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ORIGINAL ARTICLE

Malondialdehyde and Total Antioxidant Levels and Hematological Parameters of Beef Cattle with Coccidiosis

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SUMMARY

The present study evaluates the malondialdehyde (MDA) level of serum lipid peroxidation and total antioxidant capacity in order to determine the effects of coccidiosis on oxidative stress in beef cattle. It will be determined in this study whether lipid peroxidation plays a role in the pathogenesis of the disease. This study included 10 beef cattle diagnosed with coccidiosis via clinical and stool examinations (Patient group) and 10 healthy matched animals from the same barn (Control group). General clinical findings (body temperature, heart and respiration rates, and rumen movements), hematological parameters (total leukocyte count, erythrocyte count, hematocrit value and hemoglobin count), and serum MDA levels and total antioxidant capacities were determined. A significant increase was observed in the body temperature, heart and respiration rates, total leukocyte count, and serum MDA levels of the patient group compared to the controls, while the rumen movements, hematocrit and hemoglobin values, and serum total antioxidant capacities showed significant reductions ($P<0.05$). The results of the present research suggest that prophylactic measures should be taken in animals with coccidiosis, as the defense system might be infected due to the increase in MDA level. It will be shown whether these parameters can be taken as references with other parameters in order to make diagnosis as well as the efficiency of treatment.

Key Words *Coccidiosis, Beef cattle, Malondialdehyde, Total antioxidant capacity*

Koksidiyozisli Besi Danalarında Malondialdehid ve Total Antioksidan Düzeyleri ile Hematolojik Parametreler

ÖZET

Bu çalışmada, koksidiyozisin oksidatif stres üzerine etkilerini araştırmak için serum lipid peroksidasyon ürünü olan malondialdehid (MDA) düzeyi ile total antioksidan kapasitesinin değerlendirilmesi ve lipid peroksidasyonun hastalığın patojenezinde etkisinin araştırılması amaçlanmıştır**.** Klinik ve dışkı muayenesinde koksidiyozis teşhisi konan 10 hayvan (hasta grubu) ile birlikte aynı ahırda sağlıklı oldukları belirlenen benzer özellikteki 10 hayvandan (kontrol grubu) oluşan toplam 20 baş besi danası araştırma materyalini oluşturmuştur. Tüm hayvanların genel klinik muayene bulguları (vücut sıcaklığı, kalp ve solunum frekansı ile rumen hareketleri) ve hematolojik parametreleri (total lökosit sayısı, eritrosit sayısı, hematokrit değer ve hemoglobin miktarı) ile serum MDA düzeyleri ve total antioksidan kapasiteleri belirlenmiştir. Hasta grubunda vücut sıcaklığı, kalp frekansı, solunum frekansı, total lökosit sayısı ve serum MDA düzeylerinin istatistiksel olarak önemli derecede arttığı, rumen hareketi, hematokrit ve hemoglobin değeri ile serum total antioksidan kapasitesinin düştüğü görülmüştür ($P<0.05$). Sonuç olarak, MDA düzeyinin artmasına bağlı olarak savunma sisteminde bozulma olabileceğinden koksidiyozisli hayvanlarda profilaktik tedbirlerin alınmasının gerekli olduğu kanaatine varılmıştır. Bu parametrelerin tedaviyi etkin hale getirmenin yanı sıra tanı koymak için diğer parametreler ile birlikte referans olarak alınabileceğini gösterebilir.

Anahtar Kelimeler *Koksidiyozis, Besi sığırı, Malondialdehid, Total antioksidan kapasite*

INTRODUCTION

Coccidiosis is caused by parasitic protozoa belonging to the *Eimeria* and *Isospora* species of the family *Eimeridae*. Coccidiosis is relatively common around the world and is especially seen in poultry, calves, sheep, goats, dogs, cats, pigs, and rabbits. Although it occurs in calves at all ages, it can be particularly severe in calves older than 3 weeks and progresses to bloody diarrhea. Sporulated *Eimeria* oocysts enter the bodies of animals through the mouth with water or feed. The severity of the infection depends on the number of oocysts (Urquhart et al. 1987; Aiello and Mays 1998; Anderson et al. 2009; Smith 2009).

Coccidiosis is a significant and severe disease in that it causes growth retardation and death in animals, large economic losses due to treatment costs including drugs and veterinary personnel and the survivors remain carriers (preimmunization) (Aiello and Mays 1998; Anderson et al. 2009; Smith 2009).

Blood plasma includes iron-binding antioxidant proteins such as transferrin and ceruloplasmin, and chain-breaking

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antioxidants that directly scavenge free radicals. The relative contribution of each in vivo antioxidant depends on its efficiency and its concentration in biological fluids. Albumen, uric acid, and ascorbic acid contribute significantly to total antioxidant capacity (>85%). This superiority depends to a great extent on their relatively high concentrations compared to other antioxidants in the blood (bilirubin, alpha-tocopherol, beta-carotene etc.). Although individual antioxidants are crucial for the defense system, together they might result in a synergistic protection for the organs against in vivo oxidative damage. Therefore, it is significant to measure total antioxidant capacity in order to evaluate the antioxidant defense system (Erel 2004).

Measuring antioxidant capacity reflects the cumulative effect of all antioxidants present in plasma and body fluids. Therefore, this is a more complete value than the sum of separate measurable antioxidants. This method enables the sensitive balance between in vivo oxidants and antioxidants to be acknowledged, as it measures known and unknown antioxidant capacities and synergistic interaction (Ghiselli et al. 2000).

Previous studies Kolodziejczk et al. (2006), reported that some parasitic infections result in changes in lipid peroxidation. Fascioliasis was reported to reduce the activities of erythrocyte glutathione peroxidase, catalase, and superoxide dismutase in animals and humans; this reduction is suggested to be related to the declination of antioxidants as a result of liver damage or to ultra-free radicals produced during the infection. Although some studies have evaluated the changes in lipid peroxidation parameters of intestinal coccidiosis caused by *Eimeria tenella* (Eraslan et al. 2004) and *Eimeria acervulina* (Koinarski et al. 2005) in poultry; and of liver coccidiosis caused by *Eimeria stiedae* (Cam et al. 2008) in rabbits; no previous studies have evaluated the lipid peroxidation level and antioxidant activities of calves. Thus, the present study evaluates the MDA level of serum lipid peroxidation and total antioxidant capacity in order to determine the effects of coccidiosis on oxidative stress in beef cattle. It will be determined in this study whether lipid peroxidation plays a role in the pathogenesis of the disease.

MATERIALS and METHODS

A one-year old Montafon hybrid beef cattle from Yazıkonak, Turkey, was brought to the Internal Medicine Clinic of the Faculty of Veterinary, Fırat University for examination and treatment for bloody diarrhea. Following clinical and stool examinations, the animal was diagnosed with coccidiosis. The study then selected 20 out of 50 age-matched beef cattle from the same barn for the purpose of the research, 10 of which suffered from bloody diarrhea and were diagnosed with coccidiosis via stool examination (using native and flotation techniques) (Patient group) and 10 of which were matched, healthy animals (Control group).

Following general clinical examinations of the animals, stool samples were taken from the rectum for parasitological examination. The samples were examined using stool examination techniques, simple, and flotation techniques, in order to determine the *Eimeria* oocysts (Turgut 2000; Dincer 2001; Lucas et al. 2006). A standard, improved Modified McMaster method was used to detect and enumerate the coccidial oocysts, with a lower detection limit of 50 oocysts per gram of faeces.

Blood samples from *V. jugularis* were taken into EDTA tubes for hematological examinations and into sterile glass tubes to determine the total antioxidant levels. After the serum was separated from the blood samples in sterile glass tubes, it was kept at 80 °C prior to analysis. The total leukocyte and erythrocyte counts in the blood samples were determined using Thoma lamina and lamella, the hemoglobin count was determined according to Sahli's method, and microhematocrit values were determined using capillary tube method (Schalm et al. 1986). The serum MDA count used the spectrophotometric method developed by Placer et al. (1966), which was based on the principle of measuring the optical density of the color of MDA in a thiobarbituric acid environment at 532 nm.

Serum total antioxidant capacity measurement were used the total antioxidant activity method identified by Erel (2004) and it was performed using commercial kits owned by Randox (Randox Laboratories, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY). According to this method, hydroxyl radicals were produced by the Fenton reaction; they reacted with o-Dianisidine, a colorless substrate in order to produce the flavescent dianisyl radical; this reaction resulted in color changes, which were measured to determine the total antioxidant capacity. The oxidative reaction began with the present hydroxyl radicals in the reaction when the plasma samples were added; and it was prevented by the antioxidant components of the plasma, thereby inhibiting the color changes that indicate the total antioxidant capacity of the plasma. The results of the measurements were unitized as µmol Trolox equivalent/L.

The findings determined in all the samples were given as mean values (X±S.E.M). The groups were compared using the t-test, and with the SPSS/PC software (version Windows 21.0).

RESULTS

It was observed during systemic clinical examination that the animals in the patient group were sluggish and inappetent for 2–3 days, with bloody diarrhea; and frequently strained in the meantime, but excreted little or no stool. All the animals in the patient group also lost weight, their mucous membranes were pale, perineum, tails, perinea and hind legs were smudged with bloody diarrhea, and there were some symptoms of dehydration at different levels (light dehydration in 5 of the animals, medium dehydration in 3 of the animals and severe dehydration in 2 of the animals). Animals suffering from severe dehydration were observed to have difficulty in standing. While no parasite egg or oocyst was observed in the stools of the animals in the control group, animals in the patients group were found to have *Eimeria* spp. oocysts.

Arithmetic mean values of general clinical parameters (body temperature, heart and respiration rates, and rumen movements), hematological parameters (total leukocyte count, erythrocyte count, hematocrit value and hemoglobin count), serum MDA levels and total antioxidant capacities, and the significance level of the differences between the groups are shown in Tables 1, 2 and 3.

Table 1. General clinical examination findings of animals in control and patient group

Parameters	Control Group	Patient Group
Body temperature (°C)	38.51±0.091	39.13±0.10*
Heart rates (/min.)	75.50±1.18	82.00±1.51*
Respiration rates (/min.)	25.50±0.73	32.00±1.06*
Rumen movement (/5 min.)	8.63±0.18	5.00±0.26*

* Significant difference between groups are indicated by different letters on the same line (P<0.05).

Table 2. Hematological parameters of animals in control and patient group

Parameters	Control Group	Patient Group
Total leukocyte count ($x10^9$/L)	7.65±0.14	8.15±0.12*
Erythrocyte count ($x10^{12}$/L)	7.02±0.23	6.39±0.26
Hematocrit value (%)	32.00±0.62	30.38±0.41*
Hemoglobin count (g/dL)	10.18±0.088	8.55±0.13*

Table 3. Serum MDA levels and total antioxidant capacities of animals in control and patient group

Parameters	Control Group	Patient Group
MDA (nm/mL)	7.96 ± 0.25	10.29 ± 0.69*
Total antioxidant capacity (µmol Trolox equivalent/L)	11.04 ± 0.52	5.75±0.74*

DISCUSSION and CONCLUSION

Parasitic diseases are a major problem in cattle breeding, are common around the world. They result in large economic losses as they reduce efficiency and lead to mortality. Although coccidiosis, one of the most significant parasitic diseases among animals, is seen in old animals as latent infections, it is clinically significant in young animals. Although deaths mostly occur in the acute stage (3–4 days), the disease lasts for about 3–4 weeks. Mortality rate vary between 10 and 50%. Survivors suffer from growth retardation (Urquhart et al. 1987; Aiello and Mays 1998; Radostits et al. 2008).

All the mean parameters determined in the control group (Tables 1–3) were within normal physiological ranges, identified in the sources for healthy animals (Aiello and Mays 1998; Radostits et al. 2008; Smith 2009).

Coccidia species are quite common among calves in Turkey; in clinical cases, *Eimeria* spp. were present at up to 90.8% in calves, and between 38.3% and 86.4% in 6–12-month-old calves (Georgi and Theodories 1980). The age group in which the disease is most common covers the age range of the calves in the patient group.

The disease is diagnosed when the *Eimeria* oocysts are identified via clinical and stool examination, as identified in the literature (Urquhart et al. 1987; Aiello and Mays 1998; Radostits et al. 2008; Anderson and Rings, 2009; Smith 2009). Depression, anorexia, growth retardation and especially tenesmus and bloody diarrhea, as diagnosed from clinical examinations of the patient group correspond to the findings identified in the literature (Aiello and Mays 1998; Radostits et al. 2008; Anderson et al. 2009; Smith 2009).

In experimental studies, a decrease in the hematocrit values of animals infected with coccidiosis has been observed (Aumot et al. 1986; Dharmendra Kumar et al. 1999). In our study was observed to decrease in hematocrit values, hemoglobin values, while an increase in leukocytes as Mimioğlu et al. (1969), Stockdale et al. (1981), Ozer et al. (1995) and Aumont et al. (1986) reported. The significant reduction of red blood cells and hemoglobin content might be attributed to the haemorrhagic enteritis associated with coccidiosis. Decrease haematological values in infected beef cattle can be attributed to intestinal tissue damage due consequent blood loss caused by endogenous stages of Eimerian parasites. Leukocyte levels were very high in beef cattle with coccidiosis in this study. This was attributed to the severe tissue damage in the intestine and fever. Radostist et al. (2008) reported that tissue damage and fever can cause leukocytosis.

It has been reported to be a moderate changes in blood parameters in natural and experimental infections in bovine coccidiosis (Mimimoğlu et al. 1969; Daugschies and Najdrowski 2005). Mimimoğlu et al. (1969) reported coccidiosis would change the blood picture, sometimes eritropeni and decrease in the amount of hemoglobin can be seen.

Stockdale et al. (1981) reported to be decrease in the red blood cell count, hemoglobin and hematocrit values of calves experimentally infected with *E. Zuerni*. Özer et al. (1995) reported to be anemia and decrease in the hemoglobin concentration of lambs infected with coccidiosis. In a study (Aydın and Aslan 2012) were observed leukopenia in 5 of calves (33.4%), leukocytosis in 5 of calves with coccidiosis (33.4%). These findings showed to changes in blood lymphocytes levels according to intensity of infective sporozoites or time periods to intestinal settlement of the genus Eimeria. Daugschies and Bangoura (2007) have described that with the progress of the inflammation to leukocytosis seen in their experimental studies with *E. Zuerni* have described that with the progress of the fire.

Although the increase in the leukocyte count and the decrease in the hematocrit value and hemoglobin count in the patient group are statistically significant (P<0.05), they are determined to be within normal physiological ranges. The increase in the total leukocyte count is considered to result from secondary infections (gastrointestinal infections), and the decrease in the hematocrit value and hemoglobin count are considered to result from hemorrhage.

Lipid peroxidation occurs when the formation of free radicals exceeds the value compensated by the cells and the cell defense systems are insufficient to destroy these compounds (Halliwell and Chirico 1993; Halliwell 1999). The most significant symptom of lipid peroxidation caused by free radicals is MDA, causing peroxidation. Changes in the tissue and blood MDAs might reflect the progress and severity of the peroxidation (Gtterridge and Halliwell 1993; Halliwell and Chirico, 1993; Halliwell 1999; Yılmaz and Yılmaz, 2006).

In this study, while the level of MDA showed an increase, the significant decrease was detected, regarding total antioxidant capacity in animals with coccidiosis. These changes indicated that *Eimeria* led to lipid peroxidation during the specified period. This implies that the affected beef cattles are under stress condition. In a previous study, some parasitic infections produced changes in lipid peroxidation parameters. Koinarski et al. (2005), reported that plasma MDA concentration increased and superoxide dismutase activity decreased in birds infected with *Eimeria acervulina*. When the antioxidant/prooxidant balance is disrupted, oxidative stress occurs because of the

infection. The increase in the plasma MDA levels in the liver coccidiosis caused by *Eimeria stiedae* in rabbits resulted in lipid peroxidation, which causes liver parenchyma and the destruction of the bile duct. It was determined that the infection caused by *Eimeria stiedae* influenced clinical, hematological, biochemical, lipid peroxidation and pathological findings, that the toltrazuril is significant in the treatment of hepatic coccidiosis; and that ivermectin is ineffective when used alone or in combination with toltrazuril (Cam et al. 2008).

There are many cell defense systems preventing the oxidative damage caused by free radicals. Determining the plasma antioxidant capacity helps identify situations in which in vivo oxidative condition changes (exposure to ROS and intake of antioxidants). The method for total antioxidant capacity has recently been developed and reflects the total antioxidant characteristics of all antioxidants. As measuring all of these antioxidants individually and determining their inter-relationships is challenging, the total antioxidant method is used (Ghiselli et al. 2000; Ching et al. 2002).

Argenzio and Rhoads (1997) reported a decrease in the catalase activity of cryptosporidiosis in pigs. In the present study, the evident decrease in the erythrocyte catalase activity despite the increase in the plasma MDA level might indicate that oxidative stress develops during the infection and that the antioxidant/prooxidant balance then favors prooxidants.

Dede et al. (2000), determined that the MDA level increased significantly in Akkaraman sheep infected with *Fasciola* spp.*, Trichostrongylidae,* and *Eimeria* spp., and that glutathione and vitamin C levels decreased; they suggested that endoparasitic infections might be one of the significant causes of oxidative stress. MDA levels of birds infected with *Eimeria tenella* increased, whereas SOD activities decreased and catalase activity increased (Georgieva et al. 2006). In the present study, a significant increase was found for serum MDA level in the patient group, which corresponds to the findings in the literature. This increase in the plasma MDA level indicates that excessive free radicals are formed during the disease and that the coccidiosis causes lipid peroxidation. According to the present research, clinical findings and the high MDA level indicate a severe infection causing oxidative stress.

In the suffering from stressful conditions, due to parasitism, reported high level of MDA in rats following Fasiola infestation. Fascioliasis was reported to reduce the activities of erythrocyte glutathione peroxidase, catalase and of superoxide dismutase in animals and humans. This reduction was suggested to be related to the declination of antioxidants as a result of liver damage, or to ultra-free radical production during the infection (Kolodziejczyk et al. 2006). In the present research, the fact that the total antioxidant capacity in calves with coccidiosis is less than that of the healthy group indicates that free radicals are formed at a higher rate than is compensated by the cell defense systems, and therefore free radicals cannot be sufficiently transformed into less harmful or inefficient metabolites.

Dede et al. (2002) reported that the parasites (*Trichostronglylidae* sp. + *Eimeria* sp. + *Babesia* sp.), which were detected in infected goats, induced the lipid peroxidation. Pabon et al. (2003) reported that malaria, a protozoal disease, increased the MDA level, but caused the decrease in the antioxidant enzyme levels in humans. Similarly, Erel et al. (2001), showed that the antioxidant enzyme level decreased and in contrast, the LPO level increased in the patients with vivax malaria. The data obtained by the present study were consistent with the results of the studies mentioned above. Eventually, *Eimeria* oocysts provoked the lipid peroxidation. The occurrence of oxidative damage may be contributed directly to the mechanism of negative effects of the disease.

The results of the present research suggest that prophylactic measures should be taken in animals with coccidiosis, as the defense system might be infected due to the increase in MDA level. The investigated parameters, both the MDA level and total antioxidant capacity, may be taken into consideration with other parameters in the case of *E. tenella* infection, in order to determine the severity of the infection and the prognosis of the disease.

The results of the present research suggest that prophylactic measures should be taken in animals with coccidiosis, as the defense system might be infected due to the increase in MDA level. It will be shown whether these parameters can be taken as references with other parameters in order to make diagnosis as well as the efficiency of treatment.

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