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Lipid Peroxidation and Antioxidant Defence of Mammary Glands and Supramammary Lymph Nodes of Cattle Infected with Brucella abortus

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Summary

The aim of this study was to determine the levels of β-carotene, vitamin A, E, the activity of glutathione peroxidase (GSH-Px, EC. 1.11.1.9) and the levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) as indicator of lipid peroxidation in the supramammary lymph nodes and mammary glands of cattle infected with *Brucella* abortus (*B. abortus*). In this study, samples of 20 healthy and 20 heifers aborted in second trimaster of gestation found to be brucella-positive by serological tests were used. The cattle were brought from various villages in Kars region and slaughtered in two local abattoirs. Our results show that the TBARS levels in the cattle infected with *B. abortus* were significantly ($p<0.001$) increased and GSH-Px activity and GSH levels were significantly (0.01) decreased as compared to the healthy cattle. Also, vitamin E, A ($P<0.01$) and β-carotene ($P<0.001$) levels of the infected animals were significantly decreased as compared to the healthy cattle. These results indicate that during immun response against *B. abortus*- induced bovine brucellosis caused oxidative stress with the elevation in LPO was reduced the levels of the vitamin E, A and β-carotene, GSH and activity of GSH-Px in the supramammary lymph nodes and mammary glands of cattle.

Keywords: Brucella abortus, antioxidant system, lipid peroxidation, supramammary lymph nodes, mammary glands.

Brucella abortus ile Enfekte Sığırların Meme ve Meme Altı Lenf Bezlerinde Lipid Peroksidasyon ve Antioksidan Savunma

Özet

Bu çalışmada, *Brucella abortus (B. abortus)* ile enfekte olmuş sığırların meme ve meme altı lenf bezlerindeki redükte glutatyon (GSH), lipid peroksidasyon indikatörü tiyobarbiturik asit reaktif substrat (TBARS) düzeyleri, glutatyon peroksidaz aktivitesi (GSH-Px, EC. 1.11.1.9), ile β-karoten, A ve E vitaminlerinin düzeylerinin belirlenmesi amaçlanmıştır. Bunun için, Kars ve yöresinin değişik köylerinden ve bölgedeki iki kesimhaneden sağlanan 20 sağlıklı, 20 ikinci trimesterde düşük yapmış ve serolojik testlerle *B. abortus* ile enfekte olduğu belirlenen toplam 40 sığırdan alınan numuneler kullanılmıştır. *B. abortus* ile enfekte sığırların meme ve meme altı lenf bezlerinde TBARS düzeyleri istatistiksel olarak yüksek olarak ($p<0.001$) belirlenirken, eritrosit GSH-Px aktivitesi ile GSH düzeyleri sağlıklı koyunlarla karşılaştırıldığında istatistiksel olarak düşük bulundu ($p<0.01$). Aynı şekilde, E vitamini, A vitamini ($p<0.01$) ve β-karoten'in ($P<0.001$) doku düzeylerinin de sağlıklı olanlarla karşılaştırıldığında, enfekte hayvanlarda istatistiksel olarak düşük olduğu belirlendi. Sonuç olarak; sığırlarda *B. abortus*'un neden olduğu sığır brusellozunda immun cevap sonucu oluşan oksidatif strese bağlı olarak meme ve meme altı lenf bezlerinde TBARS düzeyleri artmış, GSH-Px aktivitesi, GSH, A vitamini, E vitamini ile β-karoten düzeyleri ise azalmıştır.

Anahtar sözcükler: Brucella abortus, antioksidan sistem, lipid peroksidasyonu, meme bezi, meme altı lenf bezleri.

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INTRODUCTION

Brucella abortus, the causative agent of bovine brucellosis, is a gram-negative facultative intracellular bacterium that is strictly parasitic and produces chronic infections in cattle consisting of persistent or recurrent bacteremias manifested typically by abortion[1-3]. It is capable of surviving and replicating in phagocytic leukocytes and epithelial cells[3]. Persistently infected cows, the mammary gland and supramammary lymph nodes are the most common sites for localization[2-4]. Many of these chronically infected cows continue to shed *B. abortus* in milk[5]. Cattles react to *B. abortus* by both cell-madiated and humoral immunity. In cattle, macrophages appear to be important in resolving an infection with *B. abortus*[6]. Also, protection against intramammary gland infection depends on the presence of neutrophils in milk. Mammary macrophages of cattle produces significantly higher oxidative burst and bacteriostatic activities[1,7]. It appears that immun system is important in resistance to bovine brucellosis. Neutrophils kill many phagocytosed microorganisms by reduction of molecular oxygen to toxic free radicals and by myeloperoxidase-associated oxidation. Even though bovine macrophages kill microbes only with toxic oxygen metabolites[7]. On the other hand, the antioxidant system becames very important during the immun response when neutrophils and macrophages produce large amount of H_2O_2 and superoxide from molecular oxygen to destroy ingested foreign organisms[8]. Vitamin E, affects the development and maintanence of immunocompetence through multiple function, by acting directly on the immune cell or by indirectly altering metabolic and endocrine parameters which in turn influence immune function. Retinoids are required for adequate innate and adaptive immune response to agents of infectious disease[9]. Also, studies have suggested a spesific role for β-carotene in regulating immune function. So, cattle fed β-carotene had increased mitogen-induced lymphocyte proliferation[10]. β-carotene also enhanced humoral immune response in mice[11]. Free radical formation and lipid peroxidation in tissues may be elevated in hypoxia, inflamatory reaction, infection and tissue destruction. Prostaglandins and leukotrienes are important mediators of inflamatory and immunological reactions. Prostaglandins represents a spesific form of lipid peroxidation controlled by GSH and GSHPx[12,13]. Also vitamin E, A and β-carotene preventing the peroxidation process of polyunsaturated fatty acids the precursors for prostaglandin formation[14-16]. This may be the key mechanism by which vitamins affects the inflamatory responses. In inflamatory state, reactions of these antioxidants may be changed their tissues level.

In this respect, the objective of this study were characterize the oxidative-antioxidative changes in mammary glands and supramammary lymph nodes in cattle infected with Brucella abortus.

MATERIALS and METHODS

In this study, the mammary gland and supra-mammary lymph nodes of 20 healthy and 20 heifers aborted in second trimaster of gestation found to be brucella-positive by serological tests were used. Samples were taken from the cattles which were brought from various villages in Kars region and slaughtered in two local abattories to measure the levels of vitamin E, A, β-carotene, lipid peroxidation (LPO), activity of glutathione peroxidase (GSHPX; EC: 1.11.1.9) and the levels of glutathione (GSH).

The tissues were weighed, rinsed with ice-cold deionized water, cut into small pieces and then dried on a filter paper. The tissues were homogenized using the appropriate buffer, depending upon the variable to be measured. The homogenates were centrifuged at 20000 g for 10 min at 4°C.

Lipid peroxidation contents were measured with the thiobarbituric acid reaction as the thiobarbituric-acid reactive substances (TBARS) in tissues using the method of Placer et al.[17], modified by Matkovics et al.[18]. The values of the malondialdehyde (MDA) reactive material were expressed in terms of TBARS (nmol/g tissue). To prevent artefuctual LPO during the boiling step samples analysed for MDA contained 1.0 mM butylated hydroxytoluene (BHT).

Glutathione peroxidase activity was determined using cumene hydroperoxide and reduced GSH as co-substrates and the loss of GSH following enzymatic reaction was measured spectrophotometrically with Ellman's reagent at 37°C and 412 nm according to Matkovics et al.[18]. The reduced GSH levels of the tissue homogenates were measured spectrophotometrically using Ellman's reagent[19].

The tocopherol content of the tissues was determined spectrophotometrically according to Tsen[20]

and Martinek[21]. Retinol and β-carotene in tissue homogenates were determinated according to the method of Suzuki and Katoh[22].

The protein content in the tissue homogenate was measured by the method of Lowry et al.[23] with bovine serum albumin as the Standard.

Statistical analysis: All results were expressed as the mean ± standard error (SE). Statistical analysis (Sample Dependent t-test) was performed using the SPSS software program and the values of group with brucella were compared to those of the control group. $P<0.05$ were considered to be statistically significant.

RESULTS

The levels of LPO, GSH, β-carotene, vitamin E and A and activities of GSHPx in the mammary glands and supramammary lymph nodes were shown in Table 1 and Table 2 respectively. The levels of LPO in animals infected with *B. abortus* increased 3 to 4 fold of the control levels in the mammary glands and supramammary lymph nodes, respectively ($p<0.001$). The levels of GSH were significantly lower in the mammary glands and supramammary lymph nodes of animals infected with *B. abortus* than in the control group ($p<0.01$). Also, the GSHPx activities of the mammary glands and supramammary lymph nodes were significantly ($p<0.01$) lower in the animals infected with *B. abortus* than in the healthy group.

As the levels of vitamin E and retinol were decreased ($p<0.01$) in the mammary glands of the group infected with *B. abortus*, the levels of β-carotene were significantly decreased ($p<0.001$) in the same group. Also, vitamin A, E ($P<0.01$) and β-carotene ($p<0.01$) levels of lymph nodes were significantly decreased in the infected animals compared to healthy group.

DISSCUSSION

This study shows that *B. abortus* infection may be play an important role in antioxidant defence system of mammary glands and supramammary lymph nodes in cattle. In ruminants, it is well known that *B. abortus* has a marked affinity for lymphoid and reproductive organs. The udder and supramammary lymph nodes are the most common sites for localization[4,24]. In a study by Meador et al.[2] suggest that macrophages and neutrophils transport brucella from systemic circulation into mammary gland which provide a site for intracellular application in alveoli and ducts. In addition, macrophages are capable of transporting substances from udder to regional lymph nodes. Supramammary lymph nodes likely remain persistently infected in chronically infected cows due to lymphatic drainage of brucella from infected udder. It has been speculated that mammary gland and supramammary lymph nodes of cattle infected with *B. abortus* may produce reactive oxygens and lipid peroxides (LPO)[1,6]. Besides, generation of toxic oxygen-dependent free radicals during phagocytosis is a major mechanism by which macrophages and neutrophils kill intracellular pathogens[6]. Upon infection these phagocytes suddenly increase oxygen consumption and produce oxygen intermediates, such as H_2O_2 ,superoxide, hidroxyl radical and singlet oxygen[7]. These some oxygen dependent mechanisms are apparently responsible for the destruction of *B. abortus* by phagocytic leucocytes. So, the oxidative burst capabilities of bovine mammary gland macrophages may influence the survival of *B. abortus*[5]. Thus, in this study, it is conceivable that increased LPO levels may be response of fagocytic cells producing reactive oxygen species and hidroxyl radicals that considered to be important aspects of the oxidative burst. On the other hand, all aerobic organisms have mechanisms that protect their cells against oxydative compounds. Glutathione (GSH), glutathione peroxidase (GSHPx), superoxide dismutase (SOD), and catalase (CAT) are widely distrubited in many cells and tissues[12,13]. The regulatory mechanisms that control these elements are rather different, depending on the nature of the oxidative stress and organism. During inflammation antioxidant defence become very important to neutrolizes the large amount of free radical products of neutrophil and macrophages. Glutathione peroxidase function as an antioxidant by reducing lipid hydroperoxides to less reactive alcohols. Glutathione in contrast to other antioxidant defenses, is a tripeptide composed of nonessential amino acids. Glutathione peroxidase has a critical need for GSH which itself is a key antioxidant at the hub of numerous reaction[12]. However we did not find any reports about the change in GSHPx activity and GSH levels of animals infected with *B. abortus*. In this study, increase on LPO levels may also be due to reduction in the activity of the selenoenzyme GSHPx and levels of GSH in mammary gland and

supramammary lymph nodes. It is known that important mediators of inflammation are prostaglandins and leukotriens controlled by GSH and related enzymes[25]. Reduction of GSHPx activity and GSH levels in our study represent their utilization against LPO formation.

The somatic cells consist of predominantly macrophages during inflammation, a massive invasion of neutrophils are typical at the inflammed site. There is evidence that lipid peroxidation and Vitamin E might have effects on white cell activity[26,27]. Although, vitamin E thought to act as a biological antioxidant, it has been implicated that vitamin E also protects biological membranes against by products of inflammed agents such as free radicals, lipid hydroperoxide and other oxidants[15]. As an antioxidant, vitamin E reduce free radical pathology during inflammation by modifying the activity of neutrophils to protect the cells[28]. A decline in vitamin E levels in this study might be one of the determinant of neutrophil and macrophage invasion possibly in relation to their oxidative burst activity.

Vitamin A and β-carotene are surface active agents that may alter cell membrane behaviour and cell-cell interaction. Retinoids are also required for adequate innate and adaptive immune responses to agents of infectious disease[9]. Albers et al.[26] showed that vitamin A enhance inflammatory responses accompanied by increased mucosal responses. In addition, Chen and Ross[29] suggest that retinoids may stimulate immunity by inducing the maturation/ differentiation of myeloid and lymphoid cells. Moreover, an other study showed that vitamin A supplementation have resulted in elevated antibody production to certain antigen in mice and chickens[14]. It is suggested that the oxidative burst capacity of granulocyte increased in inflammed site with vitamin A[26]. So, we found that vitamin A levels induced by *B. abortus* infection decreased both in mammary gland and supramammary lymph node. These results confirm that to remove oxydative damage caused by fagocytic cell activity against infectious agents declined vitamin A levels of tissues by its utilization with LPO formation.

β-carotene is the most efficient quencher of singlet oxygen known in nature and can also function an antioxidant[16]. Michal et al[10], suggest that β-carotene had increased mitogen-induced lymphocyte proliferation in cattle. β-carotene also enhanced humoral immune response in mice[11]. Chew et al.[30] showed that β-carotene heightened cell-mediated and humoral immune responses in dogs. In our study, decreased levels of β-carotene might be due to maintain the functional integrity of cell membranes and organelles from reactive oxygen species produced during cellular metabolism.

In conclusion, our results indicate that during immun response against *B. abortus*-induced bovine brucellosis caused oxidative stress with the elevation in LPO was reduced the antioxidant levels and activity in the supramammary lymph nodes and mammary glands of cattle.

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Table 1. Levels of investigated parameters in the mammary gland of cattle in the healthy and infected with *Brucella abortus*.
Tablo 1. Sağlıklı ve *Brucella abortus* ile enfekte olmuş sığırların meme bezlerinde araştırılan çeşitli parametrelerin düzeyleri.

PARAMETERS	GROUPS	
	Healthy Cattle $\bar{X}$±SE	Infected with *B. abortus* $\bar{X}$±SE
TBARS (nmol/g dry tissue)	4.80±1.05	12.71±2.52***
GSHPx (IU/g protein)	16.48±1.05	11.12±0.78**
GSH (nmol/g dry tissue)	2.13±0.88	1.22±0.90**
Vitamin E (μg/g dry tissue)	17.81±2.15	10.54±1.43**
Vitamin A (μg/g dry tissue)	51.23±3.11	33.75±1.38**
β-Carotene (μg/g dry tissue)	219.50±5.21	113.61±3.56***

**: p<0.01, ***: p<0.001 Values expressed as mean±SE

Table 2. Levels of investigated parameters in the supramammary lymph node of cattle in the healthy and infected with *Brucella abortus*.
Tablo 2. Sağlıklı ve *Brucella abortus* ile enfekte olmuş sıgırların meme altı lenf nodüllerinde araştırılan çeşitli parametrelerin düzeyleri.

PARAMETERS	GROUPS	
	Healthy Cattle $\bar{X}$±SE	Infected With B. abortus $\bar{X}$±SE
TBARS (nmol/g dry tissue)	2.91±0.68	8.17±0.16***
GSHPx (IU/g protein)	12.54±0.71	6.83±0.42**
GSH (nmol/g dry tissue)	1.68±0.22	0.72±0.07**
Vitamin E (μg/g dry tissue)	11.62±1.88	5.89±0.94**
Vitamin A (μg/g dry tissue)	37.53±2.28	20.74±1.02**
β-Carotene (μg/g dry tissue)	195.72±2.35	123.06±1.08***

**: p<0.01, ***: p<0.001 Values expressed as mean±SE