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Oxidative stress in calves with enzootic pneumonia

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Abstract: This study was performed to evaluate the oxidative stress in calves with enzootic pneumonia. Ninety calves that were 2–6 months old were used in this study. Ten of these calves were healthy and used as the control group, while 80 were diagnosed with enzootic pneumonia according to the clinical examinations. The biochemical results showed that total oxidant status (TOS), nitric oxide (NO), malondialdehyde (MDA), and sialic acid (SA) levels were higher in calves with enzootic pneumonia than in the control group. While there was a statistically significant increase in TOS, MDA, and SA concentrations, there was not any statistically significant change at NO levels. Furthermore, a statistically significant decrease was observed in total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) concentrations. Oxidative stress index (OSI) was calculated after TOS and TAS concentrations were obtained and it was detected to be high, but not statistically significant. In conclusion, according to these results, severe oxidative stress was detected in calves with enzootic pneumonia. These results reveal that oxidative stress may have a role and contribution in the occurrence of the disease and the obtained results might contribute to further studies. Besides, it is concluded that antioxidant supplements would be beneficial for supporting the routine treatment of the disease.

Key words: Antioxidant, calf, enzootic pneumonia, oxidant, oxidative stress

1. Introduction

One of the most significant worldwide problems in the cattle industry is respiratory system diseases, which lead to serious production losses and death, particularly in calves. Respiratory system diseases have high morbidity and mortality. They increase the cost of treatment and control [1,2]. Enzootic pneumonia is a type of pneumonia that occurs enzootically in young calves raised in closed environments. The disease is caused by various bacterial and viral agents, as well as care, nutrition, stress, and environmental factors [3]. Disrupted care, nutrition, and environmental factors predispose the calves to respiratory system diseases and these conditions can lead to the production of various free radicals, eventually resulting in oxidative stress in the animals [4–6].

Oxidative stress emerges with overproduction of oxidative substances and due to the imbalance between oxidants and antioxidants. It is reported to take part in the etiology of several diseases and to increase their severity, and to have a role in the pathogenesis of various respiratory system disorders. When the balance is not maintained by antioxidants, which serve as the primary defense against oxidative stress, severe damage in lung tissues may arise [5,7–10].

The purpose of this study was to determine the oxidative stress parameters [total oxidant status (TOS), nitric oxide (NO), malondialdehyde (MDA), sialic acid (SA), total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), oxidative stress index (OSI)] in calves with enzootic pneumonia.

2. Materials and methods

2.1. Animals

Ninety calves of different breeds (Simmental, Holstein, and crossbred) and sex that were 2–6 months old were used in this study. Of these calves, 80 were diagnosed with enzootic pneumonia according to the clinical examinations (coughing, nasal discharge, lacrimation, anemic or hyperemic mucous membranes, rough hair coats, swollen lymph nodes, various pathological lung sounds, and increase in body temperature, heartbeat, and respiratory rate), and the disease was enzootic among the examined calves. The control group consisted of 10 calves of different breeds (Simmental, Holstein, and crossbred) and sex that were 2–6 months old. They were healthy and did not have any disorder.

2.2. Obtaining samples

Before beginning the study, application to Van Yüzüncü Yıl University, Animal Researches Ethic Committee was

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made, and the final decision was received as the study did not require approval of the ethic committee (Approval Date: 25th May 2017, Decision number: 2017/05). When obtaining animal materials, the calves were properly handled and the sampling was performed very carefully to avoid stress and injury. Besides, approval and clinical examination forms were filled and signed by the animal owners.

General examination of all animals was performed, and blood samples were obtained from jugular veins into coagulant-free tubes. The samples were centrifuged at 3000 rpm (Rotofix 32 ® Hettich, Germany) for 15 min and the sera were extracted. Obtained serum samples were moved to microtubes and stored at –20 °C until biochemical analyses.

2.3. Biochemical analyses

To measure TOS, NO, MDA, SA, TAS, SOD, GPx, and CAT concentrations, commercial ELISA kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used. NO, MDA, SA, SOD, GPx, and CAT levels were measured with an ELISA device (DAS, Italy) at 450 nm wave-length according to the kit procedures. TOS and TAS were measured with another ELISA device (ELISA reader® - Anthos Zenyth 200rt) at 530 nm and 660 nm wavelengths, respectively, according to the kit procedures. OSI was calculated according to the formula: "[Total oxidant status/total antioxidant status] × 100", following the conversion of total antioxidant status unit (mmol) to μmol/L.

2.4. Statistical analyses

Definitive statistics for continuous variables were defined as mean and standard error. For comparing the group mean values, independent *t*-test was used. Statistical significance level was taken as 5% and IBM SPSS Statistics 22 was used for calculations.

3. Results

3.1. Clinical findings

Mean values of ages, body temperatures, heart rates, and respiratory rates belonging to healthy calves and calves with enzootic pneumonia are given in Table 1. According to these results, the ages of the animals were similar in both groups and there was not any statistically significant differences ($P > 0.05$). Body temperatures, heart rates, and respiratory rates had considerable increase that was also statistically significant ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). Some clinical findings such as coughing, nasal discharge, lacrimation, structure of mucous membranes, appearance of hair coat, and condition of lymph nodes are quantified in Table 2. Characteristics and quantity of pathological lung sounds in calves with enzootic pneumonia are presented in Table 3.

3.2. Biochemical findings

Mean and standard error values of oxidative stress parameters such as TOS, NO, MDA, SA, TAS, SOD, GPx, CAT, and OSI are given in Table 4. According to the obtained results; TOS, NO, MDA, and SA concentrations were higher in calves with enzootic pneumonia than in the healthy ones. The elevations were statistically significant for TOS, MDA, and SA ($P < 0.01$), and not for NO ($P > 0.05$). Besides, there was a statistically significant decrease in TAS ($P < 0.05$), SOD, GPx, and CAT ($P < 0.01$) concentrations in calves with enzootic pneumonia when compared with the healthy ones.

4. Discussion

Respiratory system disorders are one of the common problems worldwide in the cattle industry and they lead to serious production losses and death [1,11]. Enzootic pneumonia represents a different name for bovine respiratory disease complex and does not have any specific etiology. It frequently occurs during transition seasons in housed young calves [3,12]. The disease can be diagnosed according to anamnesis information and physical examination findings. Detecting clinical findings of pneumonia among several animals at crowded, congested, and unhygienic barns assist the diagnosis of enzootic pneumonia. Environmental factors, husbandry conditions, bacterial and viral agents, as well as stress, have a considerable impact on the occurrence of enzootic pneumonia in calves [2,4,13].

Stress is defined as a nonspecific response to various external factors [14,15], and oxidative stress is defined as the oxidative damage of cells and tissues due to imbalance between oxidants and antioxidants. When an imbalance occurs due to the increase in oxidants or decrease in antioxidants, eventual oxidative stress causes structural damage and function loss in cells and tissues, and may lead to a broad range of negative consequences in animals, from production losses to death. Therefore, oxidative stress is reported to have a notable role in the etiology and pathogenesis of various diseases in animals, especially in respiratory system disorders [7,8,16–18].

Several studies were performed to reveal the role of oxidative stress in respiratory system disorders in various animals, and studies related to oxidative stress are relatively recent in ruminants. Therefore, it is necessary to perform more studies to reveal the role of oxidative stress in respiratory system disorders [7–9,19]. Nevertheless, in cattle, oxidative stress is reported to have an important role in the pathogenesis of respiratory system disorders and the formation of lung lesions, and to increase the severity of the disease [20,21]. Furthermore, in calves, the risk of oxidative stress and respiratory system disorders is pointed out to be highest following weaning [8,22].

Table 1. Some clinical findings of healthy calves and calves with enzootic pneumonia.

Parameters	Groups		
	Healthy ($\bar{x} \pm S\bar{x}$) (n = 10)	Enzootic pneumonia ($\bar{x} \pm S\bar{x}$) (n = 80)	P
Age (months)	2.7 ± 0.27	3.6 ± 0.17 ^{NS}	0.065
Body temperature (°C)	38.84 ± 0.08	39.72 ± 0.10 **	0.001
Heart rate (/min)	85.60 ± 1.75	101.72 ± 3.16 *	0.028
Respiratory rate (/min)	37.60 ± 1.12	52.40 ± 2.68 *	0.018

* : $P < 0.05$; ** : $P < 0.01$; ^{NS}: Nonnsignificant

Table 2. Some clinical findings of calves with enzootic pneumonia.

Cough (n = 80)	Dry	Wet	Not present
	44	34	2
Nasal discharge (n = 80)	Seromucous	Mucopurulent	Not present
	51	19	10
Lacrimation (n = 80)	Present	Not present	
	39	41	
Mucous membranes (n = 80)	Normal	Mildly anemic	Hyperemic
	3	40	37
Hair coat (n = 80)	Normal	Rough	
	24	56	
Lymph nodes (n = 80)	Normal	Mildly swollen	
	16	64	

Table 3. Pathological lung sounds detected in calves with enzootic pneumonia.

Pathological lung sounds	Quantity
Increased vesicular sounds and dry rales	7
Increased vesicular sounds and moist rales	14
Increased vesicular sounds and pleural friction rub	19
Increased vesicular sounds, pleural friction rub, and dry rales	17
Increased vesicular sounds, pleural friction rub, and moist rales	8
Solely increased vesicular sounds	15

Oxidants and antioxidants have high reactivity and to measure their levels, both specialized equipment and laboratory experience are necessary. Measuring each oxidative stress parameter has advantages and disadvantages; however, evaluating a single parameter is not usually sufficient. Therefore, evaluating multiple parameters together is reported to be beneficial [5,23,24]. These oxidative stress parameters include TOS and TAS, free radicals (O_2^-, OH, NO, $ONOO^-$), oxidative damage biomarkers (H_2O_2, MDA, SA, 8-OHdG), antioxidant enzymes (SOD, GPx, CAT, GST, GR), and low-molecule antioxidants (alpha-tocopherol, ascorbic acid, glutathione, melatonin). Besides, measuring the levels of Cu, Zn, Mn, Se, and Fe may additionally be used for evaluating oxidative stress, since they have enzyme cofactor roles [6,8,18,25–27]. In this study, TOS, NO, MDA, and SA concentrations

Table 4. Oxidative stress parameters in healthy calves and calves with enzootic pneumonia.

Parameters	Groups		
	Healthy calves ($\bar{x} \pm S\bar{x}$) (n = 10)	Enzootic pneumonia ($\bar{x} \pm S\bar{x}$) (n = 80)	p
TOS (µmol H_2O_2 Eq/L)	3.60 ± 0.58	6.43 ± 0.37 **	0.008
NO (µmol/L)	60.08 ± 3.41	63.85 ± 1.29 ^{NS}	0.310
MDA (nmol/L)	17.62 ± 2.27	33.17 ± 1.38 **	0.001
SA (ng/mL)	1.85 ± 0.19	3.92 ± 0.12 **	0.001
TAS (mmol Trolox Eq/L)	1.21 ± 0.08	1.07 ± 0.02 *	0.044
SOD (ng/mL)	72.03 ± 4.01	49.90 ± 2.84 **	0.006
GPx (ng/mL)	217.91 ± 3.34	134.43 ± 7.34 **	0.001
CAT (ng/mL)	126.67 ± 5.16	86.18 ± 4.80 **	0.003
OSI (arbitrary unit)	0.31 ± 0.06	0.72 ± 0.11 ^{NS}	0.178

* = P < 0.05, ** = P < 0.01, ^{NS} = Nonsignificant, TOS = Total oxidant status, NO = Nitric oxide, MDA = Malondialdehyde, SA = Sialic acid, TAS = Total antioxidant status, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, CAT = Catalase, OSI = Oxidative stress index.

were higher in calves with enzootic pneumonia than in the healthy ones. The increases were statistically significant for TOS, MDA, and SA (P < 0.01), but not for NO (P > 0.05).

Reactive oxygen species (ROS) concentrations in serum and plasma can be accepted as the free radical production and indicator of oxidative stress. Total oxidant status is the oxidative stress parameter that provides collective information related to the whole reactive oxygen species in the body [5,10,23,24]. In studies performed on animals with respiratory system disorders [20,21,28], increase in TOS levels was attributed to the increase of reactive oxygen species due to respiratory system infection and inhibition of antioxidant defense system. In this study, it is thought that excessive ROS was produced in calves with enzootic pneumonia, which led to an increase in TOS levels.

Nitric oxide is a reactive nitrogen species and a free radical which is secreted by various cells and has roles as a physiological messenger [5,28,29]. There are numerous studies performed on animals to determine the relationship between NO and various disorders [28,30,31]. In studies performed on cattle with respiratory disease, NO levels were reported to be increased [32]. Moreover, in calves with pneumonia, Yurdakul and Aydogdu [33] reported that serum NO concentration was higher in sick animals than in healthy ones. Mandelker [16] and Hamp et al. [34] also reported that NO levels may increase due to hypoxia because of the elevated levels of carbon dioxide, and Kirmizigul et al. [28] stated that NO increase might be due to increased secretion of NO from stimulated monocytes. Besides, Singh et al. [32] determined an association between increased levels of NO and lung damage. In the present study, the increase in NO levels is thought to occur due to the stimulation of NO by infectious agents and lung damage, as well as hypoxia, as reported by the previous researchers.

Malondialdehyde is an end-product formed by the reaction of polyunsaturated fatty acids with free radicals [5,27,28]. MDA has high reactivity, is one of the primary indicators of lipid peroxidation, and leads to pathological disorders such as cellular deformations, aggregation of cell surface components, ion transport, and enzyme malfunctions [6,7,18,30]. In studies performed on various pneumonic animals [9,35–37], increased levels of MDA were detected. Özçelik et al. [27] and Eissa et al. [38] detected high levels of MDA in cattle with pneumonia. Likewise, Joshi et al. [2] also reported high levels of MDA in calves with respiratory system disease and stated that this increase was associated with the overproduction of free radicals and eventual lipid peroxidation. Ismael et al. [22] and Yurdakul and Aydogdu [33] similarly determined high levels of MDA in pneumonic calves and reported that excessive oxygen consumption and hypoxia may also lead to increase in MDA concentrations. In the present study, high MDA levels are also thought to be caused by hypoxia due to enzootic pneumonia. Additionally, Hermeyer et al. [29] reported that *Mycoplasma bovis* can also produce hydrogen peroxide as well as lipid peroxidation, thus lead to oxidative stress and tissue damage. In the present study, MDA increase might also be attributed to *M. bovis*, which is an etiological agent in enzootic pneumonia [12,39].

Sialic acid is a substance that is present in all tissues of mammalians and has various functions at the cellular level. It increases at the initial stage of inflammation and tissue damage. Elevated levels of total sialic acid were reported in cattle with various diseases [26,35,40]. In the present study, sialic acid levels were higher in calves with enzootic pneumonia than in the control group. The increase in this disease is assumed to occur due to tissue damage and cellular degeneration caused by increased extracellular neuraminidase activity, which breaks the bound between SA and cell membrane and ultimately causes the entrance of SA into the circulation [19,35].

Antioxidants are the first line of defense that can rapidly remove the oxidants or neutralize them [7,9,27,31]. While high levels of oxidants is an indicator of oxidative stress, low levels of antioxidants may also be an indicator [20,25]. In this study, there was a statistically significant decrease in TAS ($P < 0.05$), SOD, GPx, and CAT ($P < 0.01$) concentrations in calves with enzootic pneumonia when compared with the healthy ones.

Many studies were performed regarding TAS levels in animals with respiratory diseases [9,20], and its levels were determined to be lower in animals with disease when compared with the healthy ones. In the present study, the low levels of TAS are thought to occur due to the lowered antioxidant capacity which coped with the severe oxidative stress for protecting the cells against oxidative damage [22]. Likewise, Durgut et al. [24] also determined lower levels of TAS in pneumonic cattle and stated that the reduced levels of TAS might be due to the depletion of antioxidant enzyme activities by the severe progression of the disease.

Superoxide dismutase is an endogenous enzyme that constitutes the primary defense line against oxidants [5,7,41]. In a study performed on cattle with pneumonia, SOD concentrations were reported to be low and the researchers stated that the decrease occurred to remove high levels of superoxide radicals produced during the infection [27,33]. Similarly, Al-Qudah [8] and Ismael et al. [22] performed studies on calves with bronchopneumonia and observed that superoxide radicals were higher in sick animals than the healthy ones due to both excessive production of radicals and oxidative stress in lungs.

GPx is an enzymatic and endogenous oxidant which degrades H_2O_2 into alcohol and water [5,8]. It is reported that GPx concentration decreased in several diseases [7,33]. In the present study, the decrease in GPx is thought to occur since it is the main antioxidant ravaging lipid peroxides intracellularly, has crucial roles of protecting epithelial cells in airways, and can prevent the production of radical oxygen species until depletion, as reported by the previous researchers [8,22,33]. Besides, as it is also known that selenium is an enzyme cofactor of GPx [42], it was reported that in animals with selenium deficiency, GPx might also be detected at low levels, and lungs of these animals could be prone to oxidative stress. In the present study, another reason for detecting low levels of GPx in animals with enzootic pneumonia is believed to be the lipid peroxidation and oxidative stress in lung tissue as well as Se deficiency due to malnutrition.

Catalase is an enzymatic antioxidant and exists particularly in erythrocytes, fat and nervous tissue, liver, and kidney. CAT converts H_2O_2 to alcohol, H_2O, and O_2 by catalization. Even though GPx activity is inhibited in the body, CAT can continue its antioxidant function against oxidative stress [7,43]. Several studies have been performed associated with CAT levels in animals with respiratory system disease and low levels of CAT were detected [36,37]. Similar to the present study, Yurdakul and Aydogdu [33] reported low levels of CAT in pneumonic calves and stated that the decrease occurred due to lipid peroxidation. In other studies performed on pneumonic animals, the decrease in CAT levels were associated with the reduction in the enzyme capacity of CAT in order to prevent peroxidation and oxidative stress [8,37]. Besides, it was also reported that *Mycoplasma* spp. can lead to H_2O_2 production and may inhibit CAT enzyme in host cells [38], which may be attributed to the low concentrations of CAT measured in this study.

In recent studies, in order to reveal oxidative stress more accurately, researchers suggest evaluating TAS and TOS levels together [17,44]. For this purpose, the oxidative stress index (OSI) is used, which is an individual indicator of oxidative stress in animals and represents the current status of oxidants and antioxidants ratio [9,10,20,24]. In the present study, OSI level was higher in calves with enzootic pneumonia than in the healthy ones, similar to the findings of previous studies which also report increased levels of OSI in cattle with various diseases [10,17,20].

As a result, in this study, oxidative stress parameters were evaluated in calves with enzootic pneumonia and severe oxidative stress was detected. These findings reveal that oxidative stress may have a significant role in the occurrence of enzootic pneumonia in calves and may contribute to the progression of the disease. It is also believed that the obtained results might contribute to further studies. Furthermore, it is concluded that antioxidant supplements would be beneficial for supporting the routine treatment of the disease.

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