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Detection of Mycotic caprine mastitis through studying of Oxidant/Antioxidant state

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Abstract

The present study was conducted to evaluate the antioxidant profile in cases of subclinical mastitis (SCM) in goats during the period from June 2015 to March 2016. This study included 9 lactated goats which were divided into 3 groups. The first one was immunized by HKCn Ag in the right mammary gland. The 2nd group was infected by virulent *Cryptococcus neoformans* strain in the right mammary gland, while the 3rd group left as control. The blood and milk samples were collected at 0, 3d, 1st, 2nd, 3rd and 4th week for determination of MDA and GSH level. The objective of this study is to determine the possibility of using oxidative product levels (MDA and GSH) as markers for the diagnosis of SCM in goats. The results showed that the differences in MDA level were not significant in milk. While in the serum, the MDA level showed a significant decreasing ($P < 0.05$) in the 1st group compared with the 2nd group, and both of these groups were higher than the control group. The GSH level increased significantly ($P < 0.05$) in the immunized group as compared with the mastitic group, and both of these groups showed significant ($P < 0.05$) differences as compared with healthy group. In conclusion: this study indicated that the measurement of MDA and GSH activities in milk and serum appears to be a suitable diagnostic method for identifying SCM in dairy goat.

Keywords: MDA, GSH, *Cryptococcus neoformans*, mastitis in goat.

1. Introduction

Mastitis remains to be vital disease in the most parts of the world [1]. It has been considered as one of the major disease problems concerning the dairy industry because of reduced the milk yield, changes in milk composition which lead to loss a lot of money to adverse processing qualities of mastitic milk, high costs of treatment, discarding of milk after treatment, then death and culling of premature animals [2, 3].

It is very important to detect the presence of mastitis early in the disease syndrome especially the diagnosis of subclinical infection that is represented huge problem because the milk look like normal while it has elevation on somatic cell count [4]. This process can be performed by many ways such as measurement the level of the somatic cell count (SCC) directly (which are don't give crucial proof of mastitis in goat) or indirectly by using California Mastitis Test (CMT) on suspected animals. On the other hand, cultivation milk of suspected cases can determine the causative agents of mastitis but not give any picture about the degree of inflammation associated with the infection or to predict the prognosis in individual cases [5, 6]. Other parameters like electrical conductivity (EC), milk composition (fat, protein, lactose) can be used for detection of mastitis [7].

However, in previous study that carried out on dairy cows, it was found that the measurement of milk MDA level and other enzymes appear to be suitable diagnostic methods for identifying subclinical mastitis in cattle [8]. As there was no study conducted to determine the malondialdehyde level (MDA) and glutathione peroxidase (GSH) in milk and serum and their relation to subclinical mastitis in goat. Therefore, a present study was conducted to determine the differences of levels of oxidative product, using MDA and GSH as a marker on both cases of mastitis and immunization with Heat Killed *Cryptococcus neoformans* Ag in goats.

2. Materials and Methods

2.1 Preparation of Yeast Suspension for mastitis induction

Yeast suspension of virulent *C. neoformans* was prepared according to Al-Delemy [9] and the concentration was adjusted to about 5×10^6 live cell/ml for injection.

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2.2 Preparation of Heat Killed *C. neoformans* Ag (HKCn Ag)

This antigen was prepared according to Murphy *et al.* [10].

2.3 Experimental Design

Nine healthy goats were used after checking as free from mastitis pathogens and clinically free from any other infectious disease during the period from June 2015 to March 2016. These animals were monitored for 1 week before experiment beginning, then divided into 3 groups equally by infusion of 2ml of 2×10^8 cell/ml of HKCn Ag in the right udder only for the 1st group, 2ml of 5×10^6 viable *C. neoformans* was inoculated in the same side of the udder in the 2nd group after one week of immunization of the 1st group, while the 3rd group was left as control by giving 2ml of sterile PBS [11].

2.4 Collection of samples

Serum was obtained from collection of blood via jugular vein puncture into a clean centrifuge glass for evaluation of the level of malondialdehyde (MDA) and activity of reduced glutathione (GSH) [12]. Whereas milk samples from both halves (right and left udder) were skimmed by centrifugation at 8,000 rpm for 20 min at 4°C. Defatted milk was used for MDA level and GSH detection and were measured by spectrophotometric techniques of Yang *et al.* [4]. All samples of serum and milk were collected at 0, 3d, 1st, 2nd, 3rd and 4th week of study.

2.5 Measurement of MDA and GSH.

Malondialdehyde level was determined by the thiobarbituric acid (TBA) method according to Guidet and Shah [13] while GSH concentration was determined according to Burtis and Ashoowd [14].

2.6 Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1) [15]. Two way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ was considered statistically significant.

3. Results

In the present study, the mean concentration of blood MDA and GSH in healthy, immunized and mastitic goats are shown in Table 1 and 2. There was no significant difference in MDA level in milk between immunized and infected mastitis goats. However, MDA level in serum of immunized group with HKCn Ag was significantly ($P < 0.05$) lower than infected group, and both of them are higher than healthy goat. While, GSH increased significantly ($P < 0.05$) in the 1st group as compared with the 2nd infected mastitis group, and both of them are more than healthy goat (3rd group).

There was a significant difference in the MDA levels between immunized group (354 ± 37.5) and infected group (176 ± 58.1) in 3 day. However, after 2 and 3 weeks, the MDA level was significantly increased ($P < 0.05$) in the infected group as compared with immunized group, (289 ± 28.3) (304 ± 20.0) and (103 ± 13.3) (172 ± 39.4) respectively.

Table 1: Means \pm SE of MDA concentrations in serum of goat immunized with HKCn Ag, mastitic goat and control group

Time Group	Zero	3d	1 st week	2 nd week	3 rd week	4 th week
G ₁	B 206 \pm 50.5a	A 354 \pm 37.5a	A 365 \pm 40.0a	B 103 \pm 13.3c	B 172 \pm 39.4b	B 176 \pm 7.7a
G ₂	D 168 \pm 48.9a	D 176 \pm 58.1b	A 412 \pm 53.1a	C 289 \pm 28.3a	B 304 \pm 20.0a	D 178 \pm 41.3a
G ₃	A 166 \pm 43.3a	A 161 \pm 42.9b	A 168 \pm 45.5b	A 159 \pm 34.4c	A 163 \pm 40.0c	A 168 \pm 41.8a
LSD	105					

Means with different small letter in the same column significantly different ($P < 0.05$)

Means with different capital letter in the same row significantly different ($P < 0.05$)

Table 2: Means \pm SE of GSH concentrations in serum of goat treated with HKCn Ag, mastitic goat and control group

Time Group	Zero	3d	1W	2W	3W	4W
G ₁	B19.7 \pm 2.6a	B25.0 \pm 4.8a	AB35.3 \pm 11.3a	A 47.7 \pm 12.6a	B28.4 \pm 5.5ab	B24.3 \pm 3.0b
G ₂	C18.7 \pm 0.4a	C21.4 \pm 0.9a	BC32.6 \pm 3.3ab	AB 41.6 \pm 7.6a	AB 45.2 \pm 6.3a	A 56.1 \pm 7.4a
G ₃	A16.4 \pm 0.3a	A18.3 \pm 0.9a	A17.1 \pm 1.0b	A19.5 \pm 2.3b	A16.9 \pm 0.7b	A17.9 \pm 0.9b
LSD	15.7					

Means with different small letter in the same column significantly different ($P < 0.05$)

Means with different capital letter in the same row significantly different ($P < 0.05$)

3. Discussion

Malondialdehyde (MDA) is the end product of lipid peroxidation, therefore it is used as index of the oxidative stress [4]. The higher MDA levels in serum of infected mastitis goats reported in this study demonstrated that the oxidative stress is higher than normal and immunized goat.

Inflammatory reaction accompanying disease such as mastitis is characterized by accumulation of neutrophils in the mammary gland. More recently, reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^\cdot), are released by neutrophils and play an important role in the inflammation and cell injury [12]. The most cytotoxic effects of ROS include protein oxidation, DNA damage, inhibition of cellular metabolic pathway and lipid peroxidation. MDA and glutathione GSH are part of

intracellular defense system against oxidation as mentioned by Celi [16]. The present results agreed with the result obtained by Celi [16] and suggest that increasing level of oxidative stress in mastitis might have an essential role in the process of the inflammation and tissue damage.

To our knowledge, there are no studies related directly to mycotic mastitis with antioxidant enzyme, or in case of immunization with fungal Ags like HKCn in the udder of goat. Therefore, due to lack of references that associated with goat, we will discuss the results of our study in the light of mastitis in cows. In recent study erythrocyte MDA production increased in cow with mastitis. This might be due to excessive ROS production by activated neutrophils from inflamed mammary gland causing peroxidative damage to membranes. In fact, lipid bilayer of cellular membrane are composed of

phospholipids and polyunsaturated fatty acids which are easily oxidized leading to creation of lipid peroxides which are decomposed to aldehydes like malondialdehyde (MDA) as reported by Cetin^[17]. This is in accordance with Jambh *et al.*^[18], who reported that MDA level was significantly higher in dairy cows with acute mastitis. A hypothetical probability exists that the phagocytic cells such as macrophages and neutrophils are considered the essential cells of defense in mammary glands against microbial infection. At moment of their activation generate large amounts of ROS and reactive nitrogen species (RNS) that cause lipid peroxidation. Nitric oxide (NO) is one of the most important RNS which operate in epithelial cells and macrophages of mammary gland, producing NO that mediate inflammation during mastitis.

Glutathione, a thiol containing tripeptide, in the reduced form (GSH) it present at high level in living organism, upon reaction with ROS, it get oxidized then can be return to its reduced form by glutathione reductase^[19].

The present study revealed a significant ($P < 0.05$) decrease in GSH concentration in immunized group in 3 and 4 weeks (28.4 ± 5.5) (24.3 ± 3.0) respectively as compared with infected groups in the same periods (45.2 ± 6.3) (56.1 ± 7.4) respectively, in mastitis does as compared with healthy group. This might be due to the excessive production of ROS from inflamed gland that causing a compromise in antioxidant defense of the body which lead to convert reduced form to oxidized form (GSSH)^[17]. So, the disease in goat decrease GSH level and increase in the incidence of mastitis^[19].

5. Conclusion

The present study indicates that the measurement of MDA in the serum and milk along with the GSH activities appear to be suitable methods for identifying the immunization via fungal Ag and infection with mycotic mastitis in goat.

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