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Oxidative Stress in Akkaraman Ewes with Seropositive for Schmallenberg Virus

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Abstract: The aim of this study was to determine oxidant-antioxidant status by evaluating some oxidative stress parameters (nitric oxide (NO), malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), catalase activity) in clinically healthy Akkaraman ewes with seropositive for Schmallenberg virus (SBV). The study consisted of four positive, six suspected and seven negative-control blood sera in terms of specific antibody for SBV by using commercial ELISA kits. Even though there were no significant statistical differences (P>0.05), the highest NO level was in sera with SBV specific antibody positive while the lowest TOS level was in sera with SBV specific antibody negative. The values of TAS in sera were very similar in the groups (P=0.981). While the MDA levels of sera with SBV specific antibody positive and suspected were similar, the statistical difference was found between the MDA level of sera with SBV specific antibody positive and negative (P=0.021). The catalase activity of sera with SBV specific antibody positive was lower (P=0.030) than the catalase activity of sera with SBV suspected whereas the catalase activities in positive and negative groups and the catalase activities in suspected and negative groups were similar. In conclusion, it was determined that MDA level was affected in Akkaraman ewes that are SBV specific antibody positive without clinical symptoms.

Keywords: Oxidative stress, Schmallenberg virus, Sheep.

Schmallenberg Virüs Seropozitif Akkaraman Koyunlarda Oksidatif Stres

Öz: Bu çalışmanın amacı Schmallenberg virüs (SBV) seropozitif belirlenen klinik olarak sağlıklı Akkaraman koyunlarda bazı oksidatif stres parametrelerinin (nitrik oksit (NO), malondialdehid (MDA), total antioksidan kapasite (TAK), total oksidan kapasite (TOK), katalaz aktivitesi) değerlendirilmesiyle oksidan-antioksidan durumun belirlenmesidir. Çalışmanın materyalini, ELISA yöntemiyle SBV özgül antikorları yönünden 4 pozitif, 6 şüpheli ve 7 negatif-kontrol kan serumu oluşturdu. Gruplar arasında istatistiksel bir fark olmamasına rağmen (P>0.05) en yüksek NO seviyesi SBV seropozitif serumlarındayken, en düşük TOK seviyesi SBV negatif serumlarda bulundu. Gruplardaki TAS değerleri oldukça benzerdi (P=0.981). SBV seropozitif ve şüpheli serum MDA seviyeleri benzerken SBV seropozitif ve negatif serumların MDA seviyeleri istatistiki olarak farklı bulundu (P=0.021). SBV seropozitif olan serumların katalaz aktiviteleri, SBV şüpheli serumların katalaz aktivitelerinden daha düşük (P=0.030) iken pozitif ve negatif grupların katalaz aktiviteleri ile şüpheli ve negatif grupların katalaz aktiviteleri benzer bulundu. Sonuç olarak; hastalığa ait klinik semptom bulunmayan SBV seropozitif Akkaraman ırkı hayvanlarda MDA seviyesinin etkilendiği belirlendi.

Anahtar Kelimeler: Koyun, Oksidatif stres, Schmallenberg virüs.

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INTRODUCTION

Oxidative stress is the shift in balance between oxidants and antioxidants for the favor of the oxidants and eventually causing damage (1). Oxidative damage can cause cell death due to apoptosis or necrosis leading to destruction of structural tissues (2). The damage of biological structures such as protein, lipid, carbohydrate and DNA are closely related to many diseases including cancer, atherosclerosis, Alzheimer's disease, arthritis, diabetes, even aging (3). Some viral infections can also cause oxidative stress such as influenza virus, picarnovirus, pox virus, caraparu virus, crimean-congo hemorrhagic fever virus and Rift Valley fever virus (4-11).

Nitric oxide (NO) is within free radicals due to its odd numbered electrons and synthesized from L-arginine, which an amino acid by NO-synthase (12,13). The NO plays role in neurotransmission and regulation of blood pressure and immune system and it can be converted to peroxynitrite, which is very harmful for tissues and organs (14). Lipids, especially the polyunsaturated ones, tend to oxidation. Although there are many procedures for determining the lipid oxidation, two groups of assays are remarkable. These are malondialdehyde (MDA) and isoprostane analyses (2). MDA, used to determine of cellular damage severity, causes to disturbance of ion transport, enzyme activity and cell membrane (14).

Measurements of antioxidants are determined both approximately for all antioxidants and definitely for each antioxidant. Total antioxidant status (TAS), Trolox equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP) and ferric reducing-antioxidant power (FRAP) are measured by colorimetric or cyclic voltammetry methods for determining antioxidants capacity. The common feature of these measurements is to summarize the activity of all antioxidants with a single value (2). Total oxidant status (TOS) can be used as an indicator for the oxidants produced by the organism as well as taken by environmental factors (15).

Catalase is also among the enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase, mitochondrial cytochrome oxidase and glutation-S-transferases. The role of catalase enzyme is breaking down of hydrogen peroxide into water and oxygen (12,13,16).

Schmallenberg virus (SBV), which is a member of the *Bunyaviridae* family, was firstly detected in Europe in 2011. SBV affects ruminant animals and is transmitted by vectors (*Culicoides* spp.) (17-20). SBV causes fever, reduced milk production, watery diarrhea, abortion, and malformed stillbirths (hydranencephaly – arthrogryposis) in cows, sheep and goats (21-25).

Oxidative stress were investigated in some *Bunyaviridae* members including caraparu virus, crimean-congo hemorrhagic fever virus and Rift Valley fever virus in mice, human and human small airway lung cells, respectively (5,6,8). There are no study showing relationship between SBV and oxidative stress in sheep. Therefore, the aim of this study was to determine oxidant-antioxidant status by evaluating some oxidative stress parameters (NO, MDA, TAS, TOS, catalase activity) in clinically healthy Akkaraman ewes with seropositive for SBV.

MATERIALS and METHODS

The study consisted of four positive (3 ewes, 1 ram), six suspected (5 ewes, 1 ram) and seven negative-control (6 ewes, 1 ram) blood sera in terms of specific antibody for SBV. Blood sera were collected from clinically healthy animals ranging in age from 2 to 5. The investigation was approved by the local Ethics Committee of Kırıkkale University (14/32).

Indirect ELISA (IDEXX, Schmallenberg Virus Antibody Test Kit, Liebefeld-Bern, Switzerland) was used to detect antibody response to SBV infection according to manufacturer's instruction. Briefly, positive and negative control of sera were diluted as 1:10 in sample diluent and added coated SBV plate

incubated for 60 minutes at room temperature. After three times washing the plates conjugate was dispensed and plates were incubated for 60 minutes at room temperature. Substrate (100 µl) was added to wells and incubated for 10 minutes at room temperature after second washing step. Reaction was stopped by adding stop solution. Plates were read at 450 nm wavelenght in spectrophotometer (SIRIO S ELISA reader, Jakarta Raya, Indonesia). Results calculated according to given formula (S/P % = 100 x {sample OD – negative control OD}/{positive control OD – negative control OD}) and were interpreted as negative if S/P% <30%, suspect if S/P% in between ≥30% and <40%, positive if S/P% ≥40%.

Total Antioxidant Status Assay

TAS absorbance was spectrophotometrically in serum with a commercial kit (Rel Assay Diagnostic, Gaziantep, Turkey). The procedure was based on the reduction of colored 2,2'-azino-bis (3-ethylbenzotiazoline-6-sulfonic acid) radical to a colorless reduced form with the antioxidants present in the sample. The absorbance of standard and samples was read at 660 nm against de-ionized water with the ELISA Reader (SIRIO S ELISA reader, Jakarta Raya, Indonesia). This procedure was calibrated using Trolox which is a vitamin E analog. The data were presented as mmol Trolox equivalent per liter (mmol Trolox Eq/L). The sample to be measured was repeated more than 5 times. The coefficient of variation (CV) values were ±10% range (26).

Total Oxidant Status Assay

TOS absorbance was spectrophotometrically measured in serum with a commercial kit (Rel Assay Diagnostic, Gaziantep, Turkey). The method was based on the principle that the oxidants in the sample oxidized ferrous ions, to ferric ions. In the acidic medium of the assay, these ferric ions formed a colored complex with a chromogen. The absorbance of standard and samples was read at 546 nm against de-ionized water with the ELISA reader. This assay was calibrated with hydrogen peroxide (H_2O_2), and the results were presented as µmol H_2O_2 Eq/L. The same sample to be measured was repeated more than 5 times. The CV values were ±10% range (27).

Determination of Malondialdehyde Concentrations

Concentrations of MDA in serum were determined as thiobarbituric acid reactive substances using the spectrophotometric method as described by Buege and Aust (28). This method is based on the formation of a pink color under the acidic condition upon the reaction of MDA and thiobarbituric acid (TBA). Briefly, TBA (0.375 m/v), hidrochloric acide (0.25 N), trichloroacetic acid solution (15%, w/v) were combined to 1:1:1 (A solution) and mixed. A solution (1000µl) and butylated hydroxytoluene (10 µl) were put into centrifuge tubes of whole serum containing samples of 0.5 ml each. Whole tubes were mixed with vortex. The tubes were then put in a boiling water at 95 °C bath for 25 minutes. After this incubation, all the test tubes were cooled in an ice bath. The tubes were centrifuged at 14000 rpm, 5 minutes 4˚C. Supernatant samples were then transferred to test tubes. The absorbance of samples was measured at 536 nm using a spectrophotometer (Shimadzu UV 1700, Japan). The MDA concentration was calculated based on the absorbance coefficient of the TBA–MDA complex ($\varepsilon = 1.56 \times 105\ cm^{-1}\ M^{-1}$) and expressed µmol/L.

Catalase Activity Assay

Catalase activity was spectrophotometrically measured in serum with a commercial kit (Cayman Chemical Company, Ann Arbor MI, USA) by double reading. The method was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced was measured colorimetrically with 4-amino-3hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. This method used a formaldehyde solution as standard. The absorbance of standard and samples was read at 540 nm with the ELISA

reader. Catalase activity was presented as nmol/min/ml.

Determination of Nitric Oxide Level

NO levels in serum were determined by the spectrophotometric method described in Miranda et al. (29). Total NO concentrations (nitrate and nitrite) were determined calorimetrically by the acidic Griess reaction via reaction involving reduction of nitrate to nitrite by vanadium (III) chloride (29). Samples were briefly deproteinized before the assay. For this; serum was added to 96% cold ethanol at 1:2 (v/v) and the solution was mixed with vortex for 5 minutes. The mixture was incubated for 30 minutes at 4 °C. Then, mixture was centrifuged at 8000 × *g* for 5 minutes and the supernatants obtained were used for the Griess assay. Analysis was done in a microtiter plate. One hundred microliter of filtrated serum was mixed with 100 µl of vanadium (III) chloride and rapidly followed by the addition of the Griess reagents (50 µl Sulfanilamid and 50 µl N-(1-Naphthyl) ethylenediamine dihydrochloride). The determination was done at 37 °C for 30 minutes. The absorbance was measured by the ELISA Reader at 540 nm. Nitrite/nitrate concentration was calculated using a sodium nitrite standard curve and expressed in µmol/L.

Statistical Analysis

The SPSS v15.0 packet program (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. The normality of the data was tested by the Shapiro-Wilk test. The Kruskal-Wallis test was used to see if the group differences exist. Then, the Mann-Whitney U test was used for pairwise group comparison after the Bonferroni adjustment to determine which of the three groups differed from the others. The differences were significant when $P<0.05$ in the Kruskal-Wallis tests and $P<0.017$ in the Mann-Whitney U test with a Bonferroni adjustment.

RESULTS

The levels of NO, MDA, TOS, TAS and catalase activity in groups were summarized in Table 1.

Table 1. The levels of NO, MDA, TOS, TAS and catalase activity in groups.
Tablo 1. Grupların NO, MDA, TOK, TAK seviyeleri ve katalaz aktiviteleri.

Groups (n)		Parameters				
		NO (µmol/L)	MDA (µmol/L)	TOS (µmol H_2O_2 Eq/L)	TAS (mmol Trolox Eq/L)	Catalase (nmol/min/ml)
Positive (4)	Mean±standard error	50.47±6.58	1.13±0.05[a]	5.21±1.36	1.49±0.05	49.03±8.78[b]
	Median±range	54.17±30.28	1.12±0.23	4.95±6.16	1.49±0.26	52.11±39.42
	Mean rank	11.63	12.73	9.75	8.75	3.88
Suspected (6)	Mean±standard error	43.74±2.37	1.14±0.24[a,b]	5.32±1.57	1.48±0.04	96.46±13.33[a]
	Median±range	41.05±12.11	1.07±1.60	4.33±10.95	1.49±0.31	86.60±87.46
	Mean rank	7.83	11.17	9.17	8.83	12.50
Negative (7)	Mean±standard error	45.09±3.82	0.62±0.04[b]	4.65±0.71	1.48±0.03	79.39±12.82[a,b]
	Median±range	45.09±28.26	0.67±0.25	4.65±5.23	1.51±0.20	69.36±91.16
	Mean rank	8.5	5.00	8.43	9.29	8.93
p		0.475	0.021	0.912	0.981	0.030

a, b: Means within column with different superscripts are significantly different; P<0.05
NO: nitric oxide, MDA: malondialdehyde, TOS: total oxidant status, TAS: total antioxidant status.

DISCUSSION and CONCLUSSION

SBV, which is a member of *Bunyaviridae* family, causes fever, reduce milk production, watery diarrhea, abortion, stillbirth malformed offspring in cows, sheep and goats (21-25). The information about oxidative stress in sheep with SBV has not been reported so far.

NO is usually increased in viral infections and has got physiological roles and also functions in immune system by activating macrophages. MDA is the most important indicator of lipid peroxidation (9). In this study, the highest NO level was determined in sera with SBV specific antibody positive (50.47±6.58 µmol/L), NO value of sera with SBV specific antibody suspected (43.74±2.37 µmol/L) was lower than the SBV specific antibody negative (45.09±3.82 µmol/L). However, there were no significant differences in any groups. The MDA levels of sera with SBV specific antibody positive (1.13±0.05 µmol/L) and suspected (1.14±0.24 µmol/L) were detected to be higher than sera with SBV specific antibody negative (0.62±0.04 µmol/L). Difference was detected between MDA level of sera with SBV specific antibody positive and negative. Even though there was no study about NO and MDA in sheep with SBV, these parameters were evaluated in some viral infections including pox virus and ecthyma contagiosa (orf). Levels of NO and MDA were increased in sheep with pox virus [NO; control= 11.05±0.37 nmol/ml, affected= 36.65±1.10 nmol/ml. MDA; control= 2.77±0.10 µmol/L, affected= 5.87±0.14 µmol/L, Kirmizigul *et al.* (9) and MDA; control= 1.27±0.21 mmol/L, affected= 5.37±0.32 mmol/L, Issi *et al.* (30)] and ecthyma contagiosa [NO; control= 7.78±1.02 µmol/L, affected= 12.44±1.90 µmol/L. MDA; control= 8.58±0.80 µmol/L, affected= 11.26±1.06 µmol/L, Deveci *et al.* (11)]. The enhanced lipid peroxidation products may cause peroxidative damage which may resulted in SBV infection.

In the present study, the levels of TOS in sera with SBV specific antibody positive (5.21±1.36 µmol H_2O_2 Eq/L) and suspected (5.32±1.57 µmol H_2O_2 Eq/L) were detected higher than sera with SBV specific antibody negative (4.65±0.71 µmol H_2O_2 Eq/L). These differences were not statistically significant. The values of TAS in sera were very similar in groups (SBV specific antibody positive: 1.49 ± 0.05 mmol Trolox Eq/L, suspected: 1.48 ± 0.04 mmol Trolox Eq/L, negative: 1.48 ± 0.03 mmol Trolox Eq/L). There was no study evaluating the levels of TOS and TAS in sheep with SBV. However, the level of TOS was increased and the level of TAS was decreased in sheep with pox virus [TOS; control= 457.80±22.48 µmol H_2O_2 Eq/L, affected= 685.05±10.84 µmol H_2O_2 Eq/L. TAS; control=1.50±0.03 mmol Trolox Eq/L, affected= 1.01±0.03 mmol Trolox Eq/L, Kirmizigul *et al.* (9)] and bluetongue [TAS; control= 0.57±0.01 mmol Trolox Eq/L, affected= 0.48±0.01 mmol Trolox Eq/L, Aytekin *et al.* (31)] and a significant negative correlation was reported between TOS and TAS (27). In this study, the values of TOS and TAS in sera were not different between the groups and this could be due to the absence of clinical symptoms when blood was collected. SBV causes a short viremia of 2-4 days in goat and sheep (32).

Catalase, which is concentrated in aerobic cells such as liver and erythrocyte, plays a role in preventing damage caused by free radicals and lipid peroxidation (14,16). In this study, the catalase activity of sera with SBV specific antibody positive (49.03±8.78 nmol/min/ml) was found to lower than sera with SBV specific antibody negative (79.39±12.82 nmol/min/ml). On the other hand these level of sera with SBV specific antibody suspected (96.46±13.33 nmol/min/ml) was higher than sera with SBV specific antibody negative. Difference was detected between catalase activity of sera with SBV specific antibody positive and suspected. There was also no study about catalase activity in sheep with SBV. However catalase activity was investigated in pox virus and in Peste des petits ruminants. The catalase activity was increased in pox virus [control= 1.23±0.09 10^3 k/L, affected= 1.54±0.07 10^3 k/L, Issi *et al.* (30)] and in Peste des petits ruminants [control= 71.00±9.00 kU L^{-1}, affected= 140.00±8.00 kU L^{-1}, Kataria and Kataria

(33)]. Increased catalase activity may explained by compensatory nature responding to the increased peroxide load in SBV infection.

In conclusion, it was determined that MDA level was affected in Akkaraman ewes that are SBV specific antibody positive without clinical symptoms. The results of the evaluated parameters will contribute to literature and support to new studies about SBV.

REFERENCES

1. Sies H., 1997. Oxidative stress: Oxidants and antioxidants. Exp Physiol, 82, 291-295.
2. Lykkesfeldt J., Svendsen O., 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet J, 173, 502-511.
3. Matsufuji H., Ochi H., Shibamoto T., 2006. Formation and inhibition of genotoxic malonaldehyde from DNA oxidation controlled with EDTA. Food Chem Toxicol, 44, 236-241.
4. Han SN., Meydani M., Wu D., Bender BS., Smith DE., Vina J., Cao G., Prior RL., Meydani SN., 2000. Effect of long-term dietary antioxidant supplementation on influenza virus infection. J Gerontol A Biol Sci Med Sci, 55, 496-503.
5. Aydin H., Yildiz G., Engin A., Yilmaz A., Çelik K., Bakir S., 2010. Malondialdehyde, vitamin E, and anti-oxidant enzyme activity levels in patients with crimean-congo hemorrhagic fever. AJMR, 4, 2402-2409.
6. Narayanan A., Popova T., Turell M., Kidd J., Chertow J., Popov SG., Bailey C., Kashanchi F., Kehn-Hall K., 2011. Alteration in superoxide dismutase 1 causes oxidative stress and p38 MAPK activation following RVFV infection. PLoS One, 6, e20354.
7. Bozukluhan K., Atakisi E., Atakisi O., 2013. Nitric oxide levels, total antioxidant and oxidant capacity in cattle with foot-and-mouth-disease. Kafkas Univ Vet Fak Derg, 19, 179-181.
8. Camini FC., Almeida LT., Bernardes CS., Silva M., Pedrosa ML., Costa DC., de Lima WG., do Amaral Pinto C., Ferreira PCP., de Magalhaes JC., de Brito Magalhaes CL., 2014. Caraparu virus induces damage and alterations in antioxidant defenses in the liver of BALB/c mice after subcutaneous infection. Arch Virol, 159, 2621-2632.
9. Kirmizigul AH., Ogun M., Ozen H., Erkilic EE., Gokce E., Karaman M., Kukurt A., 2016. Oxidative stress and total sialic acid levels in sheep naturally infected with pox virus. Pak Vet J, 36, 312-315.
10. Uzlu E., Karapehlivan M., Erdoğan HM., Kızıltepe Ş., Erkılıç EE., Deveci HA., Gökçe E., Kaya İ., Çitil M., 2016. Serum and saliva sialic and oxidative stress parameters changes in bulls with foot and mouth disease. Kafkas Univ Vet Fak Derg, 22, 321-325.
11. Deveci AH., Kükürt A., Uzlu E., Sözdutmaz İ., Merhan O., Aktaş S., Alpay M., Kaya İ., Karapehlivan M., 2017. Evaluation of paraoxonase activity, total sialic acid and oxidative stress in sheep with ecthyma contagiosa. Kafkas Univ Vet Fak Derg, 23, 453-457.
12. Akkuş İ., 1995. Serbest Radikaller ve Fizyopatolojik Etkileri. 1st ed., 42-112, Mimoza Yayınları, Konya.
13. Gutteridge JMC., 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem, 41, 1819-1828.
14. Bozukluhan K., Merhan O., Öğün M., Cihan M., Gökçe G., 2016. Omfalitisli buzağılarda bazı oksidatif stres parametre düzeylerinin belirlenmesi. F Ü Sağ Bil Vet Derg, 30, 79-81.
15. Özbek Ö., 2013. Testis iskemi/reperfüzyonu oluşturulmuş ratlarda apocyninin testis dokusu üzerine koruyucu ve tedavi edici etkilerinin araştırılması. İnönü Üniversitesi, Tıp Fakültesi, Türkiye.
16. Çaylak E., 2011. Hayvan ve bitkilerde oksidatif stres ile antioksidanlar. TAD, 9, 73-83.
17. Hoffmann B., Scheuch M., Hoper D., Jungblut R., Holsteg M., Schirrmeier H., Eschbaumer M., Goller KV., Wernike K., Fischer M., Breithaupt A., Mettenleiter TC., Beer M., 2012. Novel orthobunyavirus in cattle, Europe, 2011. Emerg Infect Dis, 18, 469-472.

18. Veldhuis AMB., van Schaik G., Vellema P., Elbers ARW., Bouwstra R., van der Heijden HMJF., Mars MH., 2013. Schmallenberg virus epidemic in the Netherlands: Spatiotemporal introduction in 2011 and seroprevalance in ruminants. Prev Vet Med, 112, 35-47.
19. Azkur AK., Tonbak Ş., 2015. Schmallenberg virüsü. F Ü Sağ Bil Vet Derg, 29, 129-135.
20. Helmer C., Eibach R., Humann-Ziehank E., Tegtmeyer PC., Burstel D., Mayer K., Moog U., Stauch S., Strobel H., Voigt K., Sieber P., Greiner M., Ganter M., 2016. Seroprevalence of Schmallenberg virus infection in sheep and goats flocks in Germany, 2012-2013. VMS, 2, 10-22.
21. Lievaart-Peterson K., Luttikholt SJM., Van den Brom R., Vellema P., 2012. Schmallenberg virus infection in small ruminants – First review of the situation and prospects in Northern Europe. Small Ruminant Res, 106, 71-76.
22. Muskens J., Smolenaars AJG., van der Poel WHM., van Wuijckhuise L., Holzhauer M., van Weering H., Kock P., 2012. Diarree en productiedaling op Nederlandse melkveebedrijven door het Schmallenbergvirus. Tijdschr Diergeneeskd, 137, 112-115.
23. Hechinger S., Wernike K., Beer M., 2013. Evaluating the protective efficacy of a trivalent vaccine containing Akabane virus, Aino virus and Chuzan virus against Schmallenberg virus infection. Vet Res, 44, 114.
24. Martinelle L., Dal Pozzo F., Gauthier B., Kirschvink N., Saegerman C., 2014. Field veterinary survey on clinical and economic impact of Schmallenberg virus in Belgium. Transbound Emerg Dis, 61, 285-288.
25. Meroc E., De Regge N., Riocreux F., Caij AB., van den Berg T., van der Stede Y., 2014. Distribution of Schmallenberg virus and seroprevalence in Belgian sheep and goats. Transbound Emerg Dis, 61, 425-431.
26. Erel O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem, 37, 277-285.
27. Erel O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38, 1103-1111.
28. Buege JA., Aust SD., 1978. Microsomal lipid peroxidation. Methods Enzymol, 52, 302-310.
29. Miranda KM., Espey MG., Wink DA., 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5, 62-71.
30. Issi M., Gul Y., Yilmaz S., 2008. Clinical, haematological and antioxidant status in naturally poxvirus infected sheep. Revue Med Vet, 159, 54-58.
31. Aytekin I., Aksit H., Sait A., Kaya F., Aksit D., Gokmen M., Unsal Baca A., 2015. Evaluation of oxidative stress via total antioxidant status, sialic acid, malondialdehyde and RT-PCR findings in sheep affected with bluetongue. Vet Rec Open, 2: e000054.
32. Van der Poel WHM., Parlevliet JM., Verstraten ERAM., Kooi EA., Hakze-van der Honing R., Stockhofe N., 2014. Schmallenberg virus detection in bovine semen after experimental infection of bulls. Epidemiol Infect, 142, 1495-1500.
33. Kataria AK., Kataria N., 2012. Evaluation of oxidative stress in sheep affected with Paste des petits ruminants. JSPB, 8, 72-77.