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RESEARCH ARTICLE

Nitric Oxide, Total Antioxidant Capacity and Total Oxidant Capacity Levels in the Lambs with Pneumonia

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Abstract

In this study, it was aimed to evaluate the nitric oxide (NO), total antioxidant capacity (TAC) and total oxidant capacity (TOC) in the lambs with pneumonia. The study was conducted on a total of 40 lambs including 30 lambs with pneumonia and 10 healthy lambs. In 30 lambs with pneumonia, the antibodies against the agents of *Mannheimia haemolytica, Mycoplasma bovis, Staphylococcus aureus* and *Adenovirus* were isolated in 8 lambs and against Mycoplasma bovis in 10 lambs and against *Mannheimia haemolytica, Staphylococcus aureus* and *Adenovirus* in 12 lambs. The values of pH, HCO_3 and BE and SO_2 in the blood gases of the lambs with pneumonia before treatment (BT) were lower than those of the healthy lambs whereas PCO_2 and body temperatures were found to be higher. Of the hematological parameters, the white blood cell (WBC), lymphocyte (LYM) values of the lambs with pneumonia were found to be higher than the values of the healthy control group ($P<0.01$). The TAC and NO levels of the lambs with pneumonia before treatment were found to be lower than those of the healthy lambs ($P<0.01$) while the TOC levels were found to increase ($P<0.01$). Based on the above results, the use of drugs containing nitric oxide and antioxidants in addition to the routine treatment of the lambs with pneumonia may be beneficial for the treatment and prognosis of pneumonia cases.

Keywords: Lamb; Nitric Oxide; Pneumonia; Total Antioxidant Capacity

Introduction

Respiratory tract infection in domestic animals cause major economic losses, especially in all countries in which sheep breeding is done, due to animal productivity loss, growth retardation, treatment costs and death [1-3]. The pneumonia in lambs is regarded as a complicated disease complex including the interactions among the defense mechanisms (immunologic and physiological) of the host, multiple agents (bacterial, viral, mycoplasma) and environmental factors [4,5]. Pneumonia is classified as acute viral, moderate proliferative, acute bacterial and chronic proliferative pneumonia depending on the etiologic factors and shelter conditions [5]. Chemically, nitric oxide (NO) is an intermediate product formed as a result of hydroxylation of the guanidine nitrogen group of L-arginine by an enzyme called Nitric Oxide Synthase (NOS), which is a homologue of cytochrome P-450 reductase in living organisms [6]. Nitric oxide is produced by various cells in the lung and plays an important physiological role in the regulation of pulmonary vasomotor tone via many known mechanisms [7]. NO has many different functions such as vasodilator, neurotransmitter, immunomodulator, antioxidant and pro-oxidant in many systems [6]. However, NO plays role in important physiological events in the respiratory system and endothelium-derived nitric oxide plays an important role in the modulation of systemic and pulmonary vascular tone in health and disease [8]. NO is synthesized by alveolar macrophages in the lower respiratory tract is indicated to have important role in the prevention of respiratory system infections. NO is bioactive cell secretion with lowest molecular weight which is secreted by many cells in the organism and functions as a mediator in cells and which has the ability to pass through cell membranes without being dependent on any receptor [6]. Although NO is produced in low amounts in respiratory cells, it is stated that a large amount is generated as a result of acute and chronic inflammatory responses [8-10]. NO is reported to play a role in the pulmonary defense mechanism and has bactericidal and bacteriostatic properties [9,10]. In healthy animals, epithelial surface fluid of the alveolar compartment in combination with low molecular weight antioxidant defense system such as glutathione, ascorbic acid and uric acid, lipophilic antioxidants such as alpha-tocopherol (vitamin E), retinol (vitamin A), and plasmalogens (1-alkenyl-phospholipids) and antioxidant enzymes such as superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidases (GPx) fight against the agents causing respiratory system diseases [11,12].

In this study, it was aimed to reveal the associations of serum nitric oxide, total antioxidant capacity and total oxidant capacities

with respiratory system disease as well as their importance in prognosis and treatment by determining the levels of nitric oxide and changes in total antioxidant capacity and total oxidant capacity in the lambs with pneumonia.

Materials and Methods

In this study, a total of 40 lambs including 30 lambs with pneumonia and 10 healthy lambs were utilized. Blood samples were taken from vena jugularis in accordance with its method for hematological and biochemical analyzes before treatment (BT) and on the tenth day after treatment (AT) from 40 lambs (30 lambs with pneumonia and 10 healthy) which were determined to have pneumonia as a result of the performed clinical and laboratory examinations. All animals in the study adhered to the principle of local Ethics Committee (University of Yuzuncu Yil). Treatments of lambs with pneumonia were applied penicillin + streptomycin, vitamin C and tylosin (applied for Mycoplasma bovis cases) intramuscularly for per lamb at the beginning of the study.

For the isolation of the agents (*Mannheimia haemolytica, Mycoplasma, Streptococcus, Staphilococus* and *Adenovirus*) in the blood samples taken from the lambs which were determined to have pneumonia as a result of clinical findings, the ELISA device (ELISA reader®-DAS) was used in accordance with the procedures described in commercial test kits.

Hematological analyzes

Red blood cell (RBC), Hematocrit value (Htc), hemoglobin concentration (Hb), leukocyte count, platelet count and MCHC values of the lambs with pneumonia were measured by a blood count device (QBCvetAutoreader®-Idexx). At the same time, the blood gas analyses from the blood samples obtained from the lambs with pneumonia BT and AT were measured with Idexx model blood gas analyzer. These samples are brought to the laboratory immediately without being contacted with air and are used as venous blood gases.

Biochemical analyzes

For the analysis of biochemical parameters, blood samples taken without anticoagulant were centrifuged at 3000 rpm to obtain their serum. The obtained sera were stored at -20 °C until measurements. Serum Na, K, Cl, total antioxidant capacity (TAC) and total oxidant capacity (TOC) were measured spectrophotometrically (Photometer 5010®-Boehringer Mannheim) in accordance with the procedures described in commercial test kits. Serum NO level was determined by Griess Reagent method, a commercial colorimetric method test kit (nitrate/nitrite colorimetric assay kit, Cayman Chemical Company, Catalog no: 780001), according to the kit procedure using ELISA device (ELISA reader®-DAS) [13].

Determination of viral and bacteriological agents

Adeno virus detection: It is determined by ELISA device (ELISA reader ®-DAS) in the obtained sera in accordance with the method described in Bio-X adenovirus 3® Elisa commercial test kits. The antibody titers were graded according to the data obtained according to the test kit method. Presence of clinical findings and antibody titre grade +2 or over were considered positive and were included in the pneumonia group.

Mannheimia haemolytica detection: It is determined by ELISA device (ELISA reader ®-DAS) in the obtained sera in accordance with the method described in Bio-X Diagnostics *Mannheimia haemolytica*® Elisa commercial test kits. The obtained data were evaluated as positive (pneumonia) when optical density was higher than 0.400 and negative when it was lower than 0.300 according to the test kit method.

Staphylococcus aureus detection: It was determined by ELISA device (ELISA reader®DAS) in the obtained sera from the taken samples in accordance with the method described in the *S. aureus* HCP Elisa commercial assay (Catalog No. F320, Cygnus Technologies®). The obtained data were evaluated as positive (pneumonia) for the values between 10-500ng / mL and negative for the values below 10 ng / mL according to the test kit method.

Mycoplasma bovis detection: It is determined by ELISA device (ELISA reader®-DAS) in the obtained sera in accordance with the method described in the Bio-X Diagnostics *Mannheimia haemolytica*® Elisa commercial test kits. From the obtained data according to the test kit, the coefficient of each sample was calculated with the given method. The coefficient values higher than 37% were regarded as positive (pneumonia) and the coefficient values below 37% were regarded as negative.

Statistical Analysis

Descriptive statistics for the studied characteristics were expressed as median mean and standard deviation. Kruskal-Wallis test was used to compare the groups in terms of these characteristics. Statistical significance level was accepted as 5% and SPSS statistical package program was used for the analyses.

Results

Clinical Results

A general examination of the lambs showing respiratory system disorder according to clinical findings was performed. The lambs

with body temperature of 40 °C and over, dyspnea, increased respiratory sounds in auscultation, pleuritic friction sounds and nasal discharge were included in the study. Clinical findings were more pronounced in cases with mixed infection (*Mannheimia haemolytica, Staphylococcus aureus* and *Adenovirus*) and pleuritic friction sounds were especially more pronounced in cases of mycoplasma bovis. The lambs with clinical examination including no sign of disease and those with normal body temperature constituted the control group.

The blood pH, saturation oxygen (SO_2) and bicarbonate (HCO_3) values of the lambs with pneumonia before treatment were found to be lower than those of the control group while total carbon dioxide (CO_2) and base excess (BE) values were found to be higher ($P<0.01$). The blood pH, SO_2, HCO_3, tCO_2 and BE values taken AT from the lambs with pneumonia were found to be close to those of the control group ($P>0.05$) (Table 1).

Parameters	Pneumonia $\bar{X}$ ±SD (n=30)		Healthy $\bar{X}$ ±SD (n=10)
	BT	AT	
PH	7.48 ±0.03 [a]	7.53 ±0.02 [b]	7.50± 0.02[b]
PCO_2 (mm/Hg)	36.20±6.44	33.90 ±2.33	35.50±3.44
HCO_3 (mmol/L)	16.09 ±6.50 [a]	27.49±4.85 [b]	20.97 ±10.07 [b]
An Gap (mmol/L)	11.24±4.33	11.94±3.98	11.91±3.96
tCO_2 (mmol/L)	28.52±5.03	22.09±6.95	23.98±7.38
BE (mmol/L)	5.83 ±3.70 [a]	2.74± 1.81 [b]	3.64±2.31[b]
PO_2 (mm/Hg)	43.20±6.14	50.40±8.34	52.30±10.66
tHb (g/dL)	10.97±1,20	11.14±0.96	10.68±0.99
SO_2	67.00±7.56	73.50±10.04	73.20±11.51
Body Temperature (°C)	39.33±0.74[a]	38.93 ±0.41[b]	38.70 ±0.35[b]

The difference between the groups with different letters was significant (**ab:** P<0.05). No lettering was made for the characteristics that are not significant between the groups.
Table1. The blood gas values of the lambs with pneumonia and healthy lambs

No difference was detected between BT and AT concentrations of Na, K and Cl of the lambs with pneumonia ($P>0.05$) (Table 2).

Parameters	Pneumonia $\bar{X}$ ±SD (n=30)		Healthy $\bar{X}$ ±SD (n=10)
	BT	AT	
Na (mmol/L)	143.10±1.37	143.40±2.99	142.50±2.41
K (mmol/L)	3.86±0.13	4.09±0.54	3.74±0.68
Cl (mmol/L)	108.20±1.69	109.70±3.34	110.00±2.67

The difference between the groups with different letters was significant ($P<0.05$). No lettering was made for the characteristics that are not significant between the groups.
Table 2: The electrolyte values of the lambs with pneumonia and healthy lambs

In the analysis of hematologic parameters, RBC and Hb values of the lambs with pneumonia BT were found to be lower compared to the control group but only the decrease in percentage of haematocrit (Htc) (%) was found to be significant ($P<0.01$) while the values of WBC and LYM were found to be higher ($P<0.01$). Despite the treatment, the WBC blood values of the lambs with pneumonia on the tenth day after treatment were found to be higher compared to the control group ($P<0.05$) (Table 3).

Parameters	Pneumonia $\bar{X}$ ±SD (n=30)		Healthy $\bar{X}$ ±SD (n=10)
	BT	AT	
RBC(10^{12}/L)	9.24±2.76	9.93±3.01	11.70±3.30
HTC(%)	24.04±7.14[a]	25.80±8.59	30.05±8.77[b]
HGB (g/dl)	8.42±1.92	8.740±1.99	9.58±1.83
MCV(fL)	37.06±0.39	37.10±0.48	36.79±0.25
MCH(pg)	9.17±2.8	8.75±2.47	7.71±2.51
MCHC(g/dL)	31.55±7.78	31.12±8.90	29.02±5.80
RDW(%)	7.98±1.98	8.59±2.0	9.25±1.71
WBC(10^9/L)	11.99±2.88 [a]	9.70 ±3.64[b]	8.87 ±1.184[b]
LYM(10^9/L)	7.66±1.93[a]	6.60±2.44[a]	4.77 ±1.48[b]
GRAN(10^9/L)	1.35±0.80	0.96±0.29	0.92±0.47

The difference between the groups with different letters was significant (**ab:** $P<0.05$). No lettering was made for the characteristics that are not significant between the groups
Table 3: The hematological parameters of the lambs with pneumonia and healthy lambs

In the analysis of biochemical parameters, TAC and NO concentrations of the lambs with pneumonia BT were found to be lower than those of the control group (P<0.01) while the TOC values were found to be higher than those of the control group (P<0.01). Despite the treatment, an increase was detected in the TAC and NO concentrations of the lambs with pneumonia on the tenth day AT. Although an increase was detected in the TAC and NO concentrations, they were found to be lower than those of the control group (Table 4). In the viral and bacteriological analysis of sick lambs, the antibodies against the agents of Mannheimia haemolytica, Mycoplasma bovis, Staphylococcus aureus and Adenovirus were isolated in 8 lambs, Mycoplasma bovis in 10 lambs, Pasteurella haemolytica, Staphylococcus aureus and Adenovirus in 12 lambs with pneumonia. The NO and TAC concentrations of the lambs with pneumonia were found to be lowest in the lambs with mixed infection pneumonia (*Pasteurella haemolytica, Staphylococcus aureus* and *Adenovirus*) while the TAC concentrations of the lambs with pneumonia were found to be highest in the lambs with mixed infection pneumonia (*Pasteurella haemolytica, Staphylococcus aureus* and *Adenovirus*). The NO concentrations of the lambs with pneumonia were found to be lowest in the lambs with mixed infection pneumonia (Table 5).

Parameters	Pneumonia $\bar{X}$ ±SD (n=30)		Healthy $\bar{X}$ ±SD (n=10)
	BT	AT	
TAC (mmol/L)	1.12±0.13[a]	1.18±0.05[c]	2.20±0.05[b]
TOC (µmol/L)	2.99±1.66[a]	1.97±1.69[c]	1.49±1.22[b]
NO(µM)	0.72±0.26[a]	0.81±0.36[a]	1.32±0.47[b]

The difference between the groups with different letters was significant (**ab, cb, ac:** *P*<0.05). No lettering was made for the characteristics that are not significant between the groups

Table 4: The NO, TAC and TOC levels of the lambs with pneumonia and healthy lambs

Parameters	Pneumonia $\bar{X}$ ±SD (n=30)			Healthy $\bar{X}$ ±SD (n=10)
	n=8	n=10	n=12	
Mannheimia haemolytica	+		+	
Mycoplasma bovis	+	+		
Staphyloccocus aureus	+		+	
Adenovirus	+		+	
Nitric Oxide (µM)	0.68±0.22[a]	0.79±0.33[a]	0,72±0,24[a]	1.32±0.47[b]

The difference between the groups with different letters was significant (ab: P<0.05).
No lettering was made for the characteristics that are not significant between the groups

Table 5: The nitric oxide levels associated with agent distribution in the lambs with pneumonia

Discussion

In pneumonia patients complicated with severe respiratory failure, it is necessary to use not only the conventional therapies such as antibiotics and artificial ventilation but also specific therapies. The causative agents for pneumonia in lambs are reported to be Mycoplasma ovipneumoniae, Mannheimia haemolytica, Mycoplasma ovipneumoniae, *Mycoplasma arginini, Chlamydia psitaci, Paraenfluenza-3 virus and Adenoviruses and Respiratory syncytial virus* [14-16]. In this study, as a result of the serological studies to be performed on the lambs with pneumonia, the antibodies against the agents of *Mannheimia haemolytica, Mycoplasma bovis, Staphylococcus aureus* and *Adenovirus* were detected. Antibody against single agent was detected in 33% of the cases while the antibodies against mixed agents were detected in 67% of the cases. The etiological factors detected as a result of serologic analyses are similar to the etiologic factors indicated by the investigators [14-16].

Hematocrit value, RBC counts and Hb concentrations of the goats infected with *Pasteurella multocida* were reported lower than the healthy goats however, only the decrease in Hb was significant [17]. In this study, RBC and Hb values of the lambs with pneumonia BT were lower compared to the control group but only the decrease in Htc (%) was statistically significant (*P*<0.01) while WBC and LYM values were found to be higher (*P*<0.01). Post-treatment and control group WBC values are statistically the same .This data is similar to the data of the researcher [17].

In respiratory acidosis caused by respiratory tract infections (bronchitis, bronchopneumonia and pneumonia), upper and lower respiratory tract obstructions, asphyxia, pneumothorax and chronic obstructive pulmonary disease, pH, $p0_2$ and $S0_2$ decrease and $pC0_2$ increases in the venous blood [18]. In this study, the blood pH, SO_2 and HCO_3 levels of the lambs with pneumonia were found to be lower than those of the control group while the tCO_2 and BE values were found to be higher (P<0.01). Similarness of the blood pH, SO_2, HCO_3, tCO_2 and BE values to the values of the control group and lack of statistically significant difference support the findings of the investigators [18-20].

Nitric oxide is a bioactive cell secretion with lowest molecular weight which is secreted by many cells in the organism and functions as a mediator in cells and which that has the ability to pass through cell membranes without being dependent on any receptor [21-26]. NO has antimicrobial activity against various pathogens with its cytotoxic or cytostatic effects [27]. It is reported that NO

have important physiological and pathological effects in respiratory system similarly many systems and it is an important defensive molecule against infectious agents in the organism [1,21-26,28]. Alveolar macrophages are stated to have significant roles for the immune system in the lower respiratory tracts and NO released by alveolar macrophages has important roles in respiratory system infections [30]. The secretion of nitric oxide at basal levels plays a role in physiological events in the organism; however excessive release plays a role in pathological events by causing the destruction of cells [6]. The microbial infections of human and experimental animals results in an increase in local and systemic production of NO [31]. Exhaled excretion of NO is an indicator of respiratory tract inflammation. Although NO is produced in low amounts in respiratory tract cells, a large amount of NO is considered to be produced in acute and chronic inflammatory responses, it plays role in the pulmonary defense mechanism and it has bactericidal and bacteriostatic effects [10]. In this study, the NO concentrations of the lambs with pneumonia BT were found to be lower than the control group. The decrease in the NO concentrations was detected to be lower in the cases with mixed infections. Although an increase was detected in the NO concentrations on the tenth day BT, it was again measured at low levels compared to the control group. According to the results obtained from the data related to NO, the detection of NO at low concentrations in the cases with mixed infections and poor clinical findings as stated by the investigators of NO reveals that NO has an important role in the immune system in case of respiratory diseases and its consumption is increased in parallel with the severity of infection [1,21-26,29]. With the complex and coordinated interactions of all antioxidant compounds in healthy lungs, it protects the lower respiratory system from the hazardous effects of oxidative attacks [32]. Many investigators reported that the impairment between oxidative compounds and local antioxidant system caused inflammation in the lungs, increased alveolar capillary leakage and decrease in the functions of surfactant [12,32-34]. In this study, the etiologic factors of pneumonia were determined serologically and the factors playing role in disease formation were detected to play a mixed role (Table 5). The levels of TAC concentrations in the lambs with pneumonia due to mixed agents were found to be lower than those in the cases with single causative agent and the control group while the TOC levels were found to be higher (Table 4). In this study, the lower level of TAC in the cases with poor prognosis and mixed infections can be explained with the situations stated by the investigators [11,12,32].

As a result, it was concluded that the lambs with pneumonia had significant changes in the serum NO, TAC and TOC levels at BT and AT, that the determination of NO, TAC and TOC levels in respiratory system diseases was important for the treatment and prognosis of the disease and that in addition to the routine treatment, the use of NO and antioxidant preparations would increase the chance of success in treatment.

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