



Oxidant-antioxidant status in bovine ephemeral fever

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ABSTRACT

Bovine ephemeral fever is an economically important viral disease of cattle and water buffalo. In this study, it was aimed to investigate oxidant-antioxidant status in cattle with ephemeral fever disease. A total of 32 Holsteins aged between 12 - 18 months kept under same care and feeding conditions were used. Activities of GPx, CAT and SOD and the levels of TAC, TOS, CP, SH and MDA were measured. Levels of CP and TAC were significantly ($p < 0,05$) higher in IG compared to HG. Levels of SH and CP were significantly ($p < 0,05$) lower and CAT activity were significantly ($p < 0,05$) higher in RG compared with IG. Levels of SH and activity of GPx and were significantly ($p < 0,05$) lower and CAT levels were significantly ($p < 0,05$) higher in RG compared with HG. The results of this experiment suggest a possible relationship between oxidant/antioxidant balance in favor of antioxidants in ephemeral fever in cattle.

Key words: Antioxidant, Bovine ephemeral fever, Oxidative stress, Oxidant.

INTRODUCTION

Bovine ephemeral fever (BEF) is a viral disease and can subclinically infect cattle species. The disease is characterized by fever, acute febrile reaction, stiffness and lameness. The disease causes major economic losses due to reduction in milk production in dairy herds (Nandi and Negi, 1999; Walker, 2005).

Despite intensive clinical and pathological studies on the BEF, the pathogenesis of the disease is not sufficiently clarified (Uren, 1989). The pathogenesis of the disease, it is complex and the release of cytokines leads to formation of inflammation. Although the virus does not cause extensive tissue damage, but in all cases an early neutrophilia is observed (Nandi and Negi, 1999).

During the last two decades, it has been clearly established that many infections trigger the production of reactive oxygen (ROS) species (Ivanov *et al.* 2017). A role of oxidants in the inactivation of viruses was first shown as early as 1970, but the metabolic role of oxidant in viral infection became apparent in recent years. The role of oxidants in viral diseases is more complex than microbial diseases and autotoxic effects because it includes metabolic regulation both in host metabolism and viral replication (Peterhans, 1997).

Reactive oxygen species (ROS) are produced in metabolic and physiological processes and they are removed via enzymatic and non-enzymatic antioxidant mechanisms.

Under certain conditions, the increase in oxidants and decrease in antioxidants cannot be prevented and the oxidant-antioxidant balance shifts towards oxidants. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides (Erel, 2005).

Concentrations of oxidants in plasma or serum such as malondialdehyde (MDA) can be measured separately in the laboratory (Nath *et al.* 2014; Sangha and Kalra 2016; Manat *et al.* 2017) since the effects of the oxidant components in plasma are additive, the measurement of the total oxidant status (TOS) accurately reflects the oxidant status of plasma or serum (Erel, 2005).

Blood contains many antioxidant molecules such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), Sulphydril groups (SH) and, Ceruloplasmin (CP) that prevent and/or inhibit harmful effects of free radical reactions (Erbay *et al.* 2003; Cemek *et al.* 2006; Işık *et al.* 2007). In order to determine the total antioxidant status (TAC) of the plasma measurement methods has been developed (Erel, 2004; Kosecik *et al.* 2005).

An increasing number of studies have been reported on the role of reactive oxygen species in the pathogenesis of viral diseases (Ivanov *et al.* 2017). Furthermore, the determination of oxidative stress may be necessary for clinicians to add antioxidant drugs in their treatment regime. However, no report on oxidant/antioxidant parameters in

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cattle with Ephemeral Fever has been found. Therefore, the aim of this study was to investigate the oxidant-antioxidant balance in cattle with ephemeral fever disease.

MATERIALS AND METHODS

A total of 32 Holsteins aged between 12 and 18 month kept in same care and feeding conditions were used. The control group consisted of 15 cattle determined to be healthy after undergoing clinical and serological examinations (healthy group - HG). The study group consisted of 17 cattle diagnosed to have ephemeral fever by clinical and serological examinations. Blood samples from study group were taken before treatment (infected group - IG) and at fifth day of treatment (recovery group - RG).

After clinical examinations, blood samples from vena jugularis were taken into heparinized tubes according to procedures and transferred to the laboratory in cold chain as soon as possible. Plasma was separated by centrifugation (3000 rpm / 5 min, at +4° C). Erythrocytes washed according to the method of Winterbourn (1975). The plasma and red cell portions were stored at -80°C until examination.

Presence of antibodies against EFV in the sera of infected and healthy animals was examined serologically by ELISA. Activity of GPx was assessed according to the method of Paglia and Valentine (1967), Catalase activity was assayed by the method of Aebi (1983). Activity of SOD was determined by the method of Sun *et al.* (1988). Hemoglobin level was measured by the method of ferrosiyanomethemoglobin developed by Tietz (1987). Levels of TAC, TOS and CP were measured by the methods of Erel (2004, 2005) while MDA levels was assessed according to the method of Yoshioka *et al.* (1979). Analysis of SH groups was performed according to the method of Hu *et al.* (1993), which was a modified method of Ellman (1959).

Drugs used in treatment of disease were Oxytetracycline (Panox 300, 1 ml/10 kg, Single dose -6 days effected, @Sanovel), Meloksikam (MaxicamX4, 1ml/40kg, Sanovel), Calcium gluconate (Cal-Vet, 1ml/kg, @Vilsan), B complex Vitamins (Bekombin, 5ml/100kg, @Topkim)

Statistical analysis was performed using SPSS 10.0 package program (SPSS Inc., Chicago, IL, USA). The

differences between HG and IG as well as RG were analyzed by using independent t-test. The differences between IG and RG were determined by paired test.

RESULTS AND DISCUSSION

In control group (HG) animals were found to be clinically healthy. In the study group (IG), where animals diagnosed with EF, a significantly ($p<0,05$) higher fever (up to 41 to 41.5°C), anorexia, rumen atony, increased heart rate and breathing rate, a serous nasal discharge, subcutaneous emphysema and muscle tremors were recorded. In addition to this lameness, stiff gait and hypocalcaemia-like symptoms where some of the animals in the supine position were observed in the clinical examination.

Activity of SOD, CAT, GPx and levels of MDA, SH, TAC, TOS, CP and the mean and standard error values of these parameters obtained from HG, IG and RG groups are presented in Table 1.

In the present study, oxidant / antioxidant balance shifted towards antioxidants in BEF. In this case, it may be considered that antioxidants were effective and blocked the formation of oxidative stress. Levels of CP and TAC levels were significantly higher in IG compared to HG. Levels of SH and CP were significantly lower and CAT activity were significantly ($p<0,05$) higher in RG compared with IG. Levels of SH and activity of GPx and were significantly ($p<0,05$) lower and CAT levels were significantly ($p<0,05$) higher in RG compared with HG. To the best of our knowledge, this is the first report showing some oxidant / antioxidant parameters in BEF.

SOD, GPx and CAT enzymes play an active role in the prevention of H₂O₂ destruction. The effect of SOD is very slow. GPx plays an active role in the hydrogen peroxide scavenging at low concentrations, while CAT plays an active role at high concentrations (Dündar and Aslan, 2000; Lobanova and Tahanovich, 2006). Similar to this information, CAT activity in RG found higher (statistically significant $p<0,05$) than in HG and IG. Activity of GPx in RG found lower than HG (statistically significant). It can be thought that although these animals have clinical recovered,

Table 1: Oxidant/Antioxidant parameters in cows with ephemeral fever.

Parameters	HealthyGroup (HG)	InfectedGroup (IG)	RecoveryGroup (RG)
GPX	3000.49±13978.95 ^a	2675.11±943.90	2203.34±869.04 ^b
CAT	702.87±170.21 ^a	632.18±171.28 ^a	890.06±310.22 ^b
SOD	296.47±87.69	243.94±136.66	348.00±140.37
SH	0.26±0.02 ^a	0.28±0.06 ^a	0.23±0.02 ^b
CP	406.38±171.61 ^a	632.15±109.4 ^b	438.09±140.54 ^a
MDA	4.89±1.89	4.74±2.41	3.62±1.8
TOS	13.21±6.93	13.84±8.59	9.23±6.16
TAC	0.91±0.08 ^a	1.09±0.29 ^b	0.91±0.22

*Different letters implicates statistically ($p<0,05$) difference between the groups

CAT: k/gHb, SOD: U/gHb, GPx: nmol/NADPH+H+/dak/mg-Hb, MDA:µmol/l

enzymatic antioxidant defense system in erythrocytes keeps being active to provide O/A Balance.

SH groups in serum act as important cellular scavengers of peroxides to protect cells from damage caused by these molecules. Decrease in the level of SH not only impairs cells' response to oxidants, but also changes the functions of inflammatory cells (McKeown *et al.* 1984). Some researchers (Erbay *et al.* 2003; Kahraman *et al.* 2003) found a significant reduction of SH groups in the presence of oxidative stress, whereas Ozkurt *et al.* (2000) reported no differences. In accordance with the studies of Erbay *et al.* (2003) and Kahraman *et al.* (2003), statistically significant ($p < 0,05$) decrease was observed in SH level in HG and IG compare to RG, in this study.

Ceruloplasmin, the acute phase protein, which increases the concentration by 50% in acute reactions, is an extracellular antioxidant that neutralizes superoxide radicals by binding free oxygen radicals and blocks their function (Akkus, 1995; Ay *et al.* 1998; Çelikezen and Ertekin, 2003). The effect of CP is supported by our results. Depending on the acute phase response CP level increased in IG and decreased in RG ($p < 0,05$). Among the antioxidants, CP plays more active role before treatment in EF infected cattle in this study.

Total antioxidant capacity reflects the cumulative action of all antioxidants. Therefore, TAC is a valuable parameter to evaluation of antioxidant capacity (Ghisellia *et al.* 2000). In this study, TAC level was found to be significantly ($p < 0,05$) higher in IG than HG. The results

suggested that the oxidative damage is prevented by increased antioxidant activity in BEF.

In the present study, there was no significant ($p > 0,05$) elevation in MDA and TOS levels, denoting that oxidative stress was not developed. This suggested that oxidative stress might be compensated within increased antioxidant activity (CAT, SH, CP, TAC in IG).

Measurements of free radical and antioxidant levels periodically, makes them more specific markers and the establishment of appropriate reference values, resistance to pathogens, geriatric process, physical fitness, physiological activity, exercise, productivity, diagnosis, prognosis, therapy, preventive therapy would be an important step in identifying the disease (Dündar and Aslan, 2000). As the symptoms and pathology of viral diseases are ultimately the result of complex host reactions in addition to direct viral effects, there is a scientific basis for this strategy of viral disease therapy (Peterhans, 1997).

The findings of this study suggest that significant antioxidant (GPx, CAT, SH, CP and TAC) responses occur in the ephemeral fever disease of cattle. It can also be said that the antioxidant response prevents the formation of oxidative stress. As a result, it can be said a possible relationship between oxidant/antioxidant balance in favor of antioxidants in ephemeral fever in cattle. However, in order to delineate the oxidant/antioxidant balance on the pathogenesis of BEF, further studies are necessary.

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