

Short Paper

## Study of the relationship between oxidative stress and subclinical mastitis in dairy cattle

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### Summary

Subclinical mastitis is considered as one of the most prevalent diseases in dairy cows, causing drastic loss in the dairy industry. Oxidative stress, induced by reactive oxygen species (ROS), is believed to be a primary factor in various cattle diseases including mastitis, but there are few studies regarding the role of ROS in subclinical mastitis. This study was undertaken to i) study the changes in activities of erythrocyte glutathione peroxidase (e-GSH-Px) and its functional component, selenium (Se); ii) compare the activities of erythrocyte superoxide dismutase (e-SOD) and its functional components, Cu and Zn in cows with subclinical mastitis and normal cows. Milk and heparinized blood samples were collected from 45 normal cows and 45 cows with subclinical mastitis from dairy cows in Tehran province, Iran. Somatic cell counts (SCC), activities of GSH-Px and SOD and concentrations of Cu, Zn and Se were measured. No significant difference ( $P>0.05$ ) was shown between GSH-Px and SOD activities and also between the concentrations of Cu and Zn in the studied groups. There was a marginal significant difference ( $P=0.05$ ) between the Se concentrations in the normal cows and cows with subclinical mastitis. The correlation between SCC and SOD was positive and significant ( $P<0.05$ ). It was concluded that optimum antioxidant intake in the feed may enhance the resistance against subclinical mastitis.

**Key words:** Subclinical mastitis, Oxidative stress, Glutathione peroxidase, Superoxide dismutase, Somatic cells

### Introduction

The term mastitis describes an inflammation of the mammary gland characterized by several physical and chemical alterations of the milk and corresponding pathological changes in the mammary tissue depending on the type of the disease. Subclinical mastitis is considered as the most economically important type of mastitis because of the long term effects of chronic infections on total milk yields. Estimates put the cost of 1 case of subclinical mastitis per cow in the range of \$200/year (Bailey, 1996). In terms of the magnitude and proportions of the total

cost, losses from milk yield depression due to subclinical mastitis appear to be the most significant cost component in all herd groups. Producers generally emphasize clinical mastitis and underestimate the significance of subclinical mastitis, not realizing that for every clinical case of mastitis in the herd, there are 15-40 subclinical cases contributing to an elevated somatic cell count (SCC).

Increase in SCC during the inflammatory process in mammary glands indicates increased neutrophils in milk which result in oxidation reactions and increase in erythrocyte lipid hydroperoxidation and low milk quality (Babor,

1999; Suriyasathaporn *et al.*, 2006).

The preventive defense systems can be accomplished by enzymatic or non-enzymatic mechanisms including reduced glutathione (GSH), vitamin C and vitamin E. Antioxidant enzymes such as glutathione peroxidase (GSH-Px) and catalase (CAT) may also have important functions in alleviating the toxic effects of reactive oxygen species (ROS) (Tokuda and Takeuchi, 1995; Kale *et al.*, 1999).

Many researchers have paid attention to the role of oxidative stress in the pathogenesis of diseases in cattle recently such as milk fever, endometritis, mastitis, retained placenta, claw disease, fertility disorder and pneumonia (Erisir *et al.*, 2006; Lykkesfeldt and Svendsen, 2007). In order to optimize the performance of dairy cow and produce high quality milk, exogenous antioxidants have been added to the dairy cattle diet and hence the stimulatory effect of ROM in oxidative stress has been minimized (Mohamed, 2007).

This study has been undertaken to study the changes in the activities of erythrocyte glutathione peroxidase (GSH-Px) and its functional component, selenium (Se), and also to compare the activities of erythrocyte superoxide dismutase (SOD) and its functional components, Cu and Zn in cows with subclinical mastitis and normal cows in dairy cows of Tehran province, Iran.

## Materials and Methods

Composite milk and heparinized blood samples were collected from 45 normal cows and 45 cows with subclinical mastitis from dairy cows. Cows were examined by a clinician and heart rate, body temperature, respiratory rate, udder health and milk appearance were examined. Diseased cows, cows with any sign of clinical mastitis and cows at early lactation and late pregnancy were excluded from the study. Cows were fed with corn silage, straw, alfalfa and concentrate. Data of age, body weight, parity, number of calves born and milk production were recorded.

Somatic cell counts were measured using cell counter (Fossomatic 90, Foss Electric, Hillerød, Denmark). 210,000

cells/ml was considered as the cutoff point for subclinical mastitis. Hemoglobin (Hb) concentrations were measured by commercially available kit (Zist Chimi, Iran) based on cyanmethemoglobin method. Erythrocyte superoxide dismutase activity was measured using RANSOD Kit (Randox Laboratories, Crumlin, UK) and was expressed as units per g Hb (IU/g Hb). Chemistry analyser (HERA, REF 1803050, Spain) was used as the spectrophotometer in this study.

Erythrocyte glutathione peroxidase activity was measured using RANSEL Kit (Randox Laboratories, Crumlin, UK). The decrease in the concentration of NADPH was measured and expressed as units per g Hb (IU/g Hb).

Plasma samples were used for measurement of Cu and Zn. Samples were diluted by chloric acid (0.1 N) 1:5 and then analysed using flame atomic absorption (Spectr AA 220, Varian, Australia). Se concentration in plasma was determined using graphite furnace atomic absorption (Spectr AA 220, GTA 110, Varian, Australia).

Data on the studied parameters were processed by SPSS. When significant effects were found ( $P < 0.05$ ), the Student's t-test was used to locate significant differences between means. Correlation coefficient and regression analysis were used to study the relationship between the studied parameters and oxidative stress.

## Results

The mean ( $\pm$ SD) somatic cell count and SOD, GSH-Px, Cu, Zn and Se concentrations in the two groups are shown in Table 1.

The Pearson's correlations between the studied parameters are shown in Table 2.

## Discussion

As the term mastitis is applied to both the udder health category "unspecific mastitis" (with an increase in SCC, but no identification of pathogens) and to the category "mastitis" (increase in SCC and detection of pathogens), a diagnosis of

**Table 1: Mean ( $\pm$ SD) somatic cell count, enzymes activities and concentrations of the studied parameters in normal cows and cows with subclinical mastitis (affected cows)**

Parameter	Unit	Normal cows (n=45)	Affected cows (n=45)	P-value
SCC	Cells $\times$ 10 <sup>3</sup> /ml	26.38 $\pm$ 20.82	834.73 $\pm$ 880.19	P<0.001
SOD	IU/g Hb	1668.87 $\pm$ 867.19	1769.34 $\pm$ 755.73	P=0.42
GSH-Px	IU/g Hb	14656.17 $\pm$ 6827.84	14061.08 $\pm$ 5318.07	P=0.66
Cu	$\mu$ g/dl	65.90 $\pm$ 7.82	66.61 $\pm$ 13.77	P=0.77
Zn	$\mu$ g/dl	132.71 $\pm$ 30.98	129.80 $\pm$ 38.64	P=0.69
Se	$\mu$ g/dl	4.47 $\pm$ 1.26	3.99 $\pm$ 0.85	P=0.04

**Table 2: Pearson's correlation coefficients between the studied parameters**

Parameter	Se	Zn	Cu	SOD	GSH-Px	SCC
SCC	-0.057	0.095	0.109	0.226*	-0.001	1
GSH-Px	-0.058	0.031	0.062	0.074	1	-0.001
SOD	-0.035	0.116	0.128	1	0.074	0.226*
Cu	0.217*	0.133	1	0.128	0.062	0.109
Zn	-0.244*	1	0.133	0.116	0.031	0.095
Se	1	-0.244*	0.217*	-0.035	-0.058	-0.057

\*Significant correlation between the parameters (P<0.05)

mastitis can be made without the detection of mastitis pathogens. Inflammatory reactions can thus be used as the criteria for the characterization of mastitis cases.

In the present study, there was a significant difference (P<0.001) between SCC in the healthy group and the cows with subclinical mastitis. The cut-off point in the present study was proposed as 210,000 cells/ml.

No significant difference (P>0.05) was seen in GSH-Px activity between the healthy and affected cows. This can be explained by the low severity of inflammation in the affected mammary glands in subclinical mastitis.

Dalir *et al.* (2006) showed that the odds ratio for low activity GSH-Px on incidence of mastitis was 3.95. Atroshi *et al.* (1986) reported a substantial decrease in GSH-Px in cows with mastitis, while Cetin *et al.* (2005) reported an increase in erythrocyte GSH-Px activity in ewes with gangrenous mastitis and attributed it to the elevated need of the enzyme to boost the defensive mechanism of the animal against oxidation.

In the present study, SOD activity was found to be increased in cows with subclinical mastitis compared to the control group, but the difference was not significant (P>0.05). Since the inflammatory changes in the studied disease were not severe, the increase in SOD activity in the affected cows was not considerable. Kleczkowski *et al.* (2008) showed an increase in SOD

activity in cows with intramammary infections due to different bacteria.

There was a positive significant (P<0.05) correlation between SCC and SOD activity in our study. Since increased SCC during subclinical mastitis is correlated with oxidative stress, an increase in SOD activity during the disease is expectable.

While significant difference (P>0.05) was found between Cu and Zn concentrations in the studied groups, Se concentration showed a marginal significant difference (P=0.05) between the groups. The mild nature of the changes in subclinical mastitis may account for the slight decrease in the concentrations of Cu and Zn in the affected group.

A significant decrease in the mean blood zinc concentration in cows with subclinical and clinical mastitis, but an increase in the mean blood copper level in the clinical mastitis group has been reported (Ranjan *et al.*, 2005).

Kleczkowski *et al.* (2008) found Cu to be increased in cows with clinical mastitis, while Smith and Conard (1987) concluded that vitamin E and Se supplementation would reduce intramammary infection (subclinical mastitis) 42.2% versus unsupplemented control. Weiss *et al.* (1990) reported that there was a negative correlation between plasma Se concentration and SCC in bulk tank, i.e., as the plasma Se concentration increases, the SCC and prevalence of mastitis would decrease. No

significant correlation was found between plasma Se concentration and SCC in cows with subclinical mastitis in the present study.

Erskin *et al.* (1989) showed rapid infiltration of PMN, reduced severity and duration of *E. coli* induced mastitis while Erskin *et al.* (1990) reported no effect of selenium supplementation on the severity of *S. aureus* induced mastitis. Since mastitis affects immune system factors and Se in the ration has different effects on mastitis, the different responses to Se supplementation could be expected in the studies.

Negative significant correlations were found between Se and Cu ( $r = -0.217$ ) and between Se and Zn ( $r = -0.244$ ). The observed correlations between Se, Cu and Zn in this study might be explained by their role in oxidative stress induced by subclinical mastitis.

Worldwide drastic changes in dairying reflected in larger herds and high yielding cows now require re-evaluation of standard mastitis diagnostics. Enzymes like GSH-Px and SOD which have been shown to be increased in clinical mastitis could be considered as the biomarkers for the online mastitis monitoring. More studies should be conducted to show if these enzymes are suitable for diagnosis of subclinical mastitis as well.

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