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## Oxidative Stress and Acute-Phase Response Status During Treatment in Premature Calves with Respiratory Distress Syndrome

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**ABSTRACT:** This study aimed to determine lipid peroxide (LPO), antioxidant capacity (AOC), and acute-phase protein changes before and after different nebulization treatments in premature calves with respiratory distress syndrome (RDS).

Thirty-six premature calves were divided into equal number of 6 groups. Group 1 was labelled as negative control and received standard treatment. Group 2 was labelled as positive control; Groups 3, 4, 5, and 6 were labelled as trial groups. These groups received nebulizer treatment. Nebulizer drug combinations were as follows: Group 2 (fluticasone), Group 3 (formoterol + fluticasone), Group 4 (ipratropium bromide + fluticasone), Group 5 (fluticasone + furosemide) and Group 6 (formoterol + ipratropium bromide + furosemide + fluticasone). Venous blood was taken from all calves before (0 hour) and after treatment (24th, 48th, 72nd hour). It was concluded that total LPO levels gradually decreased while AOC levels increased during treatment but there was no difference in the serum amyloid A (SAA) and fibrinogen levels within groups.

In conclusion, supportive and nebulizer treatments to improve function of lungs were demonstrated to alleviate oxidative stress. However, in order to reveal the effects of local nebulizer applications on oxidative stress, further studies are required to investigate oxidation parameters in the bronchoalveolar fluid.

**Keywords:** respiratory distress syndrome, premature calf, oxidative stress markers, acute-phase response

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## INTRODUCTION

As a widespread complication in the world and Turkey, premature calf birth is among the leading causes of mortality (Guzelbektes et al., 2012; Aydogdu et al., 2016; Yildiz and Ok, 2017; Yildiz et al., 2019; Ok et al., 2020). The majority of perinatal calf deaths are related to premature birth which causes failing to complete organ development (Meyer et al., 2001; Johanson and Berger, 2003). Premature calves are born without completing the ordinary developmental process, thus they may have substantial problems in circulation, nervous system, metabolism, and especially respiration (Yildiz and Ok, 2017; Yildiz et al., 2019; Ok et al., 2020). The most critical problem in these cases is respiratory distress syndrome, which is caused by the insufficient development of the lungs in the absence of surfactant. In premature calves with RDS, the lungs collapse with the development of hypoxia, interstitial inflammation, pulmonary hypertension due to excessive strain, and interstitial edema as a result of impaired air exchange due to decreased pulmonary compliance and surfactant deficiency. Death occurs in a short period if the cases are left untreated (Avery and Merrit, 1991). In most premature calves with RDS (Yildiz and Ok, 2017), respiratory acidosis (Ok et al., 2020), hypercapnia (Sustronck et al., 1996; Konduri and Kim, 2009), and hypoxemia (Yildiz and Ok, 2017, Yildiz et al., 2019) develop. Respiratory difficulties also lead to significant hypoxia in tissues. This increases the production of oxidative stress factors and causes a decrease in antioxidant capacity (Mutinati et al., 2014).

Hypoxia associated with lung diseases increases oxidative stress products in tissues and massive amounts of free radicals are also formed in the organism. Lipids are the most sensitive biomolecules to the free radical effects. When the radicals develop at rates exceeding antioxidant capacity, the unsaturated bonds of cholesterol and fatty acids in the cellular membranes react easily with free radicals to produce peroxidation products (Gutteridge, 1995; Mutinati et al., 2014). Therefore, lipid peroxidation is the basis of cellular damage and can be used as an indicator of oxidative stress in tissues and cells (Lichtenstern et al., 2011, Maden et al., 2012). Total LPO measurement determines all peroxide products in the organism. Antioxidant capacity (AOC) or total antioxidant protection AOP measurement carries out a crucial role in biological events. The presence of antioxidants is effective in preventing the harmful effects of free radicals. Antioxidants include superoxide dismutase, cat-

alase, glutathione peroxidase enzymes and albumin, ferritin, ceruloplasmin, ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, and uric acid (Gutteridge, 1995, Halliwell, 1997; Carole et al., 2007; Ighodaro and Akinloye, 2018; Gulcin et al., 2018; Gulcin, 2020). Oxidative stress is the cause of impaired balance between oxidants and antioxidants in cells in favor of oxidants. In case of oxidative damage in the cell, similar to the tissues, damage or death occurs (Grobber et al., 1997; Lykkesfeldt ve Svendsen, 2007). In hypoxia-causing diseases (lung diseases), a significant amount of oxidative stress occurs and antioxidant levels decrease (Deaton, 2006). Respiratory distress syndrome is the most important cause of mortality in preterm children. As a result of surfactant insufficiency, vascular permeability due to pulmonary hypertension increases causing pulmonary edema and ischemic damage (Avery and Merrit, 1991). While the antioxidant levels decrease in hypoxia-related ischemic damage, an increase in free radical LPO levels occurs (Auten and Davis, 2009; Kim et al., 2012). In newborns and premature, free radicals are reported to bear a very high risk of creating oxidative damage and oxidative stress (Bounocore et al., 2002; Mutinati et al., 2014).

Inflammation and edema develop in the interstitial tissue due to forced functioning of the lungs and hypertension in the pulmonary vessels as a result of the surfactant insufficiency. Moreover, due to the difficulties in air exchange, breathing difficulties and hypoxia occur in tissues. Acute-phase protein profile can be changed as a consequence of inflammation, edema, and hypoxia in the lung tissue. The most important positive acute-phase proteins in ruminants are fibrinogen, haptoglobin (Hp), serum amyloid A (SAA), alpha-1 acid glycoprotein, and ceruloplasmin (Murata et al., 2004; Eckersall and Bell, 2010, Yun et al., 2014). The number of acute-phase proteins increases in inflammation, edema, hypoxia, bacterial infection, or trauma and may also increase in transplantation and other stress situations (Murata et al., 2004; Lomborg et al., 2008). Serum fibrinogen, serum amyloid A and haptoglobin levels increases significantly during the inflammation process (Martinez-Subiela et al., 2004; McGrotty et al., 2005). Acute-phase proteins are a group of glycoproteins produced primarily in the liver by leukocytes and macrophages in response to inflammatory mediators during (AOP) tissue damage. Acute-phase proteins carry out many roles in free oxygen radicals, immunoglobulin production, and tissue repairment (Martinez-Subiela et al., 2004).

This study aimed to determine changes in LPO, AOC, and acute-phase proteins before and after different nebulizer treatments in premature calves with RDS.

## MATERIALS AND METHODS

### Materials

In this study, only premature calves of spontaneous birth that fulfilled the premature criteria and at least three criteria of respiratory distress syndrome were included. The criteria for premature and respiratory distress syndrome were determined according to the studies of Yildiz and Ok (2017) and Ok et al. (2020). All the calves were admitted from those referred to the clinic of the Department of Internal Medicine at Selcuk University, Faculty of Veterinary Medicine. Study was approved (2014/05) by the Ethics Board (SUVFEK) of Selcuk University Faculty of Veterinary Medicine.

### Animals

The material of the study included 36 spontaneously born premature calves (27 Holsteins, 5 Simmentals, and 4 Montafon breeds) with respiratory distress syndromes. The calves were evenly randomly divided into 6 groups: Group 1 as a negative control, Group 2 as a positive control, Groups 3, 4, 5, and 6 as experimental groups.

### Clinical Examinations and Diagnosis

The first clinical evaluation was carried out to diagnose the premature status of the calves referred to the clinic. History of the calves and premature criteria determined in the previous related studies were taken into consideration in the evaluation of premature status. Calves that met premature criteria were evaluated whether they fulfilled the criteria of respiratory distress syndrome. As a result of these evaluations, 36 calves, which fulfilled the criteria of respiratory distress syndrome, from 43 premature calves were included in the study.

### Collection and Storage of Blood Samples

Venous blood samples with and without anticoagulants were collected for biochemical measurement from calves in all groups before (0 hour) and after treatment (24th, 48th, 72nd hour) from Vena Jugularis according to the study of Ok et al. (2020). Blood samples were centrifuged at 5000 rpm, plasma and serum samples were collected. Plasma and sera were kept at -80 °C until further analysis. Venous blood SatO<sub>2</sub>

were analysed within 15 min at 0, 24, 48 and 72 h using a blood gas analyser (GEM Premier 3000, Instrumentation Laboratory, Lexington).

## Methods

### The Treatment Protocols

Premature calves with respiratory distress syndrome were evenly and randomly divided into 6 groups and the treatment protocols below were applied.

1. Group negative control (G1): Standard treatment protocol
2. Group positive control (G2): Standard treatment protocol + fluticasone
3. Group 3 (G3): Standard treatment protocol + fluticasone + formoterol
4. Group 4 (G4): Standard treatment protocol + fluticasone + ipratropium bromide
5. Group 5 (G5): Standard treatment protocol + fluticasone + furosemide
6. Group 6 (G6): Standard treatment protocol + fluticasone + formoterol + ipratropium bromide + furosemid

### Standard Treatment Protocol for Premature Calves

Standard treatment including oxygen supplementation and supportive treatment were provided to each calf in all the groups.

### Oxygen Therapy

All the premature calves received oxygen therapy via a mask (Figure 2) at the rate of 5 - 6 L / min per calf. The flow rate of oxygen was decreased to 3 to 4 L / min after 3h. Oxygen supplementation was discontinued when SatO<sub>2</sub> was > 80% after 24 hours of intranasal oxygen administration and if SatO<sub>2</sub> was < 80%, oxygen supplementation was reinstated.

### Supportive Treatment and Clinical Care

Vitamin A - D - E combination (1 ml / day / IM - Ademin, Ceva Animal Health Limited), calcium (0.2ml / kg / day / SC - Kalsimin, Vilsan Veterinary Pharmaceuticals), phosphorus (3 ml / day / SC, Catosal, Bayer Animal Health), erythromycin (10 mg / kg / day / IM - Erivet, Mira Ilac ve Yem Katki Mad. Gida San. Tic. Ltd. Sti.), vitamin C (3 ml / day / SC

- Vitce, Sanovel Pharmaceuticals) were administered every day for 3 days, along with a single dose of selenium - vitamin E combination (1 ml / IM, Yeldif, Ceva Animal Health Limited) was administered to the each calf. Further, a single dose of hyperimmune serum (15 ml / SC, Septicol, Vetall Animal Health, containing Escherichia Coli serotypes K99, F41 and F (Y) piluses.) was administered to each premature calf. Intravenous fluids (0.9% NaCl and 5% dextrose) were administered slowly as required.

### Nebulizer Treatment Protocol for Premature Calves

While calves in the experimental group received nebulizer drugs for 5 min (Figure 3), calves in the control group received only 2.5 mL saline solution (12h, during 72h) for 5 min via a nebulizer machine. The nebulizer drug combinations were administered as follows: fluticasone for Group 2 (G2), fluticasone + formoterol for Group 3 (G3), fluticasone + ipratropium bromide for Group 4 (G4), fluticasone + furosemide for Group 5 (G5) and fluticasone + formoterol + ipratropium bromide + furosemide for Group 6 (G6). Formoterol (15 µg totally / 12h, during 72h, Ventofor, Bilim Pharmaceuticals), ipratropium bromide (2 µg / kg / 12hr, during 72h, Atrovent, Boehringer Ingelheim International), furosemide (1 mg / kg / 12h, during 72h, Lasix, Sanofi Pharmaceuticals) and fluticasone (15 µg / kg / 12h, during 72h, Flixotide, GlaxoSmith-Kline Pharmaceuticals) each diluted with 2.5 mL saline solution, was administered for 5 min using a nebulizer machine sequentially at regular intervals during the study period (72h). The 0h represents the time just before the initiation of the treatment and immediately after the collection of blood sample the nebulized drugs were administered over a course of 5-20 min depending on the drug combination used.

### Measurement of Total Lipid Peroxide

Lipid peroxidation products were determined from plasma by a spectrophotometric method with commercial LPO-586™ (Oxis Research, TM, Bioxytech, CA, 92202, USA).

*Principle:* The analysis is based on the reaction of chromogen substance N-methyl-2-phenylindole at 45 °C with MDA and 4-hydroxialkenal (LPO). One mole MDA or 4-hydroxialkenal reacts with 2 molecules of N-methyl-2-fenilindol in acetonitrile, resulting in a durable chromophore at 586 nm. The results were defined as µMol.

### Measurement of Total Antioxidant Capacity

Total AOC was determined from plasma by spectrophotometric method with antioxidant capacity kit (Antioxidant Assay Kit, Sigma Aldrich CS O790, Germany).

*Principle:* The reaction is based on the formation of ferrilmyoglobin, which is formed by hydrogen peroxide and myoglobin and constitutes radical ABTS<sup>+</sup> cation from ABTS (2,2'-azinobis 3 ethylbenzthiazoline-6-sulfonic acid). Radical ABTS<sup>+</sup> cation is a soluble green chromogen that can be determined at 405 nm. In the presence of antioxidants, radical ABTS<sup>+</sup> cation formation is inhibited and color density decreases. As standard, a vitamin E analog, trolox, was used. The results were defined as mMol.

### Acute-Phase Protein Measurement

Bovine serum Amyloid A (Cat. No: 201-04-0126) and Bovine fibrinogen (Cat. No: 201-04-0086) levels were measured on Synergy HT multi-mode microplate reader (BioTek Inc. USA) with commercial ELISA kit (SunRed®, China) method. The measurable sensitivity and test range of the SAA test was 0.15 µg/ml to 40 µg/ml and the measurable sensitivity and test range of the fibrinogen test was 0.2 mg/ml to 60 mg/ml.

### Statistical Analysis

All data were presented as mean and standard errors (SEs) values (mean ± SEM). The distribution of the data was determined using the Kolmogorov-Smirnov test. One way Anova (Posthoc Duncan) was used for statistical analyses of the parameters of within groups by using the SPSS 21.0 software (USA). Values that differed significantly from the control were indicated as P < 0.05.

## RESULTS

### Lipid Peroxide

Averages and within group statistical significance values of the LPO levels of calves in all groups are presented in Table 1.

Statistically significant decreases were detected in all groups at 24th, 48th, and 72nd hours compared to 0-hour sampling time in all groups (Table 1).

### Antioxidant Capacity

Averages and within group statistical significance values of the AOC levels of calves in all groups are

presented in Table 2.

Statistically significant increases were detected in all groups at 24th, 48th, and 72nd hours compared to 0-hour sampling time in all groups (Table 2).

### Acute-Phase Proteins

Serum amyloid A and fibrinogen levels measured in calves of all groups at all time points and their within group statistical significance values are presented in Table 3. There was no statistical difference in the SAA and fibrinogen parameters of the calves

in all groups after the treatments compared to 0-hour sampling time (Table 3).

### SatO<sub>2</sub> Percentages of Venous Blood Samples

Averages and within group statistical significance values of the SatO<sub>2</sub> percentages of venous blood samples of calves in all groups are presented in Table 4. There was no statistical difference within G1 and G6. However, statistically significant increases were detected within G2 at 24. and 48<sup>th</sup>. hour, G3, G4 and G5 at 72<sup>nd</sup>. hour after the treatments compared to 0-hour sampling time within the groups (Table 4).

**Table 1.** Lipid peroxide product levels, standard errors and statistical significance between the groups of the premature calves (Mean  $\pm$  SE).

LPO ( $\mu$ Mol / L)	0h	24h	48h	72h
G1	0.89 $\pm$ 0.15 <sup>a</sup>	0.51 $\pm$ 0.12 <sup>ab</sup>	0.42 $\pm$ 0.10 <sup>b</sup>	0.30 $\pm$ 0.06 <sup>b</sup>
G2	0.53 $\pm$ 0.06 <sup>a</sup>	0.37 $\pm$ 0.08 <sup>ab</sup>	0.43 $\pm$ 0.09 <sup>ab</sup>	0.21 $\pm$ 0.01 <sup>b</sup>
G3	1.04 $\pm$ 0.14 <sup>a</sup>	0.57 $\pm$ 0.09 <sup>b</sup>	0.50 $\pm$ 0.07 <sup>b</sup>	0.46 $\pm$ 0.06 <sup>b</sup>
G4	0.87 $\pm$ 0.12 <sup>a</sup>	0.78 $\pm$ 0.17 <sup>ab</sup>	0.47 $\pm$ 0.05 <sup>ab</sup>	0.43 $\pm$ 0.04 <sup>b</sup>
G5	1.27 $\pm$ 0.21 <sup>a</sup>	0.73 $\pm$ 0.10 <sup>b</sup>	0.69 $\pm$ 0.17 <sup>b</sup>	0.57 $\pm$ 0.06 <sup>b</sup>
G6	1.02 $\pm$ 0.20 <sup>a</sup>	0.61 $\pm$ 0.13 <sup>ab</sup>	0.47 $\pm$ 0.03 <sup>b</sup>	0.41 $\pm$ 0.02 <sup>b</sup>

<sup>a, b, c</sup>: Means in the same row with different letters differed significantly ( $P \leq 0.05$ ). Group negative control (G1). Group positive control (G2). Group 3 (G3). Group 4 (G4). Group 5 (G5). Group 6 (G6).

**Table 2.** Averages of total antioxidant capacity levels, standard errors and statistical significance between the groups of the premature calves (Mean  $\pm$  SE).

AOC (mMol / L)	0h	24h	48h	72h
G1	0.54 $\pm$ 0.02 <sup>b</sup>	0.59 $\pm$ 0.03 <sup>ab</sup>	0.65 $\pm$ 0.01 <sup>a</sup>	0.64 $\pm$ 0.02 <sup>a</sup>
G2	0.51 $\pm$ 0.04 <sup>b</sup>	0.57 $\pm$ 0.01 <sup>ab</sup>	0.62 $\pm$ 0.02 <sup>a</sup>	0.61 $\pm$ 0.02 <sup>a</sup>
G3	0.48 $\pm$ 0.02 <sup>b</sup>	0.55 $\pm$ 0.03 <sup>ab</sup>	0.55 $\pm$ 0.03 <sup>ab</sup>	0.58 $\pm$ 0.02 <sup>a</sup>
G4	0.58 $\pm$ 0.02 <sup>b</sup>	0.66 $\pm$ 0.01 <sup>a</sup>	0.73 $\pm$ 0.04 <sup>a</sup>	0.71 $\pm$ 0.03 <sup>a</sup>
G5	0.64 $\pm$ 0.03 <sup>b</sup>	0.71 $\pm$ 0.03 <sup>ab</sup>	0.74 $\pm$ 0.03 <sup>a</sup>	0.71 $\pm$ 0.02 <sup>ab</sup>
G6	0.54 $\pm$ 0.05 <sup>b</sup>	0.64 $\pm$ 0.02 <sup>a</sup>	0.71 $\pm$ 0.01 <sup>a</sup>	0.67 $\pm$ 0.02 <sup>a</sup>

<sup>a, b, c</sup>: Means in the same row with different letters differed significantly ( $P \leq 0.05$ ).

**Table 3.** Serum amyloid A and fibrinogen levels, standard errors and statistical significance between the groups of the premature calves (Mean  $\pm$  Statistics kısmında SEs yazılmıştır).

Parametreler		0h	24h	48h	72h
SAA ( $\mu$ g / ml)	G1	0.40 $\pm$ 0.03	0.50 $\pm$ 0.06	0.32 $\pm$ 0.07	0.50 $\pm$ 0.04
	G2	0.43 $\pm$ 0.03	0.49 $\pm$ 0.02	0.46 $\pm$ 0.02	0.47 $\pm$ 0.02
	G3	0.50 $\pm$ 0.14	0.47 $\pm$ 0.06	0.45 $\pm$ 0.06	0.50 $\pm$ 0.14
	G4	0.55 $\pm$ 0.03	0.60 $\pm$ 0.08	0.54 $\pm$ 0.06	0.55 $\pm$ 0.07
	G5	0.50 $\pm$ 0.10	0.62 $\pm$ 0.04	0.64 $\pm$ 0.06	0.58 $\pm$ 0.04
	G6	0.42 $\pm$ 0.11	0.48 $\pm$ 0.13	0.50 $\pm$ 0.12	0.51 $\pm$ 0.12
Fibrinojen (mg / ml)	G1	0.35 $\pm$ 0.01	0.39 $\pm$ 0.05	0.42 $\pm$ 0.04	0.42 $\pm$ 0.03
	G2	0.39 $\pm$ 0.02	0.41 $\pm$ 0.01	0.40 $\pm$ 0.01	0.39 $\pm$ 0.01
	G3	0.36 $\pm$ 0.20	0.37 $\pm$ 0.16	0.41 $\pm$ 0.03	0.44 $\pm$ 0.13
	G4	0.33 $\pm$ 0.11	0.41 $\pm$ 0.14	0.43 $\pm$ 0.05	0.35 $\pm$ 0.16
	G5	0.43 $\pm$ 0.03	0.53 $\pm$ 0.05	0.51 $\pm$ 0.05	0.48 $\pm$ 0.03
	G6	0.42 $\pm$ 0.02	0.42 $\pm$ 0.07	0.47 $\pm$ 0.02	0.43 $\pm$ 0.01

\* No statistical difference within the groups.

**Table 4.** SatO<sub>2</sub> percentages of venous blood samples, standard errors and statistical significance between the groups of the premature calves (Mean ± SE).

SatO <sub>2</sub> %	0h	24h	48h	72h
G1	15,67 ± 3,48	28,00 ± 6,86	21,00 ± 4,49	29,17 ± 5,33
G2	22,00 ± 3,08 <sup>b</sup>	41,50 ± 4,98 <sup>a</sup>	40,00 ± 6,07 <sup>a</sup>	35,00 ± 4,29 <sup>ab</sup>
G3	25,67 ± 2,95 <sup>b</sup>	37,83 ± 4,28 <sup>ab</sup>	36,67 ± 5,68 <sup>ab</sup>	41,17 ± 5,19 <sup>a</sup>
G4	26,00 ± 4,96 <sup>b</sup>	37,83 ± 3,69 <sup>ab</sup>	38,60 ± 4,4 <sup>ab</sup>	44,20 ± 6,95 <sup>a</sup>
G5	15,33 ± 6,26 <sup>b</sup>	19,50 ± 3,15 <sup>ab</sup>	26,33 ± 2,22 <sup>ab</sup>	32,83 ± 6,61 <sup>a</sup>
G6	20,50 ± 4,46	28,67 ± 6,15	27,20 ± 4,29	31,00 ± 7,82

<sup>a, b, c</sup>: Means in the same row with different letters differed significantly ( $P \leq 0.05$ ).

## DISCUSSION

In this study, changes in LPO, AOC, and acute-phase protein levels were evaluated before and after treatment in premature calves with respiratory distress syndrome (Table 1, 2, 3). Following the treatment of premature calves with RDS, the total LPO levels decreased and antioxidant levels increased gradually at 24th, 48th, and 72nd hours. A decrease in LPO levels and increase in AOC levels following the treatments (Tables 1 and 2) were probably due to improved lung function, normal gas exchange, and elimination of hypoxia in the organism. These results were in accordance with the results of Ballard et al. (2008). Premature infants are extremely susceptible to oxidative stress since they have both increased reactive oxygen species (ROS) formation and underdeveloped antioxidant defenses (Ahola et al., 2004). In this study, it was realized that premature calves were as highly susceptible to oxidative stress as premature infants (Table 1, 2). Increased need for oxygen due to immature lungs and infections is thought to contribute to increased oxygen radical formation (Moison et al., 1996; Laborie et al., 2000; Hirano et al., 2001; Denery, 2004; Kapoor et al., 2006; von Dessauer et al., 2010). Oxidative stress levels increase in premature infants in the first day and following weeks after birth (Buonocore et al. 2002; Perone et al., 2007).

While ROS formation increases, the antioxidant defense is stated to be not fully mature or present at birth in premature infants (Frank, 1992; Buonocore et al. 2002; Georgeson et al., 2002; Ahola et al., 2004; Davis and Auten, 2010). It has been reported that antioxidant consumption increases after birth and therefore AOC decreases in premature infants (Buhimschi, 2003). It has also been shown that the concentration of glutathione (GSH) in premature infants declines rapidly in the early days of life (Jain et al., 1995; Jean-Baptiste and Rudolph, 2003; Ahola et al., 2004]. This is considered to be most likely due to increased GSH consumption associated with oxida-

tive stress. Glutathione is one of the most important non-enzymatic intra-cell antioxidants. Glutathione in the epithelial layer fluid (ELF) of the lower respiratory tract is thought to be the first line of defense against oxidative stress (Lomaestro and Malone, 1995). The ELF concentration of GSH is 140 times higher than the serum concentration with a redox ratio of > 9:1 (Cantin et al., 1987). In this study, the decrease in total LPO levels and the increase in AOC levels during the treatment of calves in both standard treatment and nebulizer treatment group (Table 1, 2) and no differences in serum total LPO levels and AOC levels in the different treatment groups may be due to the nebulizer applications locally in the lung (Yildiz and Ok, 2017; Ok et al., 2020). The most satisfactory explanation of this condition can be the presence of glutathione (Cantin et al., 1987), which is found to be 140 times more in the lungs than in the serum. In the present study, bronchoalveolar fluid sampling was not possible because premature calves had severe RDS symptoms which could have risked different complications. Therefore, local effects of different nebulizer treatment applications on oxidation parameters could not be evaluated, which was considered to be the limitation of this research.

Hypertension in the pulmonary vessels and similar complications caused by surfactant insufficiency lead to the development of inflammation and edema in the interstitial tissues. As a result of inflammation, edema, and hypoxia in the lung tissue, changes in acute-phase proteins, cytokines, and coagulation profiles can occur (Murata et al., 2004; Eckersall and Bell, 2010). Acute-phase proteins may increase tissue damages caused by inflammation, edema, hypoxia, bacterial infection or trauma, and following transplantations and other stress situations (Murata et al., 2004; Lomborg et al., 2008). Significant changes in serum fibrinogen, serum amyloid A and haptoglobin levels have been reported in inflammations (Martinez-Subiela et al., 2003; McGrotty et al., 2005). In this study, SAA and

fibrinogen parameters were not statistically different at 24th, 48th, and 72nd hours compared to 0 hours in all groups. No changes in the acute-phase protein levels in the present study could be due to the fact that the treatments did not cause a significant change in serum acute-phase protein levels.

In conclusion, supportive and nebulizer treatment protocols were demonstrated to decrease the total lipid peroxide levels and improve the antioxidant capacity in premature calves with RDS. However, further researches are needed to evaluate the local effects of

these treatments on lung tissue and also to compare these effects in calves born at term without any complications.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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