

## Nitric Oxide Levels, Total Antioxidant and Oxidant Capacity in Cattle with Foot-and-Mouth-Disease

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### Summary

The aim of this study was to investigate total antioxidant (TAC), oxidant capacity (TOC) and nitric oxide levels (NO) in cattle with foot-and-mouth-diseases (FMD). Twenty Swiss Brown cattle aged between 24 and 48 months were used. Animals were divided into two groups as control (n=10) and FMD (n=10). Blood samples were collected from jugular vein and centrifuged. TOC and NO levels were found to be significantly higher in FMD group compared to those of control group. However, no significant differences were present in TAC levels between FMD and control groups. It was concluded that FMD increases serum NO levels and TOC, but do not affect TAC in cattle.

**Keywords:** Cattle, Foot-and -Mouth Disease, Oxidative stress, Nitric oxide

## Şap Hastalıklı Sığırlarda Nitrik Oksit Düzeyi, Total Antioksidan ve Oksidan Kapasite

### Özet

Çalışmanın amacı şap hastalığına (FMD) yakalanmış sığırlarda total antioksidan (TAC), oksidan kapasite (TOC) ve nitrik oksit (NO) seviyelerini araştırmaktır. Araştırmada, yaşları 24 ve 48 ay arasında olan 20 Montofon ırkı sığır kullanıldı. Hayvanlar kontrol (n=10) ve şap hastalıklı (n=10) olmak üzere iki gruba ayrıldı. Kan örnekleri Vena jugularis'ten alınarak santrifuj edildi. Şaplı hayvanlarda TOC ve NO seviyeleri kontrol grubundaki hayvanlara göre önemli düzeyde yüksekti. TAC seviyesinde ise şap hastalıklı ve kontrol grubu arasında önemli bir değişiklik saptanmadı. Sonuç olarak şap hastalığının sığırlarda serum NO ve TOC seviyesini artırırken, TAC seviyesini etkilemediği belirlendi.

**Anahtar sözcükler:** Sığır, Şap, Oksidatif stres, Nitrik oksit

### INTRODUCTION

Foot-and-mouth-disease (FMD) is a highly contagious viral disease of all cloven-footed domestic and wild animals. The disease is distributed worldwide and is a major problem for cattle and sheep farmers. The virus, picarnovirus of genus aphthovirus, has a zoonotic potential and causes mild infection in farmers, veterinary surgeons, farm workers<sup>1-3</sup>.

FMD has been shown to alter both haematological and biochemical parameters in cattle. In infected animals, erythropeni, lymphositis, monositis and reduction in the serum concentrations of total protein, albumin, glucose, cholesterol, triglyceride and calcium have been reported elsewhere<sup>3-7</sup>.

Nitric oxide is a cytotoxic factor released by a variety of cells. It is generated from the terminal guanidine nitrogen atom of L-arginine by NO synthase<sup>8-11</sup>. Despite its role in the primary defence against bacteria, viruses and parasites<sup>12,13</sup>, it has also been reported to be immunosuppressive on immune system<sup>14,15</sup>. Therefore, NO may be protective or hazardous for mammalian tissues depending on concentration<sup>16</sup>. Oxidative stress is commonly observed in different pathological events of farm animals<sup>17</sup>. When the cellular oxidant state is overwhelmed by excessive production of reactive oxygen species and the condition may end up with cellular damage due to oxidative stress and lipid peroxidation<sup>18,19</sup>.



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These reactive oxygen species are eliminated through enzymatic and non-enzymatic antioxidative mechanisms<sup>18,20,21</sup>. It has been known that picornavirus induces NO synthesis in naturally infected cattle with FMD5. However, the relationship between NO and its antioxidant capacity on free radicals is not well-known in FMD. The aim of the study was to investigate total antioxidant, oxidant capacity and nitric oxide levels in cattle with foot-and-mouth-diseases.

## MATERIAL and METHODS

In this study, 20 Swiss-Brown cattle aged between 24-48 months old were used. Initially, animals suspected of FMD were tested serologically by the Etlik Central Veterinary Control and Research Institute, Ankara for FMD. Ten cattle were diagnosed with FMD, serotype Asia 1. Then, the blood samples were collected from the animals which were confirmed to have FMD. The remaining cattle were clinically healthy and used as control in the study. The blood samples were collected from all the animals and used to prepare serum samples. These samples were then used to determine NO levels, TAC and TOC.

Serum nitric oxide concentrations were determined using a spectrophotometer (PowerWave XS, BioTek, Instruments, USA) as described by Miranda and others<sup>22</sup>. Initially, serum samples were deproteinized with 10% zinc sulphate and serum nitrate was reduced to nitrite by vanadium (III) chloride. Total nitrite, an indicator of nitric oxide, were then determined calorimetrically using acidic Griess reaction<sup>22</sup>.

Serum total antioxidant and oxidant capacities were measured colorimetrically (PowerWave XS, BioTek, Instruments, USA) using a commercial kit (Rel Assay Diagnostic, Turkey). Trolox and hydrogen peroxide were used as standards to calculate for total antioxidant and total oxidant capacities, respectively<sup>18</sup>.

All values were expressed as mean±SEM. ANOVA and then Tukey test were used to analyze the significance of differences between the groups using SPSS Windows 10.0. The differences between the groups were considered significant if P value was less than 0.05 (P<0.05).

## RESULTS

Clinical examination revealed that the animals with FMD had characteristic clinical symptoms including fever, blisters or vesicles, erosions and ulcers in the mucosa of mouth, tongue, lips, gums and palate. Lesions were also observed in foot and teats. Furthermore, picornavirus serotype Asia 1 was also isolated and identified from all the animals having lesions of FMD. In the present study, TOC and NO levels were found to be significantly higher in FMD group compared to those of control group. However, no significant differences were obtained in TAC levels between FMD and control groups (Table 1).

**Table 1.** Serum nitric oxide levels, total antioxidant and oxidant capacity in cattle with Foot-and-Mouth-Disease and control groups

**Tablo 1.** Şap hastalıklı sığırlarda ve kontrol grubunda serum nitrik oksit seviyesi, total antioksidan ve oksidan kapasite

Parameters	Control (n=10)	FMD (n=10)	P
TAC (mmolTrolox Equiv./L)	0.79±0.047	0.66±0.051	Non significant
TOC (mmolH <sub>2</sub> O <sub>2</sub> Equiv./L)	7.15±0.66	11.76±0.84	<0.001
NO (mmol/L)	4.23±0.75 <sup>b</sup>	15.80±1.53 <sup>a</sup>	<0.05

\* Different letters in the same line indicate significant differences between the groups

## DISCUSSION

Nitrate and nitrite in serum are formed by the decomposition of NO<sup>23,24</sup>. Their concentrations in serum are used as a direct measure of NO production<sup>22</sup>. In the present study, a significantly high level of nitrate was determined in serum samples obtained from FMD group. The results of the present study indicate that picornavirus induce the production of NO *in vivo*. It is well-known that NO plays an important role in the primary defence mechanism against several bacteria<sup>25,26</sup>, viruses and parasites<sup>27,28</sup>. The production of NO is known to be induced by various viruses which inhibit virus replication *in vivo* and *in vitro*<sup>29,30</sup>. On the other hand, the protective or harmful effect of NO is suggested to be associated with the NO concentration<sup>16</sup>.

Oxidative stress is generally defined as imbalance between the oxidant and antioxidant molecules<sup>31</sup>. Peroxynitrite radical, a reactive molecule, is formed due to reaction of NO and super oxide anion during inflammation<sup>32</sup>. Peroxynitrite radicals are formed from the lipid peroxides and free radicals via oxidizing long chained lipid acids located on the cell membranes<sup>33-35</sup>. In the present study, serum TOC in FMD group was shown to be increased significantly, whereas TAC in FMD group was lower than in control group. However, the difference was not statistically significant. It can be speculated that increased levels of NO found in this study might be due to the production of oxidant molecules. Establishment of TAC values in a sample allow us to determine all of the exogenous and endogenous antioxidants on a large spectrum<sup>20</sup>. However, there was no correlation between TOC and TAC values obtained from FMD group.

In conclusion, presence of FMD in cattle increase serum NO and TOC values, but does not affect TAC values in these animals.

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