



The Effect of Enteritis, Pneumonia and Omphalitis on Oxidative/Antioxidant Balance in the Calves

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| ARTICLE INFO | ABSTRACT |
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| <p><i>Research Article</i></p> <p>Received : 23/02/2019 Accepted : 05/03/2019</p> <p>Keywords: Calves Disease Oxidative stress Antioxidants Nitric oxide</p> | <p>In this study, it was aimed to determine the effects of enteritis, pneumonia and omphalitis on oxidative/antioxidant balance in the calves. In total, 27 calves with neonatal disease and 10 healthy calves (control) were used. The sick calves were divided into 3 groups according to localization of infection, omphalitis (n = 10), pneumonia (n = 8) and enteritis (n = 9). Blood samples of the calves were taken from jugular vein for analysis. Blood in tubes without anticoagulant was centrifuged at 5000 rpm for 5 min following clotting, and the sera were removed and stored at -80°C until analyzed. Serum malondialdehyde and nitric oxide levels of all groups were significantly higher, while glutathione, glutathione peroxidase, catalase and superoxide dismutase levels were significantly lower compared to the control group. As a conclusion, diarrhea, pneumonia and omphalitis are caused by oxidative damage in the calves, and antioxidant treatment may be beneficial in the treatment of these diseases.</p> |

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Introduction

Calf diseases have a major impact on the economic viability due to deaths, treatment costs and long-term effects on performance (Lorenz et al., 2011c). The most important causes of morbidity and mortality in newborns are infections. After birth, calves who do not consume enough colostrum, are more susceptible to diseases. In this situation, which is expressed as passive transfer insufficiency, diseases such as diarrhea, pneumonia and omphalitis are more frequently observed in the calves (Constable et al., 2017). Diarrhea is one of the most important causes of morbidity and mortality in the neonatal period, especially in dairy calves (Lorenz et al., 2011b). It is also pneumonia in the calves that preweaning and/or as soon as possible following the weaning is one of the most important causes of morbidity and mortality. Pneumonia may occur in the neonatal period, especially in the form of aspiration pneumonia, but it is observed in calves for older than 4 weeks of age (Lorenz et al., 2011a). And also, omphalitis is one of the causes of morbidity and mortality in newborn calves, and the absence of umbilical cord disinfection is more common in wet and dirty floor (Bozukluhan et al., 2016; Constable et al., 2017).

A free radical is a chemical compound in the molecular or atomic orbit of a free radical, which contains an unpaired electron, which is usually very reactive. Free radical reactions are necessary for the defense mechanism of immune system cells such as neutrophils and macrophages. However, the production of excess free radicals can lead to tissue damage and cell death. Reactive oxygen species in biological systems are one of the most important causes of oxidative stress such as hydroxyl radical, peroxy radical, superoxide anion, nitric oxide and non-radical hydrogen peroxide (Altan et al., 2006). Oxidative stress is often described as an imbalance between oxidants and antioxidants at the cellular or individual level. Oxidative damage is the result of such imbalance and includes oxidative modification of cellular macromolecules, cell death by apoptosis or necrosis, and also structural tissue damage (Lykkesfeldt and Svendsen, 2007; Perrone et al., 2010). This balance is deteriorating in favor of oxidants in situations such as infection, inflammation, malabsorption, stress, exercise, metabolic and environmental factors that prevent the removal of antioxidants (Adly, 2010; Tabakoğlu and Durgut, 2013).

The aim of this study was to determine the effects of enteritis, pneumonia and omphalitis on oxidative/antioxidant balance in the calves.

Materials and Methods

The material of this study consisted of 27 calves who were brought to the Sivas Cumhuriyet University Faculty of Veterinary Medicine Animal Hospital for examination and treatment, and 10 healthy calves for control without any disease. The sick calves were divided into 3 groups according to the localization of the disease: omphalitis (n = 10), enteritis (n = 9) and pneumonia (n = 8). When the sick calves were separated into groups, care was taken to avoid any concomitant disease. If concomitant disease is present, it was not included in the study. After routine clinical examinations of all the calves were carried out, 8 ml of blood was collected from the jugular vein for analysis. Blood samples in anticoagulant-free tubes were stored in room temperature and allowed to clot, then centrifuged at 5.000 rpm for 5 minutes to remove serum and stored at -80°C until analysis. Serum malondialdehyde [MDA] (Yoshioka et al., 1979), catalase [CAT] (Goth, 1991), glutathione [GSH] (Tietze, 1969), glutathione peroxidase [GSH-Px] (Paglia and Valentine, 1967), nitric oxide [NO] (Miranda et al., 2011), total protein (Lowry et al., 1951) and superoxide dismutase [SOD] (Sun et al., 1988) levels were measured with the Biotek ELISA Reader (Bio TekQuant MQX200 Elisa reader / USA). Permission was obtained from the Sivas Cumhuriyet University Animal Experiments Local Ethics Board for the study.

Statistical Analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed using SPSS software package, version 20.00. Post-hoc Tukey's test was used to compare the biochemical parameters between the groups. P<0.05 values were considered as significant. The results are expressed as mean ± standard error (SE) for each group.

Results

The calves included in the study were 2-60 days old, of different race and gender. Diarrhea continuing 1-5 days, dehydration, decrease in suckling reflex, depression and fatigue were observed in the calves with enteritis. Increased respiratory rate, tracheal tenderness, cough and mucous or mucopurulent nasal discharge, absence of suction reflex, and high fever were observed in the calves with pneumonia. The calves with omphalitis had swelling and tenderness in the umbilical cord, increased regional temperature and weakened suction reflex. The changes in oxidative/antioxidant parameters of sick groups and healthy calves were given in Table 1. Serum MDA and NO levels were significantly higher (P<0.05), while GSH, GSH-Px, catalase and SOD levels were significantly lower (P<0.05) compared to control group of enteritis, omphalitis and pneumonia groups. When the groups were examined, the levels of MDA and NO were higher and the levels of GSH, GSH-Px, catalase and SOD were lower in calves with omphalitis compared to diarrhea and pneumonia groups.

Table 1 Changes in oxidative/antioxidant parameters of sick and healthy calves (Mean± SE)

| Groups | MDA (nmol/L) | GSH (nmol/L) | CAT (U/L) | GSH-Px (U/L) | SOD (U/L) | NO (µmol/L) |
|-------------------|-------------------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| Control (n=10) | 6.43±0.71 ^c | 0.73±0.03 ^a | 34.82±0.94 ^a | 0.23±0.00 ^a | 3.46±0.12 ^a | 27.88±0.11 ^d |
| Enteritis (n=9) | 13.26±0.59 ^b | 0.55±0.01 ^b | 22.89±1.42 ^b | 0.15±0.00 ^b | 1.94±0.11 ^b | 32.15±0.53 ^c |
| Pneumonia (n=8) | 13.93±0.79 ^b | 0.54±0.01 ^b | 14.49±0.71 ^c | 0.14±0.00 ^c | 1.57±0.09 ^{bc} | 35.53±0.54 ^b |
| Omphalitis (n=10) | 17.42±0.20 ^a | 0.49±0.01 ^b | 11.58±0.49 ^c | 0.11±0.00 ^d | 1.29±0.11 ^c | 44.23±0.58 ^a |
| P value | *** | *** | *** | *** | *** | *** |

MDA; Malondialdehyde, NO; nitric oxide, GSH; glutathione, GSH-Px; glutathione peroxidase, CAT; catalase, SOD; superoxide dismutase. ^{a,b,c,d} Values followed by the different letters in the same columns are significantly different (***) P<0.001).

Discussion

The results of the presented study have shown that enteritis, omphalitis and pneumonia cause oxidative damage in the calves. In recent years, substantial evidence has been obtained of oxidative stress in farm animals. Oxidative stress is reported to play an important role in the pathogenesis of many infectious and non-infectious diseases in farm animals (Lykkesfeldt and Svendsen, 2007). Generalized or localized infections in large animal newborns are the most common causes of morbidity and mortality. The prognosis of neonatal infections varies according to the severity and type of the disease (Constable et al., 2017). There is a balance between prooxidants and antioxidants in the body. This balance is degraded in favor of prooxidants under the influence of various factors, resulting in lipid peroxidation in the organism (Perrone et al., 2010). As a result of lipid peroxidation and formation of reactive oxygen species, cellular damage occurs in the

organism. If antioxidant systems are not sufficient to counteract the oxidative stresses, oxidative damage occurs in the cells and the functions of the cells are significantly degraded (Tabakoğlu and Durgut, 2013). MDA is one of the most important indicators of lipid peroxidation. MDA is widely used to assess increased oxidant activity in oxidative stress conditions (Adly, 2010). Diseases such as coccidiosis (Yılmaz et al., 2014), foot and mouth disease (Khoshvaghti et al., 2014; Nath et al., 2014), pneumonia (Özçelik et al., 2014), arthritis (Yurdakul et al., 2013), omphalitis (Bozukluhan et al., 2016), dermatophytosis (Karapehlivan et al., 2007) and babesiosis (Salem et al., 2016) in cattle have been reported to increase MDA levels. In this study, MDA level was significantly higher in all sick groups than control group (P<0.05). It was observed that the highest group of MDA level was omphalitis and at the same time it was significantly higher than the group of

enteritis and pneumonia. This difference in MDA level may be related to the severity and duration of the disease. The increase in MDA levels suggests that enteritis, pneumonia and omphalitis in the calves cause lipid peroxidation.

Free nitrogen derivatives from free radicals are NO and peroxynitrite (Tabakoğlu and Durgut, 2013). In many physiological and pathological processes, nitric oxide acts as a biologically active molecule with different effects (Hofseth et al., 2003). Nitric oxide is a cytotoxic factor produced by the NO synthase and L-arginine terminal guanidine nitrogen atom and released by various cells (Groves and Wang, 2000). It plays a role in the primary defense against some pathogens (James, 1995; Akaike et al., 1996). However, it has also been reported that it is immunosuppressive (Rockett et al., 1994). Serum NO levels were significantly higher in reticuloperitonitis traumatica (Atakisi et al., 2010), malignant catarrhal fever (Erkılıç et al., 2017), brucellosis (Nisbet et al., 2007) and foot and mouth disease (Bozukluhan et al., 2013) in cattle and this increase was associated with an increase in endogenous NO release by stimulation of the immune system. Yurdakul and Sarıtaş (2013) found that the NO levels in synovial fluid of 20 calves with arthritis were significantly increased compared to the control group. However, in the same study, serum NO levels were not statistically different from the control group (Yurdakul and Sarıtaş, 2013). In this study, NO levels were found to be statistically significant ($P < 0.05$) higher in pneumonia, enteritis and omphalitis groups compared to the control group. According to the control group, it was determined that the group with the highest NO level in the calves was omphalitis, followed by pneumonia and enteritis, respectively. This increase has shown that NO may play an active role in body defense of the sick calves.

Defense mechanisms known as antioxidant defense systems have been developed in the body to prevent the formation of reactive oxygen species and the damage caused by reactive oxygen species. Antioxidant molecules are endogenous and exogenous, and the damage caused by oxidant molecules is rendered ineffective by both intracellular and extracellular defense. Extracellular defense consists of various molecules such as albumin, bilirubin, transferrin, ceruloplasmin and uric acid. The main antioxidant defense is provided from intracellular free radical scavenging enzymes. These enzymes are; glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase, catalase and cytochrome oxidase. Minerals such as copper, zinc and selenium are necessary for the function of these enzymes (Halliwell, 1995; Altan et al., 2006). Excessive production of reactive oxygen species and inadequacy of the antioxidant system cause harmful effects by disrupting the structure of proteins, lipids, carbohydrates, nucleic acids and useful enzymes (Cross et al., 1987; Southorn, 1988). GSH-Px and CAT are known as primary antioxidant enzymes in the protection mechanism against lipid peroxidation. GSH-Px provides reduction of hydrogen peroxide and lipid hydroperoxides. Since both enzymes play an important role in cellular protection in the body, changes in GSH-Px and CAT activities have been reported to occur in oxidative stress-related events (Gutteridge, 1995; Tabakoğlu and Durgut, 2013). SOD is an antioxidant

enzyme known for its ability to excrete toxins. Changes in SOD, CAT and GSH-Px activities are considered indicative of oxidative stress (Adly, 2010; Tabakoğlu and Durgut, 2013; Salem et al., 2016). GSH, a non-enzymatic antioxidant; it is important to protect the body against the oxidative stress by having an important role in the inactivation of free radicals and the level decreases during oxidative stress (Mates, 2000; Tabakoğlu and Durgut, 2013). Özçelik et al. (2014), reported that catalase and GSH-Px levels significantly decreased in cattle with enzootic pneumonia, whereas MDA levels increased significantly and oxidative damage developed. Nath et al., (2014), reported that GSH-Px levels fell in cattle with foot and mouth disease, and that this decrease was due to GSH-Px consumption in order to protect the resulting severe tissue damage resulting from increased production of oxidants in the animals. It has been reported that serum GSH levels in cattle with coryza gangrenosa bovum (Erkılıç et al., 2017) and calves with arthritis (Yurdakul and Sarıtaş, 2013) significantly decreased compared to the control group, while MDA levels also significantly increased. Salem et al. (2016), reported significant reductions in SOD and GSH-Px levels compared to the control group in calves with babesiosis. Bozukluhan et al., (2016), reported that GSH levels were lower than those of the control group in calves with omphalitis, whereas MDA and NO levels significantly increased and lipid peroxidation developed in calves with omphalitis. In this study, GSH, GSH-Px, SOD and catalase levels were significantly decreased in the enteritis, pneumonia and omphalitis groups compared to the control group. This decrease in the level of enzymatic antioxidants is probably due to increased lipid peroxidation.

Conclusions

In conclusion, it has been found that the balance between oxidants and antioxidants in calves with enteritis, pneumonia and omphalitis is deteriorated in favor of oxidants and that the antioxidant enzyme levels are insufficient and oxidative stress develops with the increase of free radicals. Therefore, it was concluded that administration of antioxidants could benefit the treatment of these diseases.

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