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Effect of probiotic on total antioxidant (TAS) and total oxidant (TOS) in treatment of newborn calf diarrhea

Nazmi Yüksek[1] Ege Çatalkaya[2] Yıldıray Başbuğan[1] Medine Yayan[3]

[1] *Department of Internal Medicine, Faculty of Veterinary Medicine, University of Van Yuzuncu Yil, Van, Turkey*
[2] *Department of Veterinary Pharmacology, Health Science Institute, University of Van Yuzuncu Yil, Van, Turkey*
[3] *Department of Animal Nutrition & Nutritional Diseases, Health Science Institute, University of Van Yuzuncu Yil, Van, Turkey*

Correspondence: *Nazmi Yüksek (nazmiyuksek@hotmail.com)*

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ABSTRACT

Objective: Newborn calf diarrhea causes significant economic losses. Therefore, this study was aimed to determine the effect of adding probiotics on Total Antioxidant (TAS) and Total Oxidant (TOS) levels in routine treatment of calf diarrhea.

Material-Method: The material of the study consisted of 12 calves, routine treatment (Group 1, n=6 calves) and probiotic group. (Group 2; n=6 calves).

Results: TAS values increased statistically after treatment ($P < 0.05$) in group 2 compared to pretreatment. Comparison of the groups after treatment revealed that TAS significantly increased ($P < 0.05$) in group 2. While there was no statistical difference in TOS values between the groups a statistically significant increase ($P < 0.05$) was detected after treatment compared to pretreatment.

Conclusion: It was concluded that adding probiotics may be useful in addition to routine treatment in newborn calf diarrhea.

Keywords: *Antioxidant, Diarrhea, TAS, TOS, Probiotic*

INTRODUCTION

The neonatal period is the most critical period of calf rearing, covering the days between 0 and 28 following birth (Alkan, 1998). This time is the period when the diseases are most common and the mortality rates are the highest, especially in the first 15 days of calf life. Neonatal diseases in calves includes infectious (bacterial, viral, parasitic and mycotic) and noninfectious aetiolody (vitamin, mineral substance and, trace element deficiencies, congenital anomalies, etc.) (Ok et al., 2009; Aslan 1986; Burgu et al., 1986). Diarrhea is the most common problem. Treatment of diarrhea in calves involves; nutrition and prevention of bacteremia / septicemia and, it is important to correct dehydration, acid base and electrolyte balance (Şen et al., 2013). Use of antibiotics may become a public health problem due to development of antibiotic resistance. To reduce the dose and side effects of antibiotic treatment applied in neonatal calf diarrhea and to increase the therapeutic effectiveness probiotic applications are of great importance. Probiotics used in single stomach animals aid in regulation of the normal digestive flora, reducing metabolic disorders thus increase the efficiency in a healthy way (Minssen and Nordberg, 2020).

Probiotics have been started to be used in ruminants (Nageshwar et al., 2016; Valencia et al., 2017). Probiotics protect the host's useful microorganisms (the natural microflora) which can positively effect on the health of the host, help in

food digestion and vitamin production, and inhibit the pathogenic bacteria with substances produced in the intestines (Oelschlaeger, 2011). Application of probiotics in the treatment of diarrhea, shows a progressive effect by strengthening the immune system, and it has also been observed that the gastrointestinal mucosa barrier function, which is impaired in both in vitro and in vivo models, is rearranged by probiotics (Yeoman et al., 2018; Garcia-Lafuente et al., 2001; Madsen et al., 2001). Probiotics, effects on the immune system include, antimicrobial (Lactobacillus acidophilus; Acidophilin, Lactobacillin) secretions, increasing defense release from cryptos in the small intestine, such as preventing the adhesion of pathogens (Salmonella typhimurium) and they also neutralize virulence factors by breaking down toxins (Clostridium difficile toxins). (Solis-Pereira and Lemonnir, 1993). In some studies, the use of probiotics, promotes cytokine production in blood cells and improves the activity of macrophages (Solis-Pereira and Lemonnir, 1993; Shah, 2001). In addition, the use of probiotics in ruminant's benefits from the increase in yield characteristics, it benefits from digestive regulatory effects in condition and rumen (Wallace and Newbold, 1992; Burçak and Yalçın, 2013; Yavuzarslan, 2018; Alıç Ural and Toplu, 2017).

In intestinal cells there are several enzymatic and non-enzymatic antioxidants, including superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) which plays a role in the antioxsidants system (Çadırcı et al., 2007). Reactive Oxygen Species (ROS) that actively contribute to the ongoing pathogenic cascade with oxidative stress increases with the release of different pro-inflammatory mediators and it connects to oxidative stress (Lewis et al., 2008; Moura et al., 2015). In infectious diarrhea intestinal permeability is impaired and Gram (-) bacterial agents cause sepsis which is an important complication and oxidative stress increases in sepsis (Winterbourn et al., 2000; Oldham et al., 2002). Many roles of probiotics in antioxidation have been identified, however, there is no research related to probiotic effects on antioxidant and oxidant system in ruminants and neonatal calves (Shen et al., 2011; Asemi et al., 2013; Kullisaar et al., 2002). Mechanisms of probiotics in antioxidation can be listed as: (1) metal ion chelating ability (Ahire et al., 2013), (2) antioxidant enzymes system capacity (LeBlanc et al., 2008), (3) reduction in antioxidant metabolites (Kullisaar et al., 2002; Endo et al., 2013) and (4) enhanced antioxidant effect by adjusting the microbiota composition (Doron & Gorbach, 2006)

It has been understood that diarrhea and diarrhea complications cause an increase in oxidative stress, and antioxidant capacity in the intestines decreases in conditions and in this regard, no studies have been found on the effect of adding probiotics on TAS and TOS levels in neonatal calf diarrhea. This study was aimed to investigate the effect of probiotic use on the antioxidative mechanism in neonatal calf diarrhea treatments by using a probiotic combination of 2 bacteria (*Lactobacillus spp: L. plantarum, L. casei and Bacillus spp: B. subtilis*) and 1 yeast (*Saccharomyces spp: Saccharomyces cerevisiae*).

MATERIALS and METHODS

This study was carried out with the approval of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (YÜHADYEK-code:03, 28.03.2019). The study included 12 calves with diarrhea from Van and its vicinity. The calves were those brought to the Department of Internal Medicine of the Faculty of Veterinary Medicine and / or private veterinary clinics for clinical examinations with the complaint of diarrhea. Diarrhoeic calves with moderate to severe dehydration, possible metabolic acidosis and hyperkalemia, hypothermia, arrhythmia and tachypnea were included in the study. All calves with diarrhea were divided into 2 groups.

I. Group animals (n=6); 1.3% NaHCO3, + 0.9% NaCl + routine diarrhea treatment

II. Group animals (n=6); 1.3% NaHCO3 + 0.9% NaCl + Oral supplement (Probiotic) + routine diarrhea treatment

Blood samples were taken from the groups before treatment (0 hour) and on the third day after treatment,

Serum TAS was measured using the Commercial Kit (Product Code: RL0017, REL Assay Diagnostic, Mega Tıp, Gaziantep, Turkey) developed by Erel (2004) on Biochrom Anthos Zenyth 200 rt Microplate Reader Device. Results were given as millimolar trolox equivalent per liter (mmol trolox equiv / L).

Serum TOS level was measured on Biochrom Anthos Zenyth 200 rt Microplate Reader using the Commercial Kit (Product Code: RL0017, REL Assay Diagnostic, Mega Tıp, Gaziantep, Turkey) developed by Erel (2005).

Statistical evaluation of data obtained in the research were paired t test to determine the statistical difference between the same parameter before and after treatment, variance analysis (ANOVA) to determine the significance of the difference between different groups before and after treatment and Duncan test to determine the difference between groups. SPSS 20.0 Statistical Package Program (SPSS Inc., Chicago, IL, USA) was used for this purpose. Statistical significance was determined as $p < 0.05$. All data are given as arithmetic mean ± standard error of mean.

RESULTS

Clinically, there was dehydration and hypothermia along with diarrhoe and metabolic acidosis and hyperkalemia in sick animals. There was arrhythmia on the cardiac examination. Tachypnea was detected.

Serum TAS values before treatment in 2 groups significantly increased after treatment ($P < 0.05$). When both groups were compared, a statistically significant increase ($P < 0.05$) was observed in group 2 compared to group 1 after treatment (Table 1). While TOS values insignificantly decreased after treatment in group 2, the value significantly increased ($P < 0.05$) after the treatment in group. TOS values significantly differed between the groups after treatment ($P < 0.05$) (Table 1).

Table 1. TAS and TOS levels (x ± SS) of both groups before and after treatment

Groups	TAS (mmol Trolox Eq/L)		TOS (μmol H_2O_2 Eq/L)	
	Before Treatment	After Treatment	Before Treatment	After Treatment
I. Group	1.326±0.84	1.144±0.73[a]	4.480±1.77*	7.784±1.42*
II. Group	1.116±0.59*	1.588±0.71* [a]	5.492±1.68	4.178±1.89

* :The difference between the pre-treatment and post-treatment of the same group is statistically significant ($p<0.05$).

[a] :The difference between groups is statistically significant ($p<0.05$).

DISCUSSION

The neonatal period covers the days 0 to 28 following birth and is the most critical time of calf breeding where, calf diseases especially are most common. Neonatal calf diseases and deaths cause serious economic losses (Alkan, 1998; Ok et al., 2009; Aslan, 1986). Recently, probiotic applications in addition to routine treatment are gaining importance in diarrhea treatments (Nageshwar et al., 2016; He et al., 2017; Le et al 2017). Diarrhea also triggers many local or systemic pathologies and causes an increase in oxidative stress (Lewis et al., 2008; Moura et al., 2015). Probiotics have been shown to reduce oxidative stress by many mechanisms (Ahire et al., 2013; LeBlanc et al., 2008; Endo et al., 2013; Kullisaar et al., 2002; Çatalkaya, 2020). Although there exist a few studies on the antioxidant and oxidant systems of calves. In a study conducted in healthy calves, TOS value was determined as 10.61 ± 3.82 (μmol H_2O_2 Eq/L) and TAS value was 1.72 ± 0.63 (mmol Trolox Eq/L) (Topraktaş, 2019). In another study, in calves with diarrhea, the value of TAS was 0.51 ± 0.02 (mmolTrolox Eq/L) before treatment and 0.55 ± 0.02 (mmolTrolox Eq/L) after treatment and the TOS value was 13.47 ± 0.81 (μmol), (H_2O_2 Eq / L) before treatment and 11.21 ± 0.26 (μmol H_2O_2 Eq / L) after treatment (Kabu et al., 2015). In a study conducted in calves with septicemia; TAS value was 0.50 ± 0.02 (mmolTrolox Eq/L) before treatment and 0.58 ± 0.03 (mmolTrolox Eq/L) after treatment. The TOS value was 5.30 ± 0.74 (μmol H_2O_2 Eq/L) before treatment and 2.13 ± 0.2 (μmol H_2O_2 Eq/L) after treatment in the study by Erkılıç et al., 2016. In this study, TAS value of 1,116 ± 0,59 (mmol Trolox Eq/L) before treatment increased to 1,588 ± 0,71 (mmol Trolox Eq/L) ($P < 0.05$) after treatment in the group 2 while these values did not markedly differ in group did not receive probiotics. When both groups were compared, a marked increase ($P < 0.05$) was observed in group 2 after treatment. This significant increase in TAS value might indicate that probiotics play a role in the rapid response in antioxidant capacity. Reactive Oxygen Species are 30% higher in calves on the first day of birth compared to their mothers and this is thought to cause increased oxidative stress, which is inevitably present in newborns after the first inhalation of atmospheric oxygen (Gaál et al., 2006). In our study, the value of TOS in Group 1 before treatment was 4,480 ± 1,77 (μmol H_2O_2 Eq / L) and 7,784 ± 1,42 (μmol H_2O_2 Eq / L) after treatment. This finding might suggest that probiotics were more effective along with antibiotic use when compared to the antibiotic use only.

In the group 2, the TOS value before the treatment 5,492 ± 1,68 (μmol H_2O_2 Eq / L) decreased to 4,178 ± 1,89 (μmol H_2O_2 Eq / L) after treatment. Although not statistically significant, it was concluded that this reduction may be related to increased capacity of antioxidant due to probiotic use. In many studies, including this study, differences in TAS and TOS values have been observed and this is thought to be due to measurement methods, kit differences or sensitivity of tests, but studies have reported that the TOS value increases and the TAS value decreases in disease condition (Kızıl et al., 2007; Topraktaş, 2019; Kabu et al., 2015; Erkılıç et al., 2016).

CONCLUSION

As a result, the use of additional probiotics in the treatment helps to reduce oxidative stress, if additional probiotics are not used an increase in oxidant capacity could be noted or might not cause a rapid decrease. Besides the local effects of probiotics on microbiota, they play a role in the antioxidant-oxidant mechanism systemically with symbiotic effects. It has been determined that adding the probiotics to neonatal calf diarrhea treatments protocol might cause an increase in antioxidant capacity. But there is a need for further investigation on the role probiotics in mechanism of antioxidant process.

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Author's Contributions: NY, EÇ and MY designed the study. EÇ and MY collected the materials, NY, EÇ, MY and YB performed the analysis. YB evaluated statistically. NY, EÇ, MY and YB wrote the study

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