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Evaluation of milk glutathione peroxidase and superoxide dismutase levels in subclinical mastitis in Damascus goats

İsfendiyar DARBAZ[1], Seçkin SALAR[2], Serkan SAYINER[3], İdil BAŞTAN[4], Osman ERGENE[1], Ayhan BAŞTAN[2,*]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Near East University, Nicosia, Northern Cyprus
[2]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Near East University, Nicosia, Northern Cyprus
[4]Department of Internal Medicine, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

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Abstract: The aim of this study was to evaluate milk glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels in Damascus goats with subclinical mastitis. According to the somatic cell counts (SCCs), 193 Damascus goats included in the study were divided into healthy (n = 75; SCC <1000 × 10^3 cell/mL) and mastitis (n = 118; SCC ≥1000 × 10^3 cell/mL) groups. It was determined that GPx levels were 271.76 ± 3.16 U/L and 300.47 ± 9.04 U/L and SOD levels were 2.57 ± 0.09 U/mL and 2.23 ± 0.07 U/mL in the healthy and mastitis group, respectively. Our findings show that the GPx ($P < 0.001$) and SOD ($P < 0.05$) levels were different between the groups. Weak correlations were also found between somatic cell counts and GPx levels (Spearman's $R = 0.296$, $P < 0.001$) and SOD levels (Spearman's $R = -0.163$, $P = 0.024$). The model showed that GPX ($P = 0.003$) and SOD ($P = 0.004$) levels were different according to the SCCs in healthy and mastitic milk samples, respectively. However, individual differences like age, parity, and number of offspring had no effect on milk GPx and SOD levels ($P > 0.05$). In summary, a significant increase in GPx and decrease in SOD levels, respectively, were determined in goat milk samples with subclinical mastitis, and these changing trends were correlated with milk SCCs. However, age, parity, and number of offspring were not associated with these changing patterns.

Key words: Damascus goat, glutathione peroxidase, milk, subclinical mastitis, superoxide dismutase

1. Introduction

Mastitis, defined as inflammation of the udder, is one of the most important diseases in dairy goat and cow farms globally [1]. Mastitis can present in two major forms as clinical mastitis (CM) and subclinical mastitis (SCM). The latter has more importance due to its higher prevalence compared to the clinical form, challenges in detection, and lack of clinical symptoms, remaining a reservoir of pathogens [2].

Unlike CM, SCM affects the cellular content of milk and milk yield without observable abnormalities in the udder or milk. During the inflammation, somatic cell counts (SCCs) in milk increase as a reaction of the udder defense system against pathogens [3]. SCC is effectively used as indicator of intramammary infections and the severity of inflammation in cows [3,4] and sheep [5]. However, the usage and effectiveness in goats is still controversial because SCCs in goat milk show great variations according to factors such as parity, breed, and physiological status (suckling, estrus, etc.) [4]. Resultantly, it is necessary to determine more appropriate diagnostic methods to distinguish SCM in goats.

The imbalance based on increased reactive oxygen species (ROS) and decreased antioxidant levels due to various factors disrupts the oxidative balance, which results in oxidative stress [6–9]. The significant increment of ROS against lower levels of antioxidants may lead to DNA, cell, or tissue damage [10]. The deleterious effects of ROS are effectively neutralized by enzymatic or nonenzymatic antioxidants in cellular defense mechanisms [11]. It has been reported that oxidative stress can be observed in dairy goats, similar to cows [12–14].

Reactive oxygen species have a stimulating role in the antibacterial activity of neutrophils [15], so oxidative stress has been linked with some inflammatory/infectious disease like mastitis and metritis [10,16,17]. In cow practice, the activities of some milk enzymes, e.g., catalase (CAT), N-acetyl-β-d-glucosaminidase (NAGase), and acid phosphatase (AcP), are accepted as indicators of mastitis [18,19]. Measurement of catalase activity in milk has been

* Correspondence: abastan@ankara.edu.tr

used to assess the udder health of cows [18]. Measurement of milk antioxidants to reflect the inflammation status of the goat's udder may be useful in diagnosing mastitis. However, to our knowledge, no published study exists on the glutathione peroxidase and superoxide dismutase activities in milk in dairy goats with subclinical mastitis.

The purpose of this study was to evaluate milk glutathione peroxidase and superoxide dismutase levels in Damascus goat's milk with subclinical mastitis.

2. Materials and methods

2.1. Animals, housing, and management

The study was conducted at the Ercan State Farm in Northern Cyprus, near Nicosia. One hundred and ninety-three lactating Damascus goats were included in the study. The mean levels of age, days in milk (DIM), parity, and number of offspring (NoO) were 3.46 ± 0.93, 177.77 ± 3.57, 2.42 ± 0.87, and 1.38 ± 0.03, respectively. The goats were raised under semiintensive conditions, housed in semiopen sheds. All animals were fed with a commercial compound, corn silage, barley hay, vetch–barley hay, and pasture feeding (depending on the season) and had free access to water. The goats were vaccinated against clostridial diseases (twice a year) and dewormed regularly.

2.2. Collection of milk samples

Two milk samples (8 mL each) in sterile containers were collected from each doe during morning milking for SCC analysis and enzymatic assays. Each sample was immediately placed in ice and transferred in a cold-chain to the laboratory.

2.3. Determination of somatic cell counts

SCCs were measured using a fluorescence optical cell counter (Fossomatic FC 5000, Foss Electric, Denmark) at the Cyprus Turkish Milk Board. A value of 1000×10^3 cells/mL was considered as the threshold for subclinical mastitis.

2.4. Preparing milk samples for enzymatic assays

One of the milk samples was transported to the Diagnostic Laboratory (Faculty of Veterinary Medicine, Near East University) in cold-chain. Milk samples were centrifuged at 5000 rpm for 20 min at 4 °C to produce skimmed (defatted) milk. After centrifugation, the samples were stored at 4 °C and analyzed within the same day.

2.5. Determination of glutathione peroxidase and superoxide dismutase activities (enzymatic assays)

Glutathione peroxidase activity in milk samples was analyzed using a commercial assay kit (Ransel Cat No. RS502 Lot. 384345, Randox, County Antrim, UK) and was determined indirectly by measuring the formation rate of oxidized glutathione (GSSG). Glutathione peroxidase activity was measured spectrophotometrically at 340 nm as decreased absorbance and GPx activities were expressed as U/L [20]. Glutathione peroxidase control (Ransod CTRL, Cat. SC692 Lot. 473RS, Randox) was also used as a test control. Superoxide dismutase activity in milk samples was analyzed using commercial kits (Ransod Cat No. SD125 Lot. 388463, Randox). The principle is based on the acquisition of superoxide radicals using xanthine and xanthine oxidase. The absorbance value of red-colored formazone, which is formed by reacting superoxide radicals with INT (2-(4-iodophenil)-3-(4-nitrophenol)-5-phenyltetrazolium chloride), was measured at 510 nm photometrically. The results are expressed as U/mL. Superoxide dismutase control (Ransod CTRL, Cat. SD126 Lot. 331RD, Randox) was also used as a test control. The measurements were performed using a fully automatic biochemistry analyzer BS-120 (Mindray, Shenzhen, China).

2.6. Statistical analyses

The animals included in the study were divided into healthy (SCC $<1000 \times 10^3$ cell/mL) and mastitis (SCC $\geq 1000 \times 10^3$ cell/mL) groups according to the somatic cell counts. Descriptive statistics for each variable were calculated and presented as mean ± standard error of the mean ($\overline{x}$ ± SE). The GPx (U/L) and SOD values (U/mL) were not normally distributed and so logarithmic transformation was performed. Independent sample t-tests were used to compare the groups after logarithmic conversation. Spearman's correlation was used to evaluate correlations in the raw data. The general linear model procedure was used to evaluate the effect of age, parity, number of offspring, and SCC on GPx and SOD. The Tukey test was used as a post hoc testing procedure. $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS 14.01 (License No: 98692604).

3. Results

Descriptive data of the individual properties between groups are shown in Table 1.

Somatic cell count, GPx, and SOD values between groups are shown in Table 2. It was found that GPx ($P < 0.001$) and SOD levels ($P < 0.05$) of collected milk samples were different between the groups. Weak correlations were also found between somatic cell counts and GPx levels (Spearman's $R = 0.296$, $P < 0.001$) and SOD levels (Spearman's $R = -0.163$, $P = 0.024$).

The model shows that GPX ($P = 0.003$) and SOD ($P = 0.004$) levels were different according to the SCCs between healthy and mastitic milk samples, respectively. However, individual differences like age, parity, and number of offspring had no effect on the milk GPx and SOD levels ($P > 0.05$; Table 3).

4. Discussion

The Damascus (Shami) goat, which is a native breed of the Middle East, was imported into Cyprus to breed about

Table 1. Comparison of individual properties between groups.

	Group	Mean ± SE	P
Age	Healthy	3.63 ± 0.17	0.159
	Mastitis	3.36 ± 0.10	
DIM	Healthy	182.36 ± 6.42	0.304
	Mastitis	174.82 ± 4.17	
Parity	Healthy	2.51 ± 0.15	0.379
	Mastitis	2.36 ± 0.10	
NoO	Healthy	1.40 ± 0.06	0.544
	Mastitis	1.36 ± 0.05	

DIM: Days in milk, NoO: number of offspring.

Table 2. Comparison of variables between groups.

Variables	Groups	Mean ± SE	P
SCC (×10^3 cell/mL)	Healthy	277.55 ± 30.57	<0.05
	Mastitis	5630.31 ± 530.32	
GPx (U/L)	Healthy	271.76 ± 3.16	<0.001
	Mastitis	300.47 ± 9.04	
SOD (U/mL)	Healthy	2.57 ± 0.09	<0.05
	Mastitis	2.23 ± 0.07	

SCC: Somatic cell count, GPx: glutathione peroxidase, SOD: superoxide dismutase.

80 years ago [21]. Known for its high milk yields and reproductive performance, this breed is generally used for milk production [22]. Güney et al. [22] reported the mean total lactation performance of Damascus goats as 489.4 kg milk yields in 254.7 days at the Ercan State Farm in Northern Cyprus between 2001 and 2002.

SCCs in goat milk are generally higher than the amount in cow milk and are affected by several factors such as breed, lactation, age, and farm [1]. The thresholds of SCCs between healthy and infected halves of goat udders were reported as between 2.1×10^5 and 1.12×10^6 cells/mL [23]. Doaa et al. [24] reported that 10^6 cells/mL of SCC is the best indicator threshold for the intramammary infection status of Damascus goats.

The antioxidant enzymes in milk originate in four possible ways: 1) blood plasma, 2) secretory cell membranes, 3) milk fat globule membranes, and 4) somatic cells [18]. However, the passage routes into milk and levels in milk show great inter- and intraspecies variations [25]. Many indigenous enzymes have been reported in blood and milk in various species [18], and two of them, GPx and SOD, represent the main forms of the antioxidant defense mechanism in cells [26]. It can be expected that antioxidant enzymes increase in line with the increment of SCCs since the synthesis and release of these enzymes are partly mediated by milk leukocytes due to the protecting role against oxidative burst associated with intramammary infections as part of the defense system [27].

Glutathione peroxidase, an intracellular antioxidant present in the cytoplasm [19], positively affects the activities of neutrophils and macrophages [28]. The enzyme reduces the harmful effects of peroxides and protects the cell against damage [19,29]. Some researchers [30–32] reported that GPx activity in cow milk with mastitis is higher than in healthy cows and its activity is correlated significantly with SCCs. We also found increased GPx levels in milk with higher SCCs,

Table 3. Effects of individual differences on GPx and SOD levels.

Variable	Category	GPx	P	SOD	P
Parity	1	317.24 ± 24.34	0.118	2.31 ± 0.17	0.062
	2	288.60 ± 8.24		2.49 ± 0.08	
	>3	278.80 ± 4.23		2.18 ± 0.10	
Age	2	317.24 ± 24.34	0.122	2.31 ± 0.17	0.055
	3	288.41 ± 8.313		2.49 ± 0.08	
	>4	279.28 ± 4.19		2.79 ± 0.10	
NoO	1	286.22 ± 6.48	0.461	2.43 ± 0.07	0.34
	2	296.23 ± 11.86		2.31 ± 0.10	
SCC	<×10^3 cell/mL	271.76 ± 3.16	0.003	2.57 ± 0.09	0.004
	≥×10^3 cell/mL	300.47 ± 9.04		2.23 ± 0.08	

supporting the researchers' studies including dairy cows. Glutathione peroxidase is at the same level in cow milk and human milk (12–32 U/mL and 31 ± 39 U/mL) [30]; however, our results were approximately ten times higher than those reported in cows and humans. This could be related to the rate of GPx within total peroxidase activity, which is similar for human and cow milk (29% and 27%, respectively). However, this rate is 65% for goat milk [30].

Superoxide dismutase is an enzyme associated with the oxidative functions of polymorph nuclear neutrophils [33] that converts O_2^- (superoxide anion, a free radical) to H_2O_2 (hydrogen peroxide, a nonradical oxidant) [26,30,34]. The enzyme is accepted as the first line of defense against prooxidants [35]. It is generally expected that the activities of antioxidant enzymes increase in body fluids due to increased activity in the bloodstream [32]. Unexpectedly, in this study, SOD levels in the mastitis group were lower compared to the healthy group. It is thought that the possible reasons for this outcome are unbalanced/restricted feeding [36] and unbalanced ROS production/SOD activity, where advanced superoxide production could represent depleted SOD activities [37].

The relationships between milk antioxidant levels and individual differences have not been investigated in goats. As expected, some individual factors like age, breed, physiological status, feeding, stage of lactation, and infections could affect the level and activity of antioxidant enzymes in milk, particularly in dairy cows [18,25]. However, we could not detect any differences between the individual factors and antioxidant changes. It is thought that individual differences in these parameters could be specific to the species.

In summary, we found evidence that significant increases in GPx and decreases in SOD levels were respectively determined in goat milk samples with subclinical mastitis, and these changing trends were correlated with milk SCCs. However, age, parity, and number of offspring were not associated with these changing patterns. The obtained results support the argument that SCM can cause noticeable variation in milk antioxidant levels, which might be useful for detection of the infection in goats. However, further research is needed to determine the interaction between the release of reactive oxygen species and antioxidant activities or mastitis pathogens.

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