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Research Article

Effect of Vitamin C on Oxidant and Antioxidant Parameters in Cattle Immunized by Blackleg Vaccine

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ABSTRACT

The goal of this study is to determine the effect of vitamin C on certain oxidant and antioxidant parameters in cattle immunized against blackleg. In the study, a total of 40 cattle aged 12 to 18 months, of different breeds and genders, were used in the Ardahan region. The cattle were divided into four equal groups. The Control group received 2 mL of saline, the Vitamin group received 5 mg/kg dose of vitamin C, the Vaccine-Vitamin group received 2 mL of blackleg vaccine and 5mg/kg vitamin C, and the Vaccine group received 2 ml of blackleg vaccine. Blood samples were taken from all animals before vaccination (day 0) and on the 2nd, 14th, and 28th days of administration. On the 2nd, 14th, and 28th days, malondialdehyde (MDA) and nitric oxide (NO) levels were substantially increased (P<0.05) in the Vaccine group compared to the Control and Vitamin groups, while superoxide dismutase (SOD) and catalase (CAT) levels were considerably decreased (P<0.05). It was noted that the amount of MDA and NO measured in the 2nd, 14th, and 28th days in the Vaccine-Vitamin group reduced compared to the Vaccine group, while the amount of SOD and CAT enhanced. The study concluded that the blackleg vaccine administered to cattle causes oxidative stress and lipid peroxidation, and that vitamin C acts as an antioxidant. Vitamin C administration with blackleg vaccine in cattle is believed to be beneficial for lowering oxidative stress.

Keywords: *Blackleg vaccine, cattle, lipid peroxidation, oxidative stress, vitamin C.*

Yanıkara Aşısı ile Aşılanan Sığırlarda C Vitamininin Oksidan ve Antioksidan Parametreler Üzerine Etkisi

ÖZET

Bu araştırmanın amacı Yanıkara aşısı ile aşılanan sığırlarda C vitamininin bazı oksidan ve antioksidan parametreler üzerine etkisini belirlemektir. Araştırmada, Ardahan yöresinde ırk ve cinsiyetleri farklı, 12-18 aylık toplam 40 sığır kullanıldı. Sığırlar dört eşit gruba ayrıldı. Kontrol grubuna 2 mL serum fizyolojik, Vitamin grubuna 5 mg/kg dozda c vitamini, Aşı-Vitamin grubuna 2 ml yanıkara aşısı ve 5mg/kg C vitamini, Aşı grubuna ise 2 mL yanıkara aşısı enjekte edildi. Aşı ve ilaç uygulaması öncesinde (0.gün) ve uygulamanın 2., 14. ve 28. gününde tüm hayvanlardan kan örnekleri alındı. Uygulamanın 2., 14. ve 28. gününde Aşı grubunda malondialdehit (MDA) ve nitrik oksit (NO) düzeyinin Kontrol ve Vitamin grubuna göre anlamlı oranda (P<0,05) arttığı, süperoksit dismutaz (SOD) ve katalaz (CAT) düzeylerinin ise anlamlı oranda (P<0,05) azaldığı görüldü. Aşı-Vitamin grubunda 2., 14. ve 28. gün ölçülen MDA ve NO miktarının Aşı grubuna göre düştüğü, SOD ve CAT miktarının ise yükseldiği görüldü. Bu araştırmada, sığırlara uygulanan yanıkara aşısının oksidatif strese ve lipid peroksidasyonuna neden olduğu ve C vitamininin antioksidan etki gösterdiği sonucuna varıldı. Sığırlarda yanıkara aşısı ile birlikte C vitamini uygulamasının oksidatif stresi azaltma açısından faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: *C vitamini, lipid peroksidasyonu, oksidatif stres, sığır, yanıkara aşısı.*

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Introduction

Blackleg disease is an infectious disease caused by *Clostridium chauvoei* (*C. chauvoei*). The pathogen of the disease is a gram-positive, spore-forming, anaerobic bacterium. Bacteria are very resistant to adverse environmental conditions due to their spore forms. The spores taken in the organism go into a vegetative form when favourable conditions present themselves. Certain toxins are synthesized by bacteria which pass into the vegetative form. These toxins cause injury to regional tissues and death (Nicholson et al., 2019). High mortality rates lead to significant economic losses. In animal species, cattle are the most vulnerable to illness. Cattle between 6 and 30 months old are generally affected. Cases of disease have also been reported in pregnant bovine animals and foetuses (Abreu et al., 2017). It may also be observed in sheep and other domestic animals in addition to cattle (Frey and Falquet, 2015).

The disease begins in the muscle tissue. Sick animals may experience high fever, oedema and cremation in the damaged area (cracking due to gas accumulation). The disease progresses quickly and the affected area turns dark red-black. The muscular tissue is haemorrhagic and necrotic. Additionally, areas of diffuse fibrin can be seen in the epicardium and dark red multifocal areas in the myocardium. The prognosis for the disease is bad and usually causes death (Araujo et al., 2010; Abreu et al., 2017).

Cells require oxygen to carry out their metabolic activities. In oxygen utilization, certain free radicals (reactive groups) are released. The most important of these are superoxide (O_2), singlet oxygen (O^-), and hydrogen peroxide (H_2O_2) (Heidarpour et al., 2013). These oxygen groups interact with nucleic acids, enzymes, proteins, and lipids in cells and break down their structures (Deveci and Güven, 2008). The reaction of these reactive groups with fatty acids in the cellular membrane is known as lipid peroxidation. Malondialdehyde (MDA) is released as the final product in this reaction chain. MDA is known to be an indicator of cellular membrane degradation (Atakisi et al., 2010; Esmaeilnejad et al., 2018).

Nitric oxide (NO) is a molecule produced from L-arginine. It has important physiological functions such as the immune system, blood pressure regulation and nerve conduction in living organisms. The NO produced by macrophages in inflammatory cases plays a significant role in defence against pathogens (like viruses, bacteria, and parasites) (Bozukluhan et al., 2016). When nitric oxide is produced in excess, it changes to peroxinitrite. That metabolite suppresses the immune system (Bozukluhan et al., 2013). It also causes cellular damage and lipid peroxidation (Mavangira and Sordillo, 2018).

The quantity of reactive oxygen groups increases in mastitis, metritis, tissue damage, heavy exercise, pregnancy, and infections (Jaguezeski et al., 2018). As a result of overproduction of oxidant substances or inadequate antioxidant substances, these reactive substances cause cellular damage (Kırbaş et al., 2021). Such a condition is known as oxidative stress. It is claimed that living beings are more sensitive to some diseases (like sepsis and lung diseases) in the case of oxidative stress (Jaguezeski et al., 2018). The antioxidant defence mechanism is divided into non-enzymatic substances and enzymatic. Catalase (CAT) and superoxide dismutase (SOD) are enzymatic, vitamin C (ascorbic acid) is non-enzymatic antioxidants (Mavangira and Sordillo, 2018).

Vaccination is the main way to prevent blackleg disease, which progresses quickly and leads to the death of cattle (Mamak et al., 2018). The blackleg vaccine given to cattle is thought to cause oxidative stress and vitamin C can reduce oxidative stress. The study was carried out to define the effect of vitamin C on some oxidant and anti-oxidant parameters in cattle immunized with blackleg vaccine.

Materials and Methods

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (27.08.2020, 2020/120 Number Ethics Committee Decision) and the Ministry of Agriculture and Forestry (21.08.2020-E.29486769-622.01-2331582).

The research was conducted on 40 cattle of different races and genders, aged between 12 and 18 months, in the Ardahan region. The animals used in the study were maintained in the same environment and equal feeding conditions (tap water from the same resource and grassland and *ad libitum*) were applied. The cattle were divided into four equal groups, each consisting of 10 animals. The groups were the Control, Vitamin, Vaccine-Vitamin and Vaccine groups, respectively. 2 ml saline physiological solution (Polifleks-Polifarma®) was administered to the Control group and 5 mg/kg vitamin C (Maxivit-C®-baVET) was administered subcutaneously to the Vitamin group. Vitamin C was administered within the recommended dose range (4-6 mg/kg) in the drug (Maxivit-C-baVET®) prospectus. In the Vaccine-Vitamin group, 2 ml of blackleg vaccine (VBR CHAUVOEI®-Ata-Fen) and 5 mg/kg of vitamin C were injected subcutaneously different areas using separate injectors. In the Vaccine group, only 2 mL. of blackleg vaccine was given subcutaneously. 10 mL. blood samples were taken from the vena jugularis in the serum tube (BD Vacutainer® CAT) before the administration (day 0) and on the 2nd, 14th, and 28th days of the administration from all animals in the groups. The blood samples were centrifuged at 3000 rpm for 15 minutes. Then, the obtained serum stored at -20°C until the test.

Enzymatic activities of CAT and SOD in the serum were identified by ELISA (Epoch, Biotek, USA) with commercial kits (Cayman Chemical Company, USA). MDA analysis was determined as colorimetric (Epoch, Biotek, USA) using the methodology reported by Yoshioka et al. (1979) and NO was determined by Miranda et al. (2001).

In this study, the data was statistically evaluated using the statistical package program (IBM SPSS Statistics®, Chicago, IL, USA). The normal distribution of the data was verified using the Shapiro-Wilk assay. The Group means were compared with one-way analysis of variance (ANOVA). Tamhane's T2 and Tukey HSD tests were used to perform multiple group comparisons. The results obtained are presented in the form of a mean ($\bar{X}$) and standard deviation (SD). P<0.05 was found to be statistically important in this study.

Results

The results of the analysis of some oxidant and antioxidants obtained from this study are shown in Table 1 below.

Table 1. The analysis results of the cattle groups

Parameters	Days	Control (n=10) $\bar{X}$ ± SD	Vitamin (n=10) $\bar{X}$ ± SD	Vaccine-Vitamin (n=10) $\bar{X}$ ± SD	Vaccine (n=10) $\bar{X}$ ± SD	P-value
MDA (µmol/L)	0	1.89 ± 0.27	1.84 ± 0.39	1.95 ± 0.43	1.81 ± 0.27	0.815
	2	1.81 ± 0.23 [a]	1.77 ± 0.23 [a]	3.90 ± 0.37 [b]	4.03 ± 0.45 [b]	0.001
	14	1.92 ± 0.23 [a]	1.75 ± 0.35 [a]	4.32 ± 0.30 [b]	4.66 ± 0.82 [b]	0.001
	28	1.82 ± 0.38 [a]	1.86 ± 0.40 [a]	4.14 ± 0.37 [b]	4.49 ± 0.64 [b]	0.001
NO (µmol/L)	0	27.32 ± 4.59	31.66 ± 5.72	28.50 ± 6.28	31.28±5.03	0.228
	2	41.02 ± 7.70 [a]	34.86 ±5.82 [a]	49.45 ± 10.00 [b]	53.41 ± 9.62 [b]	0.001
	14	44.36 ± 9.80 [a]	36.71±5.46 [a]	53.64 ± 6.14 [b]	57.62 ± 10.54 [b]	0.001
	28	46.78 ± 11.14 [a]	35.98 ± 8.51 [a]	55.62 ± 9.58 [b]	62.70 ± 12.45 [b]	0.001
SOD (U/mL)	0	233.00 ± 15.37	230.82 ± 15.30	224.62 ± 14.40	227.59 ± 13.96	0.608
	2	236.42 ± 12.23 [a]	233.61 ± 16.70 [a]	201.33 ±12.55 [b]	174.82 ± 36.57 [b]	0.001
	14	241.97 ± 16.30 [a]	236.42 ± 12.16 [a]	186.54 ± 18.69 [b]	182.02 ± 33.92 [b]	0.001
	28	239.23 ± 16.70 [a]	230.60 ± 17.12 [a]	200.63 ± 29.33 [b]	194.76 ± 27.04 [b]	0.001
CAT (nmol/min/mL)	0	42.62 ± 9.68	41.34 ± 4.41	39.85 ± 7.84	43.92 ± 5.83	0.632
	2	46.82 ± 10.14 [a]	36.90 ± 3.95 [a]	29.35 ± 6.70 [b]	19.57 ± 3.76 [c]	0.001
	14	47.00 ± 9.12 [a]	40.07 ± 5.53 [a]	31.20 ± 7.35 [b]	15.44 ± 4.21 [c]	0.001
	28	43.21 ± 4.89 [a]	43.58 ± 4.33 [a]	35.55 ± 5.21 [b]	20.83 ± 5.26 [c]	0.001

[a, b, c] Those with different letters in the same row were statistically significant in the range of P<0.05

As shown in Table 1 above, for the zero day, there is no difference among the MDA, NO, SOD, and CAT amounts of the groups. However, on the 2nd, 14th, and 28th days, in the Vaccine group, the mean amount of NO and MDA increased substantially (P<0.05) compared to the Control and Vitamin groups, and insignificantly (P>0.05) compared to the Vaccine-Vitamin group. It is seen that the amount of SOD measured in the 2nd, 14th, and 28th days decreased significantly (P<0.05) in the Vaccine group compared to the Control and Vitamin groups, and insignificantly (P>0.05) compared to the Vaccine-Vitamin group. It is observed that the amount of CAT in the Vaccine group decreased significantly (P<0.05) on the 2nd, 14th, and 28th days compared to the Vitamin, Control and Vaccine-Vitamin groups. There was an important boost (P<0.05) in the CAT enzyme level in the Vaccine-Vitamin group compared to the Vaccine group during the 2nd, 14th, and 28th days.

Discussion

NO is a molecule synthesized by the nitric oxide synthetase (NOS) enzyme and has important biological functions. This molecule, whose half-life is very short, has significant physiological and metabolic functions in many species, from bacteria to mammals (Atakişi and Merhan, 2017). It is reported that the level of NO increases in foot-and-mouth disease (FMD) in cattle (Bozukluhan et al., 2013; Uzlu et al., 2016), mastitis (Atakisi et al., 2010), omphalitis (Bozukluhan et al., 2016) and vaccination with anthrax vaccine (Doğan et al., 2021). In this study, on 2nd, 14th, and 28th days, NO levels in the Vaccine-Vitamin group boosted considerably (P<0.05) relative to the Vitamin and Control groups (Table 1). The findings in this study are similar to those in the studies reported by Bozukluhan et al. (2013), Atakişi et al. (2010), Uzlu et al. (2016), Doğan et al. (2021), and Bozukluhan et al. (2016). This increase in nitric oxide can be explained by the antigenic characteristic of the blackleg vaccine. The bacterial blackleg vaccine (antigenic stimulus) is thought to activate macrophages and induce the synthesis and secretion of NO. The reactions between the antigen and the macrophages explain the increase in the quantity of NO. It was found that the amount of NO of days 2, 14, and 28 in the Vaccine-Vitamin group was reduced compared to the Vaccine group. This reduction in NO is due to the reaction of vitamin C with the oxidant groups (antioxidant effect).

MDA is the final product formed when free oxygen groups react with fatty acids in the cell membrane. Detection of MDA is accepted as an indicator of lipid peroxidation. In studies conducted on cattle, MDA levels were reported to be affected in some bacterial, viral, and parasitic diseases. It is reported that the level of MDA increases in skin papilloma (Arslan et al., 2018), *Fasciola gigantica* (Bahrami et al., 2014), Neosporiosis (Glombowsky et al., 2017), Coccidiosis (Yilmaz and Issi, 2014), Babesiosis (Saleh, 2009), Anaplazmosis (Esmaeilnejad et al., 2018), *Dictyocaulus viviparus* (Değer et al., 2008), *Hypoderma* spp (Merhan et al., 2017), Theileriosis (Kızıl et al., 2011), *Toxocara vitulorum* (Bozukluhan et al., 2017), Hidatidosis (Heidarpour et al., 2013), Tuberculosis (Kızıl and Keltek, 2017), listeriosis (Jaguezeski et al., 2018), FMD (Khoshvaghti et al., 2014; Uzlu et al., 2016), Omphalitis (Bozukluhan et al., 2016), Brucellosis (Perin et al., 2017), Mastitis (Deveci and Güven, 2008), anthrax vaccine administration (Doğan et al., 2021), Dystocia (Bayyit and Merhan, 2020), and transplanted cattle (Chirase et al., 2004).

In this study, it was noted that the MDA quantity for the 2nd, 14th, and 28th days of the Vaccine group boosted significantly ($P<0.05$). The findings in this study are similar to those reported by Bahrami et al. (2014), Glombowsky et al. (2017), Perin et al. (2017), Yılmaz and Issı (2014), Saleh (2009), Arslan et al. (2018), Esmaeilnejad et al. (2018), Değer et al. (2008), Merhan et al. (2017) Kızıl et al. (2011), Heidarpour et al. (2013), Bozukluhan et al. (2017), Kızıl and Keltek (2017), Jaguezeski et al. (2018), Khoshvaghti et al. (2014), Uzlu et al. (2016), Bozukluhan et al. (2016), Deveci and Güven (2008), Doğan et al. (2021), Bayyit and Merhan (2020), and Chirase et al. (2004). This increase in the amount of MDA can be explained by the fact that oxidant substances produced during the recognition and processing of antigens by macrophages cause lipid peroxidation. Because these oxidants, which are produced in excess, react with unsaturated fatty acids in the cellular membrane. MDA, which is produced in excess through a series of reactions, is released into the bloodstream. Based on the increase in the amount of MDA in the blood, it can be suggested that the blackleg vaccine causes lipid peroxidation.

CAT is a significant enzyme whose antioxidant properties are present in the body. It is reported that the amount of CAT in cattle decreases in Tuberculosis (Kızıl and Keltek, 2017), Coccidiosis (Tufan and Çam, 2008), *Theileria annulata* (Kızıl et al., 2011), *Dictyocaulus viviparus* (Değer et al., 2008), *Listeria monocytogenes* (Jaguezeski et al., 2018), Brucellosis (Perin et al., 2017), *Anaplasma marginale* (Esmaeilnejad et al., 2018), Etiyonin (Abd Ellah et al., 2009), and administration anthrax vaccine (Doğan et al., 2021). In this study, on the 2nd, 14th, and 28th days, the amount of CAT in the Vaccine group declined substantially compared to the Control, Vitamin, and Vaccine-Vitamin groups. The study results are similar to those studies results reported by Kızıl and Keltek (2017), Tufan and Çam (2008), Esmaeilnejad et al. (2018), Değer et al. (2008), Jaguezeski et al. (2018), Perin et al. (2017), Kızıl et al. (2011), Abd Ellah et al. (2009), and Doğan et al. (2021). The reduction in the amount of CAT may be due to the depletion of the CAT enzyme reacting with the increasing oxidant groups after vaccination.

In the present study, on the 2nd, 14th, and 28th days, the amount of CAT in the Vaccine-Vitamin group boosted substantially compared to the Vaccine group (Table 1). The reason for this boost in the CAT quantity is thought to be due to the antioxidant property of vitamin c. As ascorbic acid reacts with oxidant groups and reduces their effect. This means that the CAT enzyme is less used in the Vaccine-Vitamin group than in the Vaccine group. It is reported that the amount of CAT in cattle increases when FMD vaccine is administered (Kızıl and Gül, 2004) while does not change significantly in Papillomatosis (Arslan et al., 2018) and FMD (Khoshvaghti et al., 2014). These differences in the CAT level are thought to be because of the content of the vaccines used (such as viral, bacterial, live, attenuated, inactive, and adjuvant), care and nutritional conditions.

One of the antioxidant enzymes is SOD. It transforms superoxide into hydrogen peroxide (H_2O_2). The resulting hydrogen peroxide loses its harmful effects by transforming into water and oxygen by means of the CAT enzyme (Aslankoç et al., 2019). It is reported that the amount of SOD in cattle decreases in Coccidiosis (Tufan and Çam, 2008), FMD (Khoshvaghti et al., 2014), *Fasciola gigantica* (Bahrami et al., 2014), *Anaplasma marginale* (Esmaeilnejad et al., 2018), Hidatidosis (Heidarpour et al., 2013), *Dictyocaulus viviparus* (Değer et al., 2008), administration anthrax vaccine (Doğan et al., 2021), and Bovine Leukemia Virus (Ali et al., 2019).

In this study, on the 2nd, 14th, and 28th days, the SOD quantity in the Vaccine group decreased substantially ($P<0.05$). Results from this study are similar to those reported by Bahrami et al. (2014), Tufan and Çam (2008), Doğan et al. (2021), Khoshvaghti et al. (2014), Esmaeilnejad et al. (2018), Heidarpour et al. (2013), Değer et al. (2008), and Ali et al. (2019). Based on the decrease in the amount of SOD in the Vaccine group, it can be claimed that the blackleg vaccine enhanced the amount of superoxide. The amount of SOD that reacts with the excess superoxide radical is reduced. Naturally, serum level also decreases. This reaction explains the decrease in the amount of SOD in the Vaccine group.

It is reported that the amount of SOD increases in Brucellosis (Perin et al., 2017), *Tenia saginata* (Łuszczak et al., 2011), septicemia (Meral et al., 2017), and cattle administered Ethionine (Abd Ellah et al., 2009). In another study, it is reported that vitamin c does not affect the amount SOD in cattle vaccinated with FMD vaccine (Kızıl and Gül, 2004). Variable amount of SOD in studies conducted on cattle is thought to be caused by the formulation of the vaccine, the kind of infection (like bacterial, viral, and parasitic), course of infection (acute, subacute or chronic), and care and food conditions.

Conclusion

In conclusion, in cattle vaccinated with blackleg vaccine, on the 2nd, 14th, and 28th days following the vaccination, it is observed that the amounts of NO and MDA boosted, the amount of CAT and SOD reduced. Based on the findings, it could be argued that blackleg vaccines in cattle lead to oxidative stress and peroxidation of lipids. It was noted that the amounts of MDA and NO in the Vaccine-Vitamin group reduced and the amounts of SOD and CAT boosted. It is recommended to use vitamin c with the blackleg vaccine in cattle.

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Conflict of interest

The authors declare that they have no conflict of interest.

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