

Investigation of Oxidative Stress Index and Lipid Profile in Cattle with Brucellosis

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Abstract

The aim of this study was to define the oxidative-antioxidative capacity and lipid profile in cattle with brucellosis. A total of 32 cattle of Simmental breed were used in the study, among those, 22 demonstrated microbiologically positive for brucellosis while 10 were negative. Biochemical analysis included total oxidant capacity (TOC), total antioxidant capacity (TAC), oxidative stress index (OSI), triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C). TAC value was found to be statistically significantly lower ($P=0.007$), and TOC and OSI values were found to be significantly higher [respectively ($P=0.013$) and ($P=0.002$)] in cattle with brucellosis compared to the control group. TC ($P=0.012$) and LDL-C ($P=0.0004$) values were significantly increased, and TG ($P=0.004$), HDL-C ($P=0.023$) and VLDL-C ($P=0.004$) values were significantly decreased compared to the control group. As a conclusion, it was determined that *Brucella* infection leads to important changes in the oxidative-antioxidative capacity and lipid profile in cattle. These data may contribute to the diagnosis of the disease and especially to the determination of inflammation severity.

Keywords: *Brucellosis, Cattle, HDL, LDL, Total antioxidant capacity (TAC), Total oxidant capacity (TOC)*

Brusellozisli Sığırlarda Oksidatif Stres İndeksi ve Lipid Profilinin İncelenmesi

Özet

Bu çalışmanın amacı, brusellozisli sığırlarda oksidatif-antioksidatif kapasite ve lipid profilinin belirlenmesidir. Çalışmada mikrobiyolojik analiz sonrası brusellozis tespit edilen 22 ve negatif olan 10, toplamda 32 Simmental ırkı inek kullanıldı. Biyokimyasal olarak total oksidan kapasite (TOK), total antioksidan kapasite (TAK), oksidatif stres indeksi (OSİ), trigliserid (TG), total kolesterol (TC), yüksek yoğunluklu lipoprotein-kolesterol (HDL-C), çok düşük yoğunluklu lipoprotein-kolesterol (VLDL-C) ve düşük yoğunluklu lipoprotein-kolesterol (LDL-C) değerleri belirlendi. Brusellozisli sığırlar ile kontrol grubu karşılaştırıldığında TAK değerinin istatistiksel olarak anlamlı bir şekilde düştüğü ($P=0.007$), TOK ve OSİ değerlerinin ise istatistiksel olarak anlamlı bir şekilde artmış olduğu, [sırasıyla ($P=0.013$) ve ($P=0.002$)] tespit edildi. Yine TC ($P=0.012$) ve LDL-C ($P=0.0004$) değerlerinin kontrol grubuna göre istatistiksel olarak anlamlı arttığı, TG ($P=0.004$), HDL-C ($P=0.023$) ve VLDL-C ($P=0.004$) değerlerinin istatistiksel olarak azalmış olduğu belirlendi. Sonuç olarak, brusella enfeksiyonunun sığırlarda oksidatif-antioksidatif kapasitede ve lipid profilinde önemli değişikliklere neden olduğu belirlendi. Elde edilen bu bulgular özellikle hastalığıdaki yangı şiddetinin belirlenmesine ve diagnozuna katkıda bulunabilir.

Anahtar sözcükler: *Brusellozis, HDL, LDL, Sığır, Total antioksidan kapasite (TAK), Total oksidan kapasite (TOK)*

INTRODUCTION

Brucellosis is an infectious disease of farm animals that leads to important economic losses, characterized by chronic inflammation of various organs and abortion ^[1]. In cattle,

disease agent is a gram negative, facultative intracellular bacterium, *B. abortus*. Cases originated from *B. melitensis* have been also reported scarcely ^[2]. Veterinarians, farm workers, butchers and shepherds are under risk since they are in close contact with the animals ^[3]. Infection is caught



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through the digestive system, conjunctiva, respiratory system or through the skin, and is carried to the regional lymph nodes, then to the spleen, liver, bone marrow, central nervous system and urogenital organs via lymph and blood circulation [4]. Although brucellosis is a multi-systemic disease, most common symptoms are abortion, retentio secundinarum, metritis-endometritis, reduced milk yield, weak and/or dead infant birth, infertility, fever, arthritis, bursitis, epididymitis and orchitis [2]. The most risky situation currently is caused by animals infected with *Brucella*. Contaminated food obtained especially from infected ruminants (milk, cheese, etc) forms risk for human health [1,4].

Free oxygen radicals lead to damage in biological molecules (lipid, proteins and DNA), prevent normal functioning of the cells and lead to increased level of various metabolites which are final products of lipid peroxidation [5,6]. Cells and tissues have antioxidant systems that inhibit radical products and reactions. Studies have demonstrated altered total oxidant (TOC) and total antioxidant capacity (TAC), or oxidative stress index (OSI) in case of local and systemic inflammation or infection [5,7,8].

Lipids contribute to the formation of energy, steroid hormones and biliary acids. They are formed of lipoproteins, esterified/non-esterified cholesterol, triglycerides (TG), phospholipids, lipids and proteins. Lipoproteins such as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) have various functions in the organism. These lipoproteins carry cholesterol and triglycerides at different degrees. Cholesterol is mostly carried by LDL and triglyceride is mostly carried by VLDL [9]. LDL and HDL hydrolyse lipid peroxide deposits and have an important role in preventing against oxidation. Furthermore, this effect has been reported to be inversely related to oxidative stress [10]. Moreover, various enzyme activities related to lipid profile have been known to reduce during infection and inflammation, and acute phase HDL has been known to be unable to protect LDL against oxidation [11].

The aim of this study was to determine the oxidative-antioxidative capacity and lipid profile in cattle with brucellosis. For this purpose, concentrations of serum TAC, TOC, OSI, TG, total cholesterol (TC), HDL-cholesterol (HDL-C), VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C), and the correlative relationship between each other were determined.

MATERIAL and METHODS

This study has been conducted in Kars between September and December 2015, after approval by Kafkas University Animal Experiments Local Ethical Committee (KAU-HADYEK-2015/59). The region was 1756 m high, and the gps coordinates were, 40°36'4.8132" North and 43°5'50.8344" East. Material of the study was formed by 270 Simmental

breed cattle with brucellosis-like clinical symptoms and reproductive problems such as abortion, retentio secundinarum, and infertility within the farms of Kars. Biochemical analyses were performed in 22 serum samples with microbiologically detected brucellosis. Control group included 10 Simmental breed cattle with normal findings in the general clinical examination (body temperature, respiration, pulse, etc), demonstrating no genital system infection and were confirmed to be *Brucella* negative in the microbiological examination. Animals included in the study had no history of vaccination against *Brucella* agents. Cattle were between 2 and 3 years of age, and their body condition scores varied between 2.5-3.25 according to 1-5 scoring system that increase by 0.25 [12]. Animals fed similarly in similar farm conditions were selected for the study. Blood samples of the animals were collected from *vena coccygea* into 10 mL sterile vacuum tubes without anticoagulant, using holder needles. Blood samples were separated from the serum by centrifugation at 3000 rpm for 15 min. Serum samples were kept at -20°C until analysis.

Biochemical Analysis

Total oxidant capacity (TOC), total antioxidant capacity (TAC) were analyzed using commercial test kits (Rel Assay Diagnostics, Turkey) as previously described in [13]. Briefly, hydrogen peroxide and trolox were used as standards to calculate for TOC and TAC, respectively. Oxidative stress index (OSI) which is the indicator of the degree of oxidative stress was calculated by using (Arbitrary Unit) = [TOC (mmol Trolox equivalent/L)/10xTAC (μmol H₂O₂ equivalent/L)] formula [13].

Colorimetric analysis (Epoch®, Biotek, USA) was performed for the detection of TG, TC and HDL-C levels in the serum samples examined for lipid profile, using commercial kits (IBL®, Turkey). VLDL-C and LDL-C levels were calculated according to the formula of Friedewald et al. [14]: LDL-C = (TC) - (HDL) - (TG/5) [If TG (mg/dL) <400 mg/dL, VLDL (mg/dL) = TG (mg/dL)/5].

Detection of *Brucella* Antibodies

Presence of *Brucella* specific antibodies in serum samples was investigated via Rose Bengal Plate Test (RBPT) and Serum Tube Agglutination Test (SAT), and the tests were performed in company with *Brucella* positive and negative control sera. Test antigens were provided from Pendik Veterinary Control and Investigation Institute. RBPT and SAT tests were performed according to the method described by Alton et al. [15]. Equal amounts of (20 μL) antigen and serum samples were mixed on a slide for the RBPT test, and the agglutination observed within 3-4 min was assessed as positive. In the SAT test, serial dilutions of 0.5 mL antigen was performed between 1:10 and 1:320, and was added onto tubes including 0.5 mL serum dilutions, overnight incubated at 37°C, the lace-like agglutinations at the bottom of the tubes were assessed

as positive outcome. (++) or more severe reactions in RBPT, and positive samples in dilutions of 1:40 or more in SAT after analysis were evaluated as diagnostic dilutions^[16,17].

Statistical Analysis

Statistical analysis was performed using SPSS® (SPSS 18.0, Chicago, IL, USA) program package. Distribution of the data within groups was evaluated using Shapiro-Wilk test. Parametrically distributed groups were compared using T test (Independent-Samples T-Test). Correlations between variables were determined using Pearson correlation test. Obtained values were expressed as mean ± standard error of mean (SEM). $P \leq 0.05$ was accepted as statistically significant.

RESULTS

RBPT and SAT Results

In 22 of 270 serum samples in the study group, (+++++) positivity was obtained with RBPT, and in all of these 22 samples, *Brucella* positivity was detected at 1:320 dilution with SAT. All 10 samples within the control group were detected to be RBPT negative. In these samples, non-specific antibodies for *Brucella* and negative reactions were detected for 1:10 or lower dilutions in SAT.

Clinical Examination

While the mean temperature, heart rate and respiratory rate were $39.27 \pm 0.25^\circ\text{C}$, 80.0 ± 4.0 bpm and 29.0 ± 1.00 bpm, respectively, in the clinical examination of brucella-infected cattle, these values were found to be $38.6 \pm 0.02^\circ\text{C}$, 64.0 ± 1.2 bpm and 21.0 ± 0.30 bpm, respectively, in healthy animals.

Biochemical Results

Oxidant-antioxidant capacity was compared between cattle with brucellosis and the control group, and TAC value was determined to be statistically significantly reduced ($P=0.007$), whereas TOC and OSI values were found to be significantly increased ($P=0.013$) and ($P=0.002$), respectively (Table 1).

The lipid profile revealed statistically significantly higher TC and LDL-C values compared to the control group ($P=0.012$) and ($P=0.0004$), respectively; and significantly lower TG ($P=0.004$), HDL-C ($P=0.023$) and VLDL-C ($P=0.004$) values (Table 2).

A significantly high positive correlation was detected between TOC and OSI ($r = 0.78$, $P < 0.001$) and TG and VLDL-C ($r = 1.00$, $P < 0.001$) in the control group, and a significantly negative correlation was determined between TAC and HDL-C ($r = -0.66$, $P < 0.05$), and LDL-C and HDL-C ($r = -0.84$, $P < 0.01$). A significantly positive correlation was detected between TOC and OSI ($r = 0.71$, $P < 0.001$), TG and VLDL-C ($r = 1.00$, $P < 0.001$), and TC and LDL-C ($r = 0.60$, $P < 0.01$), and

Table 1. Changes in TAC, TOC and OSI concentrations in cattle with brucellosis and healthy animals (mean ± SEM)

Parameters	Control (n=10)	Brucellosis (n=22)	P value
TAC (mmol Trolox Eq/L)	1.23±0.19	0.77±0.06	0.007
TOC (µmol H ₂ O ₂ Eq/L)	26.41±4.57	41.47±3.33	0.013
OSI (Arbitrary Unit)	2.57±0.60	6.16±0.69	0.002

TOC: Total oxidant capacity, TAC: Total antioxidant capacity, OSI: Oxidative stress index

Table 2. Lipid profile concentrations in cattle with brucellosis and healthy animals (mean ± SEM)

Parameters	Control (n=10)	Brucellosis (n=22)	P value
TG (mg/dL)	15.12±0.89	11.60±0.64	0.004
TC (mg/dL)	92.49±1.33	97.34±1.08	0.012
HDL-C (mg/dL)	43.01±2.28	37.20±1.27	0.023
VLDL-C (mg/dL)	3.02±0.18	2.32±0.13	0.004
LDL-C (mg/dL)	46.47±2.23	57.82±1.63	0.0004

TG: Triglyceride, TC: Total cholesterol, HDL-C: High-density lipoprotein-cholesterol, VLDL-C: Very low-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol

significantly negative correlation was determined between TAC and OSI ($r = -0.68$, $P < 0.001$), TG and OSI ($r = -0.47$, $P < 0.05$), VLDL-C and OSI ($r = -0.48$, $P < 0.05$), and HDL-C and LDL-C ($r = -0.76$, $P < 0.001$) in the *Brucella* group (Table 3).

DISCUSSION

In this study, we investigated the effect of brucellosis on changes in oxidative-antioxidative capacity and lipid profile in cattle. Brucellosis, which is a zoonotic disease with chronic progression, not only threatens public health but leads to economic loss of important extent by causing abortion, retentio secundinarum, postpartum infections and infertility^[1]. While eradication of brucellosis has been an important economic value, it is important to determine its biochemical and cellular changes for treatment purposes^[18,19]. This study determines the changes in TOC, TAC and lipid profile that may play a role in the pathogenesis of brucellosis. Although there are many studies in the medicine^[10,11,20-22], the number of studies in the veterinarian field, especially on cattle is limited.

In the case of inflammation or various infections, antioxidant defense gets weakens, and increased reactive oxygen species react with macromolecules, including proteins, lipids and DNA; and ultimately, lead to oxidative damage^[8,23,24]. In the current study, parameters of oxidative stress were measured to determine the severity of the cellular damage and the effectiveness of the treatment likewise reported formerly^[5,6,25]. Oxidative stress parameters have been reported to be stimulated while the antioxidant concentration decreased in brucellosis, a chronic

Table 3. Pearson coefficient of correlations between groups of brucellosis and control

Groups	Parameters	TOC	OSI	TG	TC	HDL-C	VLDL-C	LDL-C
Brucellosis (n=22)	TAC	-0.101	-0.680***	0.437	0.120	-0.058	0.438	0.090
	TOC		0.709***	-0.211	-0.064	-0.165	-0.214	0.103
	OSI			-0.482*	-0.231	0.088	-0.484*	-0.183
	TG				0.178	0.303	1.000***	-0.196
	TC					0.064	0.178	0.598**
	HDL-C						0.303	-0.760***
	VLDL-C							-0.197
Control (n=10)	TAC	0.034	-0.525	-0.400	-0.184	-0.655*	-0.398	0.590
	TOC		0.783**	0.445	-0.239	-0.254	0.444	0.082
	OSI			0.365	-0.192	0.298	0.362	-0.447
	TG				0.381	-0.091	1.000***	0.241
	TC					0.287	0.381	0.273
	HDL-C						-0.093	-0.841**
	VLDL-C							0.243

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed); *** Correlation is significant at the 0.001 level (2-tailed)

infection [7,24,26]. In studies conducted on patients with diagnosed brucellosis, serum TOC and OSI concentrations were reported to significantly increase, and TAC concentration was reported to decrease [10,11,21]. In this study, we found that *Brucella* significantly increase TOC (P=0.013) and OSI (P=0.002) concentrations in cattle. TAC concentration, on the other hand, was observed to decrease (P=0.007) which is in parallel with the previous studies. These findings indicate that *Brucella* infection impairs the oxidant-antioxidant balance in cattle. Brucellosis may lead to severe inflammation and cellular damage, and the phagocytic activity that is formed against the infection may alter TOC and OSI concentrations as well. These reactions formed are tried to be compensated by the organism, and that may be the reason of reduction in TAC concentration. The correlation observed between the oxidant-antioxidant mechanisms is the evidence of this situation (Table 3).

Infection and inflammations may lead to changes in lipid and lipoprotein metabolism similar to that of the cytokines. Therefore, serum TC, HDL-C, LDL-C and TG concentrations are affected as well [25,27,28]. Especially, after the introduction of an infection or inflammation agent into the host, serum oxidized lipids are increased and LDL oxidation is started [29]. Changes in cholesterol levels observed during infections are demonstrated. However, the underlying mechanisms of these changes are yet uncertain [11,25]. *Brucella* species are facultative, intracellular pathogens among the phagocytic cells that can stay alive and reproduce. Oxidative catabolism by polymorphonuclear leukocytes and macrophages plays the primary role in the elimination of brucella [11]. At the same time, these bacteria can prevent the apoptosis of macrophages and weaken the immune response of the host organism [10]. As a result, infection and inflammation are related to the reduction in HDL-C levels [11] because it

is well known that acute phase HDL cannot protect LDL against oxidation during infection and inflammation [10,11]. In a study conducted on sheep infected with *B. melitensis*, serum cholesterol and LDL levels were detected to increase [9]. In human, brucellosis has been reported to cause an increase in TC and LDL levels and a reduction in HDL levels [10,11,20]. In our study, lipid profile was observed to change as a result of brucellosis that TG, HDL-C and VLDL-C levels were decreased, and that TC and LDL-C were increased in cattle. These outcomes were in accordance with the data in literature.

There are several enzyme activities that protect HDL and LDL against oxidation such as paraoxonase (PON1). These enzyme activities were detected to be inversely related to oxidative stress. PON1 is a HDL-related antioxidant enzyme and there is positive correlation between them [10,11]. Furthermore, especially LDL was detected to be in correlative relationship of LDL with oxidative stress markers [25]. In our study, negative correlation was observed between TG and OSI (r= -0.47), and between VLDL-C and OSI (r= -0.48) in the group with brucellosis (Table 3). These findings indicate that lipid metabolism is related to oxidative damage and oxidative stress markers.

In conclusion, *Brucella* infection was detected to cause important changes in the oxidative-antioxidative capacity and lipid profile in cattle. These data obtained may contribute to define the severity of the inflammation and to its diagnosis in particularly this disease.

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