 April 2025. The individual calf was considered the experimental unit. A total of 40 neonatal calves aged 0–28 days were included in the study. The calves were classified into two groups according to clinical status and fecal immunochromatographic test results: calves diagnosed with cryptosporidiosis constituted the diseased group (n=30), and clinically healthy calves with negative fecal test results constituted the control group (n=10). All calves were Simmental or Simmental-crossbred. The diseased group consisted of diarrheic calves with clinical findings compatible with neonatal enteric disease and confirmed Cryptosporidium positivity. The control group consisted of clinically healthy calves without diarrhea or evident systemic clinical abnormalities at the time of examination and with negative results in the same fecal immunochromatographic test panel. Written informed consent was obtained from the animal owners before inclusion in the study.

Clinical Examination and Diagnostic Criteria

All calves were subjected to clinical examination before blood and fecal sampling. For the diseased group, the inclusion criteria were as follows: neonatal calves aged 0–28 days, presence of diarrhea at admission, compatible clinical findings including anorexia, weakness, and depression, no previous treatment before sampling, and fecal Cryptosporidium positivity. Calves were excluded from the diseased group if they had received antibiotics, antidiarrheal drugs, oral electrolyte solutions, or intravenous fluid therapy before admission. Calves with positivity for any other enteric agent evaluated in the same diagnostic test panel were also excluded in order to minimize the confounding effect of mixed enteric infections.

The clinical status of each calf was evaluated according to general behavior, alertness or depression, willingness to stand, suckling reflex, appetite status, hydration status, and the presence of diarrhea. Fresh fecal samples were collected directly from the rectum using clean disposable materials to prevent contamination. Cryptosporidiosis was diagnosed using a commercial immunochromatographic test kit (BoviD-5 Ag Test Kit, BioNote Inc., Korea) in accordance with the manufacturer's instructions. Only calves that were positive for Cryptosporidium and

negative for the other enteric agents included in the same test panel were assigned to the diseased group.

The control group consisted of neonatal calves aged 0–28 days that were clinically healthy at the time of examination. Control calves had no diarrhea, anorexia, weakness, depression, or other evident systemic clinical abnormalities. In addition, fresh fecal samples obtained from control calves were evaluated using the same immunochromatographic test panel, and only calves with negative fecal test results were included as healthy controls.

Collection of Blood Samples

Blood samples were collected once from each calf during the initial clinical evaluation and, for diseased calves, before any treatment was administered. Sampling was performed from the jugular vein (V. jugularis) using appropriate restraint and a sterile vacuum blood collection system (Vacuette®, Greiner Bio-One GmbH, Austria). Blood samples were transferred into serum tubes for biochemical analyses. Serum was separated by centrifugation at 3000 rpm for 10 min using a Hettich Rotina 380R® centrifuge (Hettich, Germany). The obtained serum samples were aliquoted and stored at −20°C until the measurement of malondialdehyde, reduced glutathione, and thiol–disulfide homeostasis parameters.

Biochemical Analyses

Serum malondialdehyde (MDA) levels were determined spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) method described by Yoshioka et al. (1979). Serum reduced glutathione (GSH) levels were measured according to the method of Beutler et al. (1963). Total thiol (TT) and native thiol (NT) concentrations were determined spectrophotometrically using the automated dynamic thiol–disulfide homeostasis method described by Erel and Neşelioğlu (2014). Disulfide (Ds) levels and TDH-derived ratios were calculated from TT and NT values using the following formulas: Ds=(TT−NT)/2; Ds/TT(%)=(Ds/TT)×100; Ds/NT(%)=(Ds/NT)×100; and NT/TT(%)=(NT/TT)×100.

Statistical Analysis

Data were assessed for normality using the Shapiro–Wilk test before statistical comparisons. Descriptive results were presented as mean ± standard deviation (SD) to maintain consistency with the graphical presentation of the data. Variables that met the normality assumption (MDA, TT, and NT) were compared between the cryptosporidiosis and control groups using the unpaired independent-samples t-test. Variables that did not meet the normality assumption (GSH, disulfide, Ds/TT, Ds/NT, and NT/TT) were analyzed using the Mann–Whitney U test. Exact p values were reported to three decimal places, and p values <0.05 were considered statistically significant.

Because this was a clinical study based on naturally occurring cases, the final sample size was determined by the number of calves that met the predefined inclusion and exclusion criteria during the study period. The study included 30 calves with cryptosporidiosis and 10 healthy control calves. To evaluate the detectable standardized difference with the available sample size, a sensitivity power analysis was performed using G*Power version 3.1.9.7. For a two-tailed independent-samples t-test with α=0.05, power=0.80, and the actual group sizes of 10 control and 30 diseased calves, the minimum detectable standardized difference was Cohen's d=1.05. This indicated that the available sample size was appropriate for detecting large between-group differences, although smaller differences may not have been detected. All statistical analyses were performed using GraphPad Prism version 8.1 (GraphPad Software, San Diego, CA, USA).

RESULTS

The demographic, clinical, and diagnostic characteristics of the calves included in the study are presented in Table 1. The mean age was 11.4±3.2 days in the control group and 13.8±4.5 days in the cryptosporidiosis group. The age range was 4–21 days in the control group and 2–28 days in the cryptosporidiosis group. The sex distribution consisted of 6 males and 4 females in the control group and 16 males and 14 females in the cryptosporidiosis group. Simmental calves constituted 70.0% of the control group and 63.3% of the cryptosporidiosis group. Diarrhea was present in all calves with cryptosporidiosis and absent in all control calves. In the cryptosporidiosis group, anorexia, weakness, and depression were observed in 26/30, 28/30, and 22/30 calves, respectively. All calves in the cryptosporidiosis group were positive for fecal Cryptosporidium antigen and negative for the other enteric agents included in the same test panel, whereas all control calves were negative in the fecal test panel.

Serum oxidative stress biomarkers and thiol–disulfide homeostasis parameters are shown in Figure 1. Serum MDA concentration was numerically higher in calves with cryptosporidiosis than in healthy controls (1.31 vs. 1.24 nmol/mL); however, this difference was not statistically significant (p=0.150). In contrast, serum GSH concentration was significantly lower in the cryptosporidiosis group than in the control group (4.98 vs. 5.20 μmol/mL; p=0.010).

Regarding thiol–disulfide homeostasis parameters, total thiol (TT) and native thiol (NT) concentrations were significantly reduced in calves with cryptosporidiosis. The mean TT concentration was 221.32 μmol/L in the cryptosporidiosis group and 295.60 μmol/L in the control group (p=0.010). Similarly, the mean NT concentration was 200.96 μmol/L in the cryptosporidiosis group and 267.00 μmol/L in the control group (p=0.030).

No statistically significant differences were detected between the groups for disulfide concentration or TDH-derived ratios. The mean disulfide concentration was 10.18 μmol/L in calves with cryptosporidiosis and 14.30 μmol/L in controls (p=0.750). The Ds/TT ratio was 4.80% in the cryptosporidiosis group and 5.15% in controls (p=0.640), while the Ds/NT ratio was 5.67% and 6.63%, respectively (p=0.640). The NT/TT ratio was also similar between groups, with values of 90.40% in calves with cryptosporidiosis and 89.70% in controls (p=0.640). Overall, calves with cryptosporidiosis showed significantly lower GSH, TT, and NT levels, whereas MDA, disulfide, and TDH-derived ratios did not differ significantly between groups.

The evaluated parameters were malondialdehyde (MDA, nmol/mL), reduced glutathione (GSH, μmol/mL), total thiol (TT, μmol/L), native thiol (NT, μmol/L), disulfide (Ds, μmol/L), and the ratios Ds/TT, Ds/NT, and NT/TT (%). Data are presented as mean ± standard deviation (SD). MDA, TT, and NT were analyzed using the unpaired independent-samples t-test, whereas GSH, Ds, Ds/TT, Ds/NT, and NT/TT were analyzed using the Mann–Whitney U test. p values are indicated above the bars, and the corresponding exact p values are reported in the Results text.

Table 1: Demographic, clinical, and diagnostic characteristics of healthy control calves and calves with cryptosporidiosis.

Parameter	Control group (n=10)	Cryptosporidiosis group (n=30)
Age, days, mean ± SD	11.4 ± 3.2	13.8 ± 4.5
Age range, days	4–21	2–28
Sex, n (%)		
Male	6 (60.0)	16 (53.3)
Female	4 (40.0)	14 (46.7)
Breed, n (%)		
Simmental	7 (70.0)	19 (63.3)
Simmental-crossbred	3 (30.0)	11 (36.7)
Clinical findings, n (%)		
Diarrhea	0 (0.0)	30 (100.0)
Anorexia	0 (0.0)	26 (86.6)
Weakness	0 (0.0)	28 (93.3)
Depression	0 (0.0)	22 (73.3)
Diagnostic test results		
Fecal Cryptosporidium test	Negative	Positive
Other enteric agents in the test panel	Negative	Negative
Medical treatment before sampling	No	No

Data are presented as mean ± standard deviation (SD) for age and as n (%) for categorical variables. The control group consisted of clinically healthy calves with negative fecal immunochromatographic test results, whereas the cryptosporidiosis group consisted of diarrheic calves positive for Cryptosporidium and negative for the other enteric agents included in the same test panel.

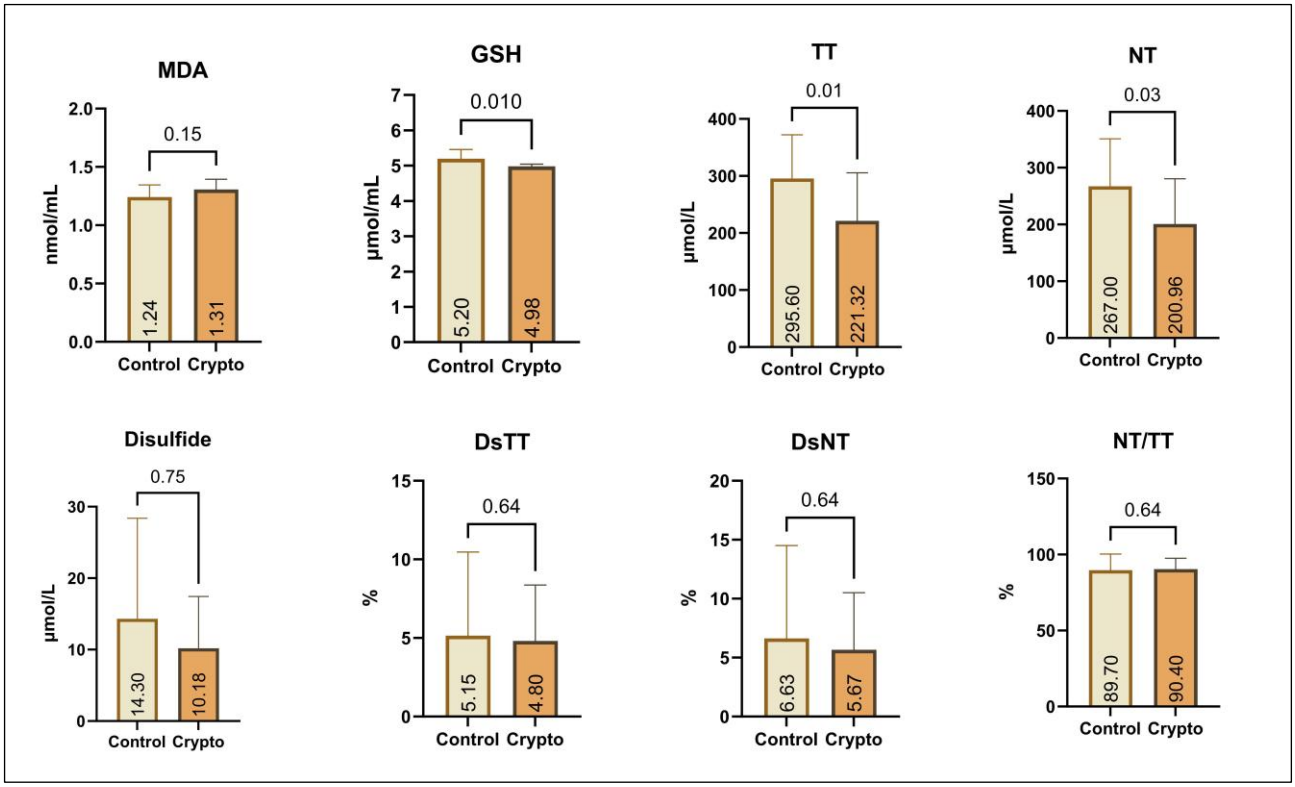

Figure 1: Comparison of serum oxidative stress biomarkers and thiol–disulfide homeostasis parameters between healthy control calves (n=10) and calves with cryptosporidiosis (n=30).

DISCUSSION AND CONCLUSION

In this study, serum MDA levels were numerically higher in calves with cryptosporidiosis than in healthy controls; however, this difference was not statistically significant. MDA is a secondary aldehyde generated during the oxidative degradation of polyunsaturated fatty acids and is widely used as an indicator of lipid peroxidation (Ayala et al. 2014). The absence of a significant increase in MDA suggests that systemic lipid peroxidation was not clearly detectable in the present cases. Similar findings have been reported by Isik and Ilhan (2018), who did not observe a significant change in oxidative status parameters, including MDA, in calves with cryptosporidiosis. In addition, Çenesiz et al. (2017) reported no significant difference in MDA levels before and after treatment in calves infected with *C. parvum*. Therefore, although MDA remains a useful marker of lipid peroxidation, the present

findings indicate that it may not be sufficiently discriminative as a standalone indicator of cryptosporidiosis-associated redox alterations. In this context, assessment of oxidative stress using complementary biomarkers rather than a single parameter may provide a more balanced biological interpretation (Celi 2011).

In this study, serum GSH levels were significantly lower in calves with cryptosporidiosis than in healthy controls. This finding suggests that antioxidant reserves may have been consumed in response to infection-associated oxidative and inflammatory burden. GSH is one of the major non-protein thiols in mammalian tissues and plays an important role in intracellular antioxidant defense, redox regulation, detoxification reactions, and immune-related cellular processes (Lu 2013; Aquilano et al. 2014). In ruminants, oxidative stress may develop as a result of increased oxidant production, reduced antioxidant availability, or accelerated antioxidant consumption; therefore, lower GSH levels in diarrheic enteric disease may reflect impaired oxidant–antioxidant balance (Celi 2011). Similar to our findings, El-Deeb et al. (2022) reported decreased GSH levels in calves with natural *C. parvum* infection, which was interpreted in relation to insufficient antioxidant capacity against free radical production. In addition, lower GSH levels reported in diarrheic calves of different etiologies support the view that intestinal inflammation can influence systemic redox status (Sağıroğlu et al. 2025). Since the calves in the present study were positive for Cryptosporidium and negative for the other enteric agents included in the test panel, the observed decrease in GSH may be interpreted more specifically as a component of the host redox response to cryptosporidiosis. However, this finding should be considered a supportive biochemical indicator rather than a standalone clinical marker.

In this study, TT and NT levels were significantly lower in calves with cryptosporidiosis than in healthy controls, suggesting depletion of the circulating thiol pool and reduced systemic redox buffering capacity. Thiol groups, particularly the –SH groups of albumin and other plasma proteins, constitute an important component of plasma antioxidant defense and can rapidly react with oxidants under conditions of increased oxidative burden (Young and Woodside 2001; Neşelioğlu et al. 2020). Within the TDH system, TT and NT provide information on the availability of reduced thiol reserves and protein-based redox status, thereby complementing conventional oxidative stress markers (Biswas et al. 2006; Circu and Aw 2010; Erel and Neşelioğlu 2014). In calves with cryptosporidiosis, intestinal epithelial injury and the associated inflammatory host response may increase oxidant production and antioxidant consumption (Thomson et al. 2017). In calves infected with *C. parvum*, alterations in antioxidant capacity and oxidative stress-related parameters have also been reported (Çenesiz et al. 2017). More generally, inflammatory and oxidative conditions are known to induce antioxidant responses and cellular redox adjustments (Espinosa-Diez et al. 2015). Therefore, the reduction in TT and NT observed in the present study may reflect consumption of thiol-dependent antioxidant reserves during cryptosporidiosis-associated neonatal diarrhea.

In contrast to the decreases in TT and NT, no statistically significant differences were observed in Ds levels or in the Ds/TT, Ds/NT, and NT/TT ratios. This finding suggests that the reduction in the circulating thiol pool was not accompanied by a clear shift toward increased disulfide formation. Since Ds and the related ratios are calculated from TT and NT values, parallel decreases in TT and NT may limit marked changes in these proportional indices (Erel and Neşelioğlu 2014). In addition, thiol oxidation under inflammatory or oxidative conditions may involve different redox pathways, and therefore changes in thiol availability may not always be reflected by an isolated increase in Ds concentration (Erel and Erdoğan 2020). Similar variability in TDH-derived responses has been reported in neonatal calf diarrhea, where NT and TT may decrease while disulfide and ratio responses differ according to disease etiology and severity (Terzi et al. 2023). Findings from a parasitic inflammatory condition in sheep also support that reductions in thiol-related parameters may occur without a consistent shift in TDH ratios (Çamkerten et al. 2019). Therefore, the present results indicate that cryptosporidiosis in neonatal calves was associated mainly with depletion of TT and NT rather than a pronounced disulfide-dominant shift in TDH.

The main strength of the present study is the combined evaluation of MDA, GSH, and TDH parameters in naturally occurring Cryptosporidium-positive neonatal diarrhea before treatment. This approach highlighted GSH, TT, and NT as the most relevant altered indicators, suggesting depletion of antioxidant and thiol reserves, while MDA, Ds, and TDH-derived ratios did not show significant between-group differences.

This study also has some limitations. The sample size was relatively small and unbalanced, with 30 calves in the cryptosporidiosis group and 10 calves in the control group. In addition, this was a single-center clinical field study, and management-related factors such as colostrum intake, feeding conditions, housing, and environmental exposure could not be fully standardized. Blood samples were collected at a single time point; therefore, temporal changes in oxidative/redox parameters during the course of disease or after treatment could not be evaluated. Moreover, although disease severity was assessed clinically, biomarker concentrations were not analyzed according to severity scores. Etiological classification was based on a commercial immunochromatographic rapid diagnostic test kit, and molecular confirmation, fecal oocyst quantification, and detailed pathogen load assessment were not performed. Therefore, the diagnosis should be interpreted as a practical field-based diagnostic classification rather than molecular confirmation of Cryptosporidium infection. The study included only calves with Cryptosporidium-positive diarrhea and healthy controls; therefore, no additional disease-control group, such as calves with E. coli, coronavirus, rotavirus, or other etiologies of neonatal diarrhea, was available for comparison. Accordingly, the present findings should be interpreted as redox alterations associated with Cryptosporidium-positive neonatal diarrhea rather than as changes specific only to cryptosporidiosis when compared with other enteric diseases. In addition, acute-phase proteins and inflammatory cytokines were not measured in the present study. Therefore, the relationship between oxidative/redox alterations and systemic inflammatory responses could not be directly evaluated. These limitations should be considered when interpreting the biological relevance, etiological specificity, and practical applicability of the findings.

In conclusion, neonatal calves with cryptosporidiosis showed a redox alteration pattern characterized mainly by reduced GSH, TT, and NT levels, whereas MDA, Ds, and TDH-derived ratios did not differ significantly from healthy controls. These findings indicate that GSH, TT, and

NT were the most informative supportive indicators of antioxidant and thiol reserve depletion in Cryptosporidium-positive neonatal diarrhea. In contrast, MDA, Ds, and TDH-derived ratios appeared less discriminative in the present study. However, considering the small and unbalanced sample size, single time-point sampling, field-related variability, and the absence of additional disease-control groups, these biomarkers should not be interpreted as standalone disease-specific clinical markers. Larger longitudinal studies are needed to validate their biological relevance and potential research utility in neonatal calf cryptosporidiosis.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: MK
Supervision/Consultancy: EA
Data Collection and/or Processing: YUB, MS
Analysis and/or Interpretation: MM
Writing the Article: YUB
Critical Review: AHK

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