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Antioxidants Status and Degree of Oxidative Stress in Mastitic and Healthy Camel (*Camelus dromedarius*)

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Abstract: Oxidative stress is an imbalance between oxidant and antioxidant in favour of the former. The aim of this investigation was to assess the antioxidant status and degree of oxidative stress in the blood of camels (*Camelus dromedarius*) in the sub clinical form of mastitis. The research was conducted on 46 camels. The results indicated a reduction in both individual and Total Antioxidant Status (TAS) and increase in lipid peroxidation, manifested by an increase in Malondialdehyde (MDA) in mastitic dairy camels compared with healthy ones. A similar trend of reduction ($p<0.05$) was observed in copper and zinc levels in mastitic camels compared with healthy.

Key words: Antioxidant, camel, mastitis, oxidative stress, TAS, MDA

INTRODUCTION

Mastitis is the most costly disease in dairy cattle and is usually caused by bacterial infections that damage the udder tissues, thus initiating an inflammatory response. It is paramount importance to develop and utilize properly all marginal resources of animal production to withstand the problem of increasing human population in the world. Camel milk is one of the underutilized resources and it is a valuable source of energy, water and vitamins for the camel herders and the calves. The information available so far on mastitis in the camelidae is scarce, as people firstly believed that this disease is not common in the camels. However, in the recent years reports indicated the occurrence of the disease in Somalia (Abdurahman *et al*., 1995), Sudan (Obied *et al*., 1996) and Israel (Guliye *et al*., 2002). Free radical and non free radical oxidants can produce damaging effects in animal tissues if antioxidants are deficient (Nockels, 1996). Important infectious diseases in farm animals, such as mastitis are thought to be associated with the so-called oxidative stress (Lykkesfeldt and Svendsen, 2007). Levels of lipid hydroperoxide were increased in erythrocytes isolated from d airy cows with acute mastitis. The detection of increased lipid peroxidation and lowered ascorbate levels suggest that the disease caused udderrelated oxidative stress (Bernabucci *et al*., 2005; Castillo *et al*., 2006). The involvement of oxidative stress in mastitis is further substantiated by data from experimental mastitis induced either by intramammary infusion of *E. coli* bacteria (Weiss *et al*., 2004) or *E. coli* or staphylococcal endotoxin (Komine *et al*., 2004).

Oxidative stress has been associated in several inflammatory conditions and incriminated in the pathogenesis of many diseases in human and animals. However, little information is available on the status of plasma antioxidant levels, essential components of important antioxidant enzymes in camel blood in inflammatory udder conditions.

MATERIALS AND METHODS

Blood samples were collected from three groups of dairy camels (Camelus dromedarius) from Sudan, with 25 in each group: Camels with healthy udder, clinical mastitis and subclinical mastitis. The camels were randomly selected from a herd on the basis of the California mastitis test, somatic cell count and total bacterial count.

Camels were milked by hands and samples were taken for the detection of mastitis. Somatic cell counts were determined using a Zm coulter counter (Coulter electronics Ltd, Uk). Plasma TAS was measured using kit (Randox Laborateries Ltd, UK), by the incubation of 2,2'-azino-di-(3-ethyl-benzthiazoline sulphonate) with a peroxidase (metamyoglobin) and H_2O_2 to produce the cation ABT^+, which produces a relatively stable green colour, that can be measured at wavelength of 600 nm. Malondialdehyde plasma level was measured by using a colometric assay kits. A solution containing 2.55% (v v^{-1}) (metaphosphoric acid; plasma: acid; 2:1) was added to the

plasma and the mixture was frozen until thawed just prior the analysis. The storage period was less than1 week. The plasma ascorbic acid was measured by HPLC using the method adopted by Lykkesfeldt (2000). Plasma alpha tocopherol was measured using isocratic higher performance liquid chromatography with florescent detection (Miller *et al*., 1984). The plasma copper and zinc were measured by the atomic absorption.

RESULTS AND DISCUSSION

Table 1 outlines the various methods, grossly or microscopically to detect whether the camel milk is healthy, with clinical or sub clinical mastitis. Significant increase in lipid peroxidation ($p < 0.05$) in both camel with clinical or sub clinical mastitis compared to healthy camels. On other hand a decrease in both total and individual antioxidant in observed (Table 2). As for trace mineral examined in this study, both copper and zinc showed reduction in their plasma levels due to both forms of mastitis as compared with non-mastitic camels (Table 3).

The study indicated that antioxidants are very important for the mammary glad health the resistance against mastitis and enhances the immune system. Vitamin E status of dairy cows is related to mammary gland health (Weiss *et al*., 1990). Plasma and milk levels of vitamin E were determined in mastitic and healthy cows and compared with erythrocyte GSH and GSH-peroxidase, selenium, silicon, prostaglandins and parameters commonly used for diagnosing mastitis.

The mean plasma ascorbic acid concentration was significantly lower in cows with sub-clinical and clinical mastitis and the erythrocytic lipid peroxide levels were significantly higher in clinical mastitis as compared to controls. There was a significant decrease in mean blood zinc concentration in sub-clinical and clinical mastitis, but an increase in mean blood copper level in the clinical mastitis group (Ranjan *et al*., 2005).

Free radicals, especially superoxide and hydroxyl radicals, play critical role in inflammatory reactions, with over production of oxidants. In cows with subclinical form of inflammation of the mammary gland, a decreased potential of antioxidant protection in the blood was noticed, which is manifested in lower ascorbic acid (Kleczkowski *et al*., 2005). The blood antioxidant status declines in inflammatory udder conditions, suggesting that incorporation of antioxidants may help in better management of mastitis in dairy cows (Ranjan *et al*., 2005).

Antioxidants like copper (Scaletti *et al*., 2003), ascorbic acid (Chaiyotwittayakun *et al*., 2002) and vitamin E (LeBlanc *et al*., 2002) increase the resistance against

Table 1: Criterion for mastitis detection in the camels (camelus dromedarius)

Change in colour of the milk
Clinical observation of pain reflex on pressure
Swelling of the udder
California Mastitis Test (CMT) and Somatic Cell Count (SCC)
N-Acetyl-B-D-glucosaminedase (NAGase)

Table 2: The level of antioxidant and oxidative stress biomarkers in the plasma of healthy camels (*Camelus dromedarius*) and camels with sub clinical or clinical mastitis

Biomarkers	Healthy	Subclinical	Clinical
Malondialadehyde (MDA) ($\mu M\ L^{-1}$)	32.56 ± 3.45^{a}	37.43 ± 2.98^{b}	42.44 ± 1.99^{c}
Total Antioxidant Status (TAS) ($mmol\ L^{-1}$)	0.213 ± 0.008^{a}	0.164 ± 0.006^{b}	0.111 ± 0.009^{c}
Ascorbic Acid (AA) ($mg\ L^{-1}$)	3.98 ± 0.01^{a}	2.68 ± 0.02^{b}	1.88 ± 0.09^{c}
Alpha tocopherol	2.08 ± 0.03^{a}	1.72 ± 0.05^{b}	1.46 ± 0.06^{c}

Values are expressed as means±Sd, a,b,c, Means on the same raw having different superscripts are significantly different at $p<0.05$

Table 3: The levels of copper and zinc in the plasma of healthy camels (*Camelus dromedarius*) and camels with sub clinical or clinical mastitis

Biomarkers	Healthy	Subclinical	Clinical
Cu ($\mu g\ 100\ mL^{-1}$)	58.62 ± 3.4^{a}	52.80 ± 2.6^{b}	48.24 ± 4.2^{c}
Zn ($\mu g\ 100\ mL^{-1}$)	50.86 ± 2.0^{a}	44.08 ± 4.2^{b}	42.06 ± 2.2^{c}

Values are expressed as means±Sd, a,b,c, Means on the same raw having different superscripts are significantly different at $p<0.05$

mastitis. To optimize performance, oxidative stress in high producing cows must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate reactive oxygen metabolites.

In line with the current findings in dairy camels, the plasma and milk levels of vitamin E in plasma was significantly lower in mastitic than healthy cows and there was a negative correlation of mastitis status with vitamin E levels in plasma and milk (Batra *et al*., 1992; Atroshi *et al*., 1987). Although intramammary infections develop in llamas, inflammation (mastitis) appears to be rare. Values for mastitis indicator tests used for cows cannot be directly extrapolated to llamas.

CONCLUSION

The present investigation indicated a reduction in total and individual antioxidant status and an increase in lipid peroxidation in camels subjected to clinical or sub clinical mastitis. Optimum antioxidant intake in the feed may enhance the resistance against mastitis, by augmenting the immune system.

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