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Comparative evaluation of total antioxidant-oxidant, oxidative stress index and ischemia modified albumin levels in calves with neonatal diarrhea

Evaluación comparativa de los niveles totales de antioxidantes-oxidantes, índice de estrés oxidativo y albúmina modificada por ísquemia en terneros con diarrea neonatal

Doğan Can HANEY[1*], Mehmet Ali ERFİDAN[2], Turan CİVELEK[3]

[1] Muğla Sıtkı Koçman University, Faculty of Milas Veterinary Medicine, Department of Internal Medicine, Milas, Mugla, Türkiye.
[2] Afyon Kocatepe University, Emirdağ Vocational School, Department of Veterinary Medicine, Emirdağ, Afyonkarahisar, Türkiye.
[3] Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Internal Medicine, Afyonkarahisar, Türkiye.
[*]Corresponding author: dogancanhaney@mu.edu.tr

ABSTRACT

The first 28 days of a calf's life, during which calf losses and diseases are most common, constitute the most critical phase of its development. Among neonatal disorders, diarrhea remains a major challenge in bovine medicine, causing substantial health and economic losses. Oxidative stress plays a significant role in the pathogenesis of disease by disrupting the balance between reactive oxygen and nitrogen species and antioxidant defenses. Though earlier research mostly looked at single oxidant or antioxidant agents, few comprehensive analyses including several oxidative indicators in neonatal calves exist. Calculated as the ratio of total oxidant capacity) to total antioxidant capacity, the oxidative stress index gives a broad index of redox balance. Further, ischemia-modified albumin, has been found to be a sensitive oxidative protein changes indicator. To characterize the oxidative condition associated with total oxidant capacity, total antioxidant capacity, oxidative stress index, albumin, and ischemia-modified albumin levels in both diarrheic and normal neonatal calves, this study investigated diarrheic calves had considerably higher ischemia-modified albumin levels ($P < 0.05$), showing more oxidative stress. Total oxidant capacity, total antioxidant capacity, and oxidative stress index also exhibited upward trend. This trend following an oxidative shift. Positive correlations existed between total oxidant capacity and oxidative stress index; negative correlations between total antioxidant capacity and oxidative stress index as well as between albumin and total oxidant capacity. These correlations revealed significant connections among these parameters.

Ultimately, this research presents a combined oxidative stress profile for neonatal calf diarrhea and stresses ischemia-modified albumin as a possible biomarker for assessing oxidative instability. These findings point to oxidative changes that contribute to the onset of neonatal diarrhea and therefore support the use of IMA as an alternative diagnostic approach for neonatal calves.

Key words: Albumin; diarrhea; ischemia modified albumin; neonatal calf; total oxidant-antioxidant.

RESUMEN

Los primeros 28 días de vida de un ternero, durante los cuales son más frecuentes las pérdidas y las enfermedades, constituyen la fase más crítica de su desarrollo. Entre los trastornos neonatales, la diarrea sigue siendo un gran reto en la medicina bovina, ya que provoca importantes pérdidas sanitarias y económicas. El estrés oxidativo desempeña un papel significativo en la patogénesis de la enfermedad al alterar el equilibrio entre las especies reactivas de oxígeno y nitrógeno y las defensas antioxidantes. Aunque las investigaciones anteriores se centraron principalmente en agentes oxidantes o antioxidantes individuales, existen pocos análisis exhaustivos que incluyan varios indicadores oxidativos en terneros neonatales. Calculado como la relación entre la capacidad oxidante total y la capacidad antioxidante total, el índice de estrés oxidativo proporciona un indicador general del equilibrio redox. Además, se ha descubierto que la albúmina modificada por isquemia es un indicador sensible de los cambios oxidativos en las proteínas. Para caracterizar el estado oxidativo asociado a los niveles de capacidad oxidante total, capacidad antioxidante total, índice de estrés oxidativo, albúmina e albúmina modificada por isquemia tanto en terneros neonatos con diarrea como en los sanos, este estudio investigó a terneros con diarrea, que presentaban niveles de albúmina modificada por isquemia considerablemente más altos ($P < 0,05$), lo que indicaba un mayor estrés oxidativo. La capacidad oxidante total, la capacidad antioxidante total y el índice de estrés oxidativo también mostraron una tendencia al alza. Esta tendencia se produce tras un cambio oxidativo. Existían correlaciones positivas entre el capacidad oxidante total y el índice de estrés oxidativo; correlaciones negativas entre el capacidad antioxidante total y el índice de estrés oxidativo, así como entre la albúmina y el capacidad oxidante total. Estas correlaciones revelaron conexiones significativas entre estos parámetros. En definitiva, este estudio presenta un perfil combinado del estrés oxidativo en la diarrea neonatal del ternero y destaca el albúmina modificada por isquemia como posible biomarcador para evaluar la inestabilidad oxidativa. Estos hallazgos apuntan a cambios oxidativos que contribuyen a la aparición de la diarrea neonatal y, por lo tanto, respaldan el uso del albúmina modificada por isquemia como método diagnóstico alternativo para los terneros neonatos.

Palabras clave: Albúmina; diarrea; dlbúmina modificada por isquemia; ternero neonatal; oxidante-antioxidante total.

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INTRODUCTION

The neonatal stage is crucial for the development of calves, spans the first 28 days and is the period during which calves are most vulnerable to morbidity and mortality [1].

Diarrhea is a significant disease affecting 5–23 % of calves (*Bos taurus*) on dairy farms [2, 3, 4, 5]. Both infectious and non-infectious causes can induce neonatal calf diarrhea, which is characterized by excessively watery feces [6, 7, 8].

According to reports, it is the main cause of sickness and death, especially in dairy calves [9, 10, 11]. A study conducted in Türkiye suggests that the financial loss resulting from neonatal calf deaths amounts to approximately 525 million euros annually [12].

When metabolic systems are unable to neutralize or totally remove free radicals and reactive intermediates, oxidative stress develops [13]. To evaluate the body's oxidative stress and associated antioxidant levels, holistic methods have been developed [14, 15]. While total oxidant capacity (TOC), measured using the method formulated by Erel, indicates the level of oxidants in plasma or serum [16], total antioxidant capacity (TAC) reflects the antioxidant load in plasma or serum [14].

Numerous studies recommend calculating the oxidative stress index (OSI) in addition to evaluating TOC and TAC levels [14, 17, 18].

Recognized as a crucial serum protein, albumin also contributes significantly to the detoxification of free radicals [19]. It has been reported to be responsible for at least 70 % of the processes involved in the elimination of free radicals [20].

Under ischemic conditions, reduced blood flow leads to tissue hypoxia and a decrease in pH, resulting in the formation of hydrogen peroxide and superoxide radicals [21]. In turn, these elevated reactive oxygen species linked to ischemia are strongly correlated with ischemia-modified albumin [22].

This study aims to explore the potential of ischemia-modified albumin (IMA) as an additional biomarker that reflects the oxidative stress status in diarrheal newborn calves. To this end, TOC, TAC, OSI, albumin, and IMA levels are being examined in a comparative analysis between neonatal calves with diarrhea and healthy calves.

MATERIALS AND METHODS

Animals and study design

The study population consisted of calves aged 0–28 days (d) that were brought to Kocatepe Animal Hospital with complaints of diarrhea and diagnosed with neonatal diarrhea. Calves exhibiting similar clinical signs were included in the study. Pathogen identification was performed using the Anigen Rapid BoviD-5 Ag Test Kit (Bionote, Inc., Korea) on fecal samples collected from the calves. The rapid test kit detected infections with *E. coli* (7), rotavirus (5), and coronavirus (1), and a total of 13 calves were included in the study. A control group consisting of 6 healthy calves of the same age range and subjected to similar care and feeding protocols was established. Written informed consent was acquired for the calves' owners to participate in the study once they were informed about it.

Sample collection and analysis

Each calf in the experimental and control groups had blood drawn from its jugular vein. Samples were collected into yellow-top serum separator tubes with gel, left at room temperature for approximately 15 minutes (min) before being centrifuged (M4808 P, Elektromag, Türkiye) at 3000 rpm (1613 G Unit) for a duration of 10 min. After that, the sera were put into Eppendorf tubes and kept at -20 °C (UDF 7 SL, Uğur Cooling, Türkiye) until the assays were performed. All parameters were assessed concurrently using the identical serum sample.

The measurement of TAC in serum samples was conducted using the Rel Assay Diagnostics Total Antioxidant Status Assay Kit (Mega Tıp San ve Tic Ltd Sti, Sahinbey/Gaziantep/Türkiye, LOT No: EK21122A). Results were expressed as mmol Trolox Equivalent per liter.

The TOC was assessed using the Rel Assay Diagnostics Total Oxidant Status Assay Kit (Mega Tıp San ve Tic Ltd Sti, Sahinbey/Gaziantep/Türkiye, LOT No: EK21136O). Results were reported as µmol H2O2 Equivalent per liter.

Serum IMA levels were measured using the Rel Assay Diagnostics brand IMA Assay Kit (Mega Tıp San ve Tic Ltd Sti, Sahinbey/Gaziantep/Türkiye, LOT No: TZ20107A). Results were presented in absorbance units (ABSU); 1 ABSU = 100 * ΔOD (Optical Density of Test minus Blank).

Absorbance readings for TAC, TOC, and IMA analyses were taken on a ChemWell 2910 ELISA reader (Awareness Technology, Inc. Martin Hwy. Palm City, USA).

Albumin levels in serum samples were determined using the Roche Cobas Integra 400 Plus, an automated analyzer, with a ROCHE brand kit.

To compute the OSI, which indicates the level of oxidative stress, the TAC measurement was converted from millimol Trolox per liter to micromol per liter. OSI was calculated using the following formula: OSI (Arbitrary Units) = TOC (µmol/L) / TAC (µmol/L) × 100.

Statistical analysis

The statistical assessments were conducted utilizing the MINITAB 18. A significance level of $P < 0.05$ was established for all analyses. Two samples t-test was used to examine the differences between the two groups. Additionally, a pearson correlation analysis was executed to explore the relationships among the parameters in the study group.

RESULTS AND DISCUSSION

The results of the statistical analysis based on the TAC, TOC, OSI, albumin, and IMA data obtained in the study are presented in TABLE I. We found that the study group had higher TAC levels compared to the control group (Study: 0.779 mmol/L; Control: 0.601 mmol/L), but this difference was not statistically significant ($P = 0.227$). Similarly, we observed that TOC levels were also higher in the study group compared to the control group (Study: 18.92 µmol/L; Control: 16.30 µmol/L), however, there was no statistically significant change ($P = 0.473$).

Although the study group's OSI values were higher than those of the control group (Control: 2.606; Study: 3.30), the difference was not statistically significant (P = 0.357). As for albumin levels, a numerical decrease was observed in the study group compared to the control group (Control: 3.117 g/dL; Study: 2.562 g/dL), but this difference was not statistically significant (P = 0.057). In contrast, the study group's IMA values were statistically significantly higher than those of the control group (Control: 50.8; Study: 173.0 ABSU) (P = 0.0001). IMA levels were approximately 3.4 times higher in calves with diarrhea (FIG. 1).

TABLE I

Comparison of Total Antioxidant Capacity, Total Oxidant Capacity, Oxidative Stress Index Albumin and Ischemia-Modified Albumin measurements by groups

Parameters	Group	Mean	SD	P
TAC	Control	0.601	0.189	0.227
	Study	0.779	0.429	
TOC	Control	16.30	7.72	0.473
	Study	18.92	5.10	
OSI	Control	2.606	0.893	0.357
	Study	3.30	2.27	
Albumin	Control	3.117	0.376	0.057
	Study	2.562	0.805	
IMA	Control	50.8	21.3	0.0001*
	Study	173.0	75.9	

*P <.0 05; P: Significance Level; SD: Standard Deviation. TAC: total antioxidant capacity. TOC: total oxidant capacity. OSI: oxidative stress index. IMA: ischemia-modified albumin

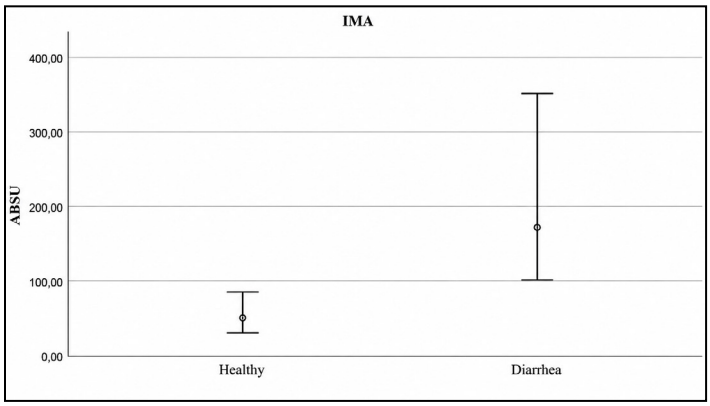

FIGURE 1. Albumin levels altered by ischemia in study groups with neonatal diarrhea and healthy controls. IMA: ischemia-modified albumin. ABSU absorbance units

The correlation matrix illustrating the relationships between TAC, TOC, OSI, albumin, and IMA values from both the study and control groups is detailed in TABLE II. This table's analysis revealed a substantial (P = 0.006) negative correlation between TAC and OSI, a significant (P = 0.012) positive correlation between TOC and OSI, and a significant (P = 0.042) negative connection between albumin and TOC.

TABLE II

Correlation Matrix of Total Antioxidant Capacity, Total Oxidant Capacity, Albumin and Ischemia-Modified Albumin measurements

Parameters		TOC	OSI	Albumin	IMA
TAC	r	-0.036	-0.606	-0.071	0.166
	P	0.883	0.006*	0.772	0.497
TOC	r	-	0.566	-0.470	0.147
	P	-	0.012*	0.042*	0.549
OSI	r		-	-0.199	0.041
	P		-	0.413	0.867
Albumin	r			-0.012	0.194
	P			-	0.962

*P < 0.05; P: Significance Level; r: Correlation Coefficient. TAC: total antioxidant capacity. TOC: total oxidant capacity. IMA: ischemia-modified albumin.

Diseases occurring during the neonatal period in calves and the resulting deaths account for some of the most significant economic losses in the livestock industry [23]. Numerous studies have shown that diarrhea is associated with increased oxidative stress and that this plays a significant role in the pathogenesis of these diseases [24, 25, 26, 27, 28].

Unlike previous studies, this study collectively evaluated IMA, TOC, TAC, and OSI parameters in a group of neonatal calves with diarrhea. Thus, it offers a more comprehensive approach. This combined evaluation provides a new perspective that facilitates a broader understanding of oxidative balance in naturally infected animals. Although a review of the literature identifies studies that test IMA in conjunction with oxidative stress indicators in a variety of disorders, studies evaluating IMA in conjunction with TOC, TAC, and OSI in neonatal calf diarrhea are quite limited. The study adds something special to the body of current literature in this area.

Both TAC and TOC levels showed a numerical rise based on the study's findings. This observed increase is not statistically significant. However, the fact that the numerical increase in TOC levels is higher than that in TAC levels indicates an increase in OSI.

Previous studies have demonstrated a link between neonatal calf diarrhea and oxidative stress [29, 30, 31].

Similarly, in their study, Akyüz and Kükürt [1] found an increase in OSI and detected oxidative damage in calves with diarrhea. OSI is an important parameter used as an indicator of oxidative stress in diseases [32, 33]. Calculated as the TOC/TAC ratio, this value represents the numerical expression of the balance between oxidants and antioxidants [34, 35]. It also provides a more comprehensive assessment compared to individual measurements [16]. In a study by Baltacıoğlu *et al.* [36], it was also suggested that OSI reflects oxidative stress more clearly than other parameters.

In this study, consistent with the literature, we found that the statistically a non-significant increase in the OSI parameter was due to a shift in the ratio of antioxidants to oxidants, and that this was linked to the emergence of oxidative stress in neonatal

calves experiencing diarrhea. A statistically significant negative connection between OSI and TAC was also found in our current investigation.

Additionally, we found a statistically significant positive association between TOC and OSI. These findings, consistent with the literature, support the importance of OSI in the assessment of oxidative stress.

Calf diarrhea is typically accompanied by symptoms such as loss of appetite and a weakened suckling reflex [37]. The resulting nutritional deficiency, when combined with malabsorption, leads to hypoalbuminemia [38]. Another factor contributing to hypoalbuminemia is inflammation [37, 39]. In infected calves, a decrease in albumin synthesis can occur for various reasons [38, 40]. During inflammation, albumin synthesis is suppressed [41].

Serum albumin and total protein levels significantly decreased in a study on E. coli-infected calves with diarrhea [42]. Similarly, studies on animals infected with rotavirus and coronavirus also observed a decrease in albumin levels [43, 44]. Additionally, serum albumin levels may be affected by dehydration caused by diarrhea [45]. Studies have reported that albumin levels in calves experiencing dehydration are higher than those in healthy animals [46, 47].

A considerable tendency toward a drop in albumin levels was noted in this study due to oxidative stress brought on by diarrhea, even if it was not statistically significant. A limitation of this study is that the degree of dehydration was not determined. However, although an increase in albumin levels associated with dehydration has been observed, previous studies have demonstrated that the resulting decrease in albumin levels is related to oxidative stress [48, 49].

Therefore, independent of dehydration, we attributed the drop in serum albumin levels observed in our study to oxidative stress. Furthermore, we found a strong inverse relationship between albumin and TOC. This finding supports the view, consistent with the literature, that a decrease in albumin levels is observed when oxidative stress occurs.

Oxidative stress causes changes in the structure of albumin, leading to the formation of IMA [50]. For this reason, increases in IMA levels are considered a sensitive indicator of oxidative stress [51]. IMA levels were higher in dogs (Canis lupus familiaris) with distemper than in dogs in good condition, according to a study [48]. Another study conducted on neonatal calves also demonstrated elevated IMA levels [31]. Furthermore, it was suggested that IMA is a helpful signal for evaluating oxidative stress in a study where elevated IMA levels were seen in sheep (Ovis aries) infected with Toxoplasma [29].

These works show that animals under oxidative stress had much greater IMA levels, which is in line with earlier findings. According to this research, IMA may be a useful indicator of oxidative stress in neonatal calf diarrhea.

Another innovative aspect of our study is its emphasis on the clinical applicability of IMA in ruminants during the neonatal period. Since IMA tests are rapid, cost-effective, and easily adaptable in laboratories, the current findings suggest that IMA can serve as a practical biomarker for monitoring oxidative stress under field conditions where advanced oxidative stress tests are not available.

CONCLUSION

In this study, we assessed the values of TAC, TOC, OSI, albumin, and IMA in newborn calves with diarrhea. The findings show that oxidative stress is linked to diarrhea in newborn calves, highlighting the superiority of OSI over TOC and TAC alone.

Calves with diarrhea showed a considerable rise in IMA levels, which increased in tandem with the rise in OSI. OSI is a reliable indicator of oxidative stress because of the positive association between OSI and TOC and the negative correlation between OSI and TAC.

Diarrhea-induced oxidative stress is believed to be linked to the decrease in blood albumin levels, even though it was not statistically significant. The interpretation of this result is, however, constrained by the paucity of information on the level of dehydration in the study animals. Still, the findings point to albumin as a potential indicator of oxidative stress.

While previous studies have evaluated isolated oxidative stress markers, our study simultaneously assessed IMA, TOC, TAC, and OSI, thereby providing a more comprehensive profile of oxidative stress in naturally infected newborn calves.

This thorough evaluation offers a fresh diagnostic perspective and reveals IMA can be a rapid and medically helpful biomarker for oxidative stress in neonatal calf diarrhea.

Therefore, this research offers fresh knowledge on the interpretation of oxidative imbalance in neonatal diarrhea and introduces IMA as a prospective indicator in veterinary neonatology.

Ethical approval

The authors confirm that all ethical guidelines of the journal have been followed. This study was conducted with the approval of the Afyon Kocatepe University Veterinary Ethics Committee (AKUHADYEK) (No. 49533702/23).

Author contributions

D.C. Haney conceptualized and designed the study, performed methodology, collected the data, conducted formal analysis, and prepared the original draft. M.A. Erfidan carried out the investigation, provided resources, curated the data, and contributed to writing – review & editing. T. Civelek supervised the study, validated the results, administered the project, and contributed to writing – review & editing. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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