

# Dynamic thiol-disulphide homeostasis and ischemia modified albumin levels in neonatal calf diarrhea

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## ABSTRACT

The aim of this study was to determine dynamic thiol-disulphide homeostasis (TDH) parameters and ischemia modified albumin (IMA) levels in calves with neonatal diarrhea and compare with healthy controls. A total of 50 calves were included in the study. There were 25 calves in both diarrhea and healthy groups. Serum native thiol, total thiol, disulfide and IMA levels were measured using new methods. Native thiol (P=0.025) and total thiol (P=0.041) values decreased significantly in calves with neonatal diarrhea compared to the healthy control group. Disulphide (P= 0.133), disulphide/native thiol ratio (P=0.001) and IMA (P=0.0018) parameters were lower in healthy group, and the difference between the two groups was significant for the parameters other than disulphide. This study shows that TDH is impaired in neonatal calf diarrhea and IMA levels are increased due to oxidative stress.

## Introduction

Calf diarrhea is one of the most important problems of cattle breeding in the neonatal period when the levels of maternal antibodies transferred by passive immunity decrease rapidly. Neonatal calf diarrhea cases characterized by excessive and watery defecation due to infectious and non-infectious causes (5, 15, 16). Calf losses are in the range of 10-15% in developed European countries. Even in farms with very good management conditions, this rate generally does not fall below 5%. It has been reported that in herds where these conditions are not well provided, mortality and morbidity rates can reach 50% and 100%, respectively. Diarrhea causes 60% of the deaths during the neonatal period (32, 34). Reasons such as calf death, costs for treatment and prophylaxis, growth retardation during the growth period, and the sale of

animals below their value can be considered the most important economic problems in farms. The economic loss in the neonatal period depends on calf mortality in Türkiye is reported to be around 525 million euros annually (36).

Thiols are organic compounds that react with oxidants to form disulfide bonds. (6, 30). These bonds can turn back into thiols, resulting in dynamic thiol-disulfide homeostasis (TDH) (18). Dynamic TDH has important effects on detoxification, protection with antioxidants, apoptosis, regulation of enzymatic activity, and signaling mechanisms of cells (4). In recent years, many studies have been carried out in the field of human medicine, including gastrointestinal system diseases (20, 23, 30, 40) related to TDH (10). Oxidative stress, especially in farm animals, can be seen in many diseases, including calf diarrhea (24, 26, 40).

In acute ischemic cases, the metal binding capacity of albumin decreases, resulting in the formation of the metabolic substance known as ischemia modified albumin (IMA) (22). New evidence has been provided that the concentrations of IMA, one of the biomarkers associated with oxidative stress clinically, differ in the diagnosis of acute intestinal disease (28, 33).

The aim of the study was to analyse the TDH and serum IMA levels in calves with neonatal diarrhea. Considering the literature, it is the first study evaluating TDH and IMA levels in newborn calves with diarrhea.

## Materials and Methods

**Study population and location:** A total of 50 calves were included in the study; 25 calves with diarrhea (15 males, 10 females) and 25 healthy calves (14 males, 11 females) between the ages of 3-28 days. Animals with diseases other than calf diarrhea in the patient group and animals that were subjected to non-standard care and feeding conditions in both groups were excluded. The study was carried out in Bala Tarım Dairy Farm, which has a capacity of approximately 1000 dairy cows in the Bala district of Ankara. This study was approved by Kırıkkale University Animal Experiments Local Ethics Committee (Date:24/12/2020).

**Sample Collection and Analysis:** Blood samples were taken from the jugular vein for TDH and IMA tests from diarrhea and healthy control groups. The samples were centrifuged at 3000 rpm for 10 minutes, after which the serum was separated and stored at -80 °C. Later, all parameters were studied in the same session and in the

same serum sample. Serum native thiol, total thiol, disulphide and % disulfide/native thiol ratio were measured with the newly developed method by Erel and Neselioğlu (9). IMA levels were evaluated with the new method created by Bar-Or et al. (2).

**Statistical Analysis:** Statistical analyses were performed using GraphPad Prism version 8.4.2. (GraphPad Software, La Jolla California USA, www.graphpad.com). For all analyses,  $P < 0.05$  was considered significant. Normality assumption was evaluated using the Shapiro-Wilk Test. Descriptive analysis, mean and standard deviation data were presented. Due to the normal distribution of data, an independent sample t test was used to compare parameters between groups.

## Results

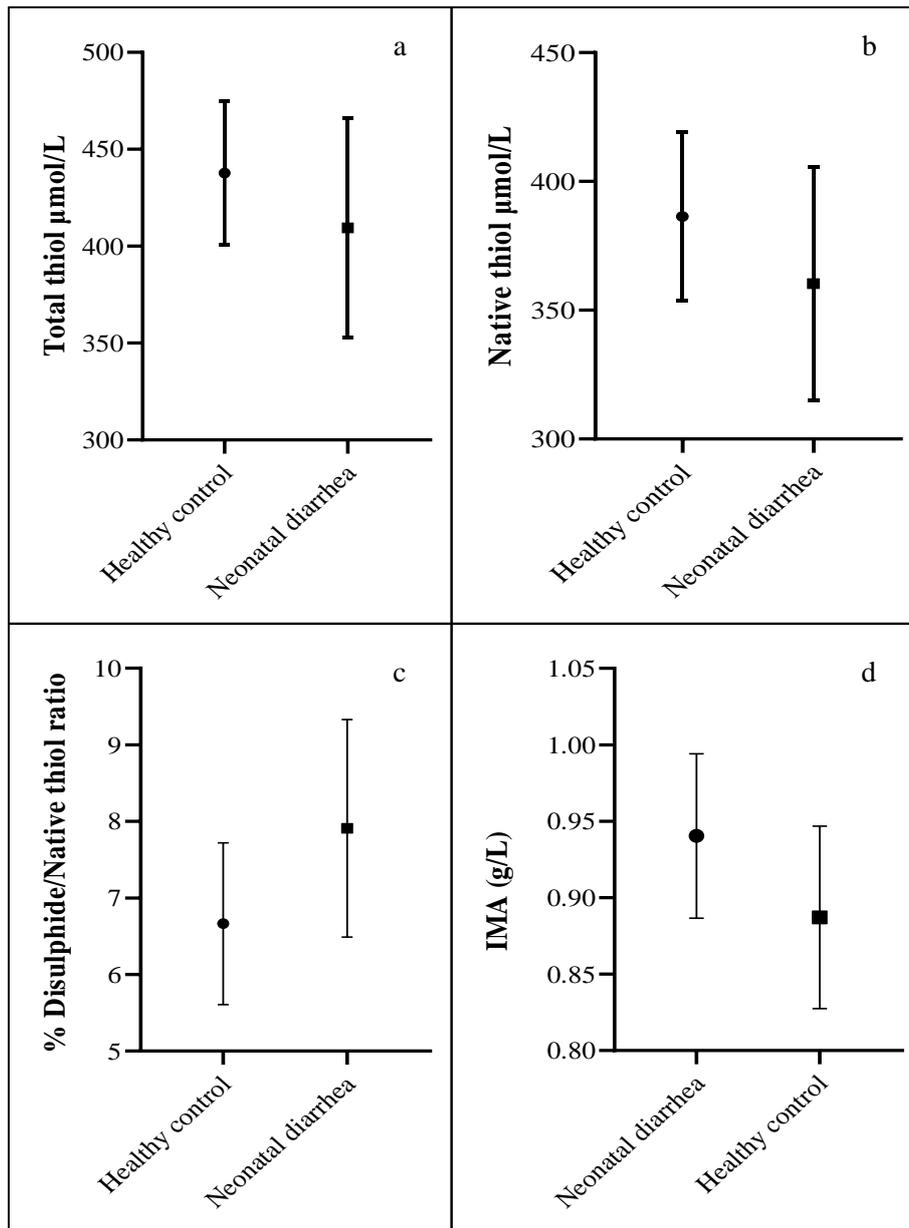
Demographic characteristics, TDH and IMA values of healthy and diarrhea groups are given in Table 1. There was no statistical difference between the two groups in gender ( $P > 0.9999$ ) and age ( $P = 0.4222$ ) data.

In this study, neonatal diarrhea group was compared with healthy controls by measuring TDH and IMA parameters. Native thiol and total thiol values decreased significantly in calves with neonatal diarrhea compared to the healthy control group (Table 1, Figure 1a, Figure 1b). Disulphide, % disulphide/native thiol ratio and IMA parameters were lower in the healthy group, and the difference between the two groups was significant for the parameters other than disulphide (Table 1, Figure 1c, Figure 1d).

**Table 1.** Demographic characteristics, TDH parameters and IMA values of the control and diarrhea groups.

Variable	Healthy Control group mean±SD (n=25)	Diarrhea group mean±SD (n=25)	P
Native Thiol	386.40±32.74	360.3±45.33	<b>0.0251*</b>
Total Thiol	437.8±37.19	409.4±56.60	<b>0.0416*</b>
Disulphide	25.71±4.534	27.57±4.062	0.1331
% Disulphide/native thiol ratio	6.663±1.056	7.910±1.422	<b>0.001*</b>
IMA	0.887±0.0597	0.940±0.0537	<b>0.0018*</b>
Age	17.84±8.107	16.04±7.607	0.4222
Gender			>0.9999
Male (n/%)	14/56	15/60	
Female (n/%)	11/44	10/40	

\*Indicates a significant statistical difference with  $P < 0.05$ .



**Figure 1.** Thiol-disulphide homeostasis parameters and ischemia-modified albumin levels in healthy control and neonatal diarrhea groups. a) Total thiol ( $\mu\text{mol/L}$ ) values b) Native thiol ( $\mu\text{mol/L}$ ) values c) % Disulphide/native thiol ratio d) IMA (Ischemia-modified albumin) (g/L) values.

## Discussion and Conclusion

High levels of free oxygen radicals in blood samples taken from healthy newborn calves indicate the occurrence of oxidative stress immediately after birth. Although the level of oxygen radicals in the blood decreases within the first week, it tends to increase again after change of care and feeding conditions (13). In the study conducted by Topaktaş (39), there are different opinions. In the studies in which the changes in the total oxidant level (TOS) and total antioxidant level (TAS) in newborn calves in the first month period were followed; Blood samples were taken from 20 calves (10 males and 10 females) at 0, 24, 72

hours and 14, 28 days in total 5 times after birth and analyzed in terms of TOS and TAS values (39). It was revealed that there was no significant change in TOS and TAS values in blood serum in the first 28-day period, which corresponds to the neonatal period in calves. Even though newborn calves can cope with oxidative stress, calf diarrhea, which is frequently encountered in the first 28 days of age, makes this struggle difficult. In diarrhea cases, intestinal permeability is impaired and oxidative stress increases, often the 2<sup>nd</sup> and 3<sup>rd</sup> weeks as a result of the acceleration of oxidative metabolism with the resulting in sepsis, by triggering many local or systemic

pathological units (25, 29, 31, 38). Physiologically native thiol, disulfide and total thiol are in a balance. This system, also known as TDH, is affected by various situations where oxidative stress occurs as a result of oxidation and reduction reactions (9). In order to evaluate the effects of oxidative stress on TDH in calf diarrhea, serum TDH parameters of 3-28 days old calves with diarrhea were compared with those of healthy ones.

Before the new method (Erel and Neselioglu 2014) was found, only the levels of some oxidant or antioxidant substances were determined in the studies (12, 14, 24). However, in our study, the levels of TDH parameters (native thiol, total thiol, disulfide and % disulfide/native thiol ratio) were measured with the aforementioned new method and TDH status was evaluated.

Reactive oxygen species (ROS) are highly toxic to cells, and the formation of oxygen radicals above the physiological level can initiate oxidative damage in the gut, damaging the intestinal antioxidant defense system (34, 35, 37). Thiol protein groups form an important antioxidant group and account for 52.9% of total serum antioxidant capacity in healthy people (9). Therefore, it can be expected that compounds containing thiol will decrease in plasma due to oxidation in the inflamed intestine (30). In the studies carried out; it was observed that oxidative stress occurred in calves with diarrhea, antioxidant values were significantly decreased and oxidant levels increased compared to healthy calves (1, 24, 34). Similar results are also seen in treatment studies (14, 19).

In the study of Neselioglu et al. (30) in people with ulcerative colitis; native thiol, total thiol and disulfide levels were significantly reduced when compared with healthy controls. According to our results, it is seen that native thiol and total thiol levels decrease in calves with diarrhea, and disulfide levels increase, although not statistically significant. In this ulcerative colitis study, it is stated that the reason for the reduction of disulfide bonds may be the conversion of thiol groups to disulfide bonds. Thus, it was observed that the TDH system did not shift to thiol group or disulfide bond formation and TDH system weakened. Similar to this study in humans, Çamkerten et al. (3) in their study in sheep with sarcoptic mange, it was revealed that the levels of native thiol, total thiol and disulfide were significantly lower in the patient group. According to the literature, it is known that while disulfide levels are higher in people with degenerative diseases, these values are lower in some immunological and oncological diseases (9). In our study, we see that disulfide levels in calves with diarrhea are higher than the healthy control group, although it is not statistically significant.

In the current literature, there is no study on the status of the TDH system in gastrointestinal system diseases in the veterinary field. In a study by Erdoğan et al. (11); the effects of dehorning with hot iron under the effect of

general or local anesthesia and painkillers on TDH were investigated. Native thiol and total thiol levels decreased without any significance in all groups throughout the study period. Disulfide levels did not show any significant changes in group, time, and group by time interactions. In this dehorning study (11), the decrease in thiol concentrations without an increase in disulfide levels was attributed to either insufficient uptake or increased degradation. Another reason for this situation is that changes in thiol and disulfide levels are thought to be caused by factors related to nutrition, not oxidative stress. In our study, low thiol and high disulfide (not statistically significant) levels were observed in the diarrhea group and it is thought that the reason might be directly oxidative stress.

Oxidative stress strongly affects albumin. Oxygen radicals bind to albumin and change its structure and IMA is formed. For this reason, IMA has been accepted as an indirect marker of increased oxidative stress. (27). In human medicine, studies conducted on people with inflammatory bowel disease, one of the gastrointestinal system diseases; It was concluded that IMA levels were higher than healthy controls (17) and positively correlated with disease activity levels (21). In a study conducted on children with irritable bowel disease; it was observed that TDH was impaired and IMA levels increased in the patient group (8). Although many parameters, indicate oxidative stress in gastrointestinal system diseases, have been evaluated in the field of veterinary medicine in the current literature, there is no study investigating IMA levels. In a study on dogs with Canine Distemper (7), IMA, disulfide, % disulfide/native thiol ratio and % disulfide/total thiol ratio levels were higher, while native thiol, total thiol, % native thiol/total thiol ratio and albumin levels were significantly lower than the control group, and this was interpreted as canine distemper increased oxidative stress in dogs. The results obtained in our study are parallel to the studies conducted in the field of human and veterinary medicine, both in terms of TDH parameters and IMA levels.

In conclusion, this study shows that TDH is impaired and IMA levels are increased due to oxidative stress in neonatal calf diarrhea. More studies should be conducted to detail our results in etiological terms and to evaluate the effectiveness of antioxidants in prevention or treatment options for neonatal calf diarrhea.

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This research received no grant from any funding agency/sector.

### Conflict of Interest

The authors declared that there is no conflict of interest.

## Author Contributions

OST, EK, YŞ and EC undertook the planning of the study, forming the groups and collecting the samples. SN and EE performed biochemical analyzes. All authors contributed equally to the evaluation of the results and the creation of the article.

## Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

## Ethical Statement

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee. (Date: 24/12/2020).

## Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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