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ANTIOXIDANT TRACE ELEMENTS AND OXIDATIVE STRESS STATUS IN DAIRY CATTLE SUFFERING FROM MASTITIS

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ABSTRACT

Mastitis is an inflammation of the mammary glands caused by several pathogens. Reactive oxygen species are natural end products of the intensive cellular metabolism. Oxidative stress occurs when there is a disturbance of homeostasis causing inflammation of the mammary gland. This study was performed to evaluate blood antioxidant profile and trace elements in dairy cows with clinical mastitis. For this aim, venous blood samples were collected from 116 cows (100 mastitic, 16 control). In cows with clinical mastitis, there was a significant ($p<0.05$) increase in the clinical index score compared with control group. Biochemically, there was a significant ($p<0.05$) decrease in super oxide dismutase, catalase and total antioxidant capacity as well as in the level of zinc. However, there was a significant ($p<0.05$) increase in the levels of malondialdehyde and glutathione reductase as well as in that of copper. The results of the current study indicate that the body antioxidant defense system was compromised in dairy cows with clinical mastitis creating a state of oxidative stress.

INTRODUCTION

Mastitis may be defined as an inflammation of all structures forming the mammary tissue and the surrounding connective tissue. The disease is the reaction of the mammary gland to irritants and significantly influences the quality and quantity of mammary tissue and milk **(Yüksel *et al.*, 2009).** It is mainly resulting in pathological, physical and chemical changes in milk and glandular tissues, resulting from an invasion of mammary tissues by pathogenes through the teat canal as mentioned by **Quinn *et al.*, (2002) andRadostits *et al.*, (2007).** The disease results in some changes in the composition of milk and shortening of the productive lives of cows **(Gürbulak *et al.*, 2009).** It is important to improve productivity and quality of milkby reducing mastitis incidence **(Halasa *et al.*, 2007;Cha *et al.*, 2011andHogeveen *et al.*, 2011).**

Mastitis was found to induce economic losses including value of discarded milk as milk from affected cows may become unsuitable for human consumption due to

bacterial contamination resulting in food poisoning, interference with manufacturing process and in rare cases providing mechanism of spread of zoonotic diseases amongpopulation. Zoonotic diseases transmitted via raw cow milk included Staphylococcal food poisoning, brucellosis, tuberculosis, toxoplasmosis and leptospirosis as previously stated by**Mungube *et al.*, (2005) and Radostits *et al.*, (2007)**.

Oxidative stress is usually described as an imbalance between antioxidant and oxidant levelsas mentioned by**Lykkesfeldt and Svendsen, (2007).** A condition of oxidative stress was produced when the production of oxidants exceeded the capacity of antioxidant defense resulting in oxidative damage to macromolecules such as lipid, proteins and DNA**(Sordillo and Aitken, 2009).**

In this regard, **Jin *et al.*, (2014)** mentioned thatMammary epithelial cells exhibit a high metabolic rate during lactation and therefore large amounts of lipid peroxides and reactive oxygen species (ROS) are produced in vivo**.**

A few hours after the udder infection with pathogenic microorganisms, the number of somatic cells in the milk, was found to be increased in response to activation of the inflammatory processes. When the mammary gland was invaded and was colonized by bacteria, the macrophages responded by initiating the inflammatory response, one that attracted polymorphonuclear cells in milk to kill the bacteria. More than 90% of SCC in infected glands was composed of neutrophils **(Groza, 2006; Andrieu, 2008).**More over **Rinaldi *et al.*, (2007)** stated thatReactive oxygen species (ROS) mediatedthe antibacterial activity of neutrophils**.**

An excess of oxidative reactions of bacterial processes might cause damage to the tissues. The absence of optimal amounts of antioxidants and an excess of ROS resulted in oxidative stress development **(Andrei, 2010b)**. Free radicals are natural end products of the intensive metabolism in cells of the living organism, including high-yielding dairy cows. When the homeostasis was disturbed mainly by generation and accumulation of these free radicals, oxidative processes resulted in oxidative stress causing mastitis in dairy cows **(Poławska *et al.*, 2012)**.

The objective of the present study was toevaluate the role of oxidative stress biomarkers, antioxidant enzymes and metabolites as possible biomarkers for clinical mastitis in dairycows.

MATERIALS AND METHODS

A total number of 116 dairy cows aged between three to ten years were included in this study. Off all, 100 cows exhibited the clinical signs of mastitis. Besides, 16 apparently healthy cows were selected as a control group. All samples were collected in the period between October, 2014 and January, 2015, where 96 samples were collected from Dakahlia while 20 samples were collected from Damietta Governorate, Egypt.

According to **Radostits *et al.*, (2007),** detailed clinical examination of the animals was carried out. Physical examination of the cows was concerned with evidence of systemic illness (on which fever, ruminal stasis, inappetence to anorexia, tachycardia, dullness and recumbency was observed and recorded). Also, local physical examination of the udder via palpation and inspection of the quarters as well as the supramammary lymph nodes was conducted. It was directed toward detection of the inflammatory cardinal signs or evidence of fibrosis or atrophy. For observation of any abnormalities in the examined milk, visual examination of the milk was done including (milk flakes, clots, pus, watery and bloody secretion).

Sampling:

1. Milk sample

Under complete aseptic precautions, sampling of the examined milk was taken using standard methods described by **Hogan *et al.* (1999).**About (20 ml) of milk was obtained from each affected quarter in separate sterile and well corked falkon tubes. The obtained milk samples were incubated at 37 ^{o}c for 12-24hours, then centrifugate the tubes at 3000 rpm for 15 minutes for concentration of the bacterial cells at the sediment and discard the supernatant. From the sediment, a loopful of about (0.01ml) was mixed with 5 ml of TryptoneSoya Broth **(Oxoid, LTD, Batingstock, Hampshire, England)** in sterile corked test tubes. Then, the tubes were incubated for 18 hours. Each sample was streaked on three different selective media. For isolation of *Staphylococcus aureus*, Baird parker (Bp.) medium **(Oxoid)** supplemented with 5% egg yolk- telluriteemulsion was used. Edward's medium (EDW.) **(Oxoid)** supplemented with 6% defibrinated sheep blood was used for the selective isolation *of Streptococcus spp.* For *Esherichiacoli*selective isolation, Eosin methelene blue medium (EMB.) was used **(Quinn *et al.*, 2002)**.

2. Blood Samples Collection

Two types of venous blood samples (ten ml for each) were collected via puncture of jugular vein from each cow. **Blood plasma samples,** were collected into Eppendorf tube which was mixed with Ethylenediaminetetraacetic acid (EDTA) as anticoagulant for biochemical estimation of level and activity of SOD, CAT and GR. hematological studies.**Blood serum sample,** were collected in clean dry tube without anti-coagulant. The collected blood samples were left to coagulate, thencentrifuged at 3000 rpm for 10 minutes to obtain blood serum forbiochemical analysis of TAC, MDA, Zn and Cu levels according to the method described by manufacture **(Biogiagnostic, Cairo,Egypt).**

3. Statistical analysis

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA). Means and standard deviation for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Correlations between different parameters were carried out using Pearson correlation coefficient. Differences between means at $p < 0.05$ were considered significant.

RESULTS

Concerning the bacteriological isolation and identification, *Staphylococcus aureus* appeared as black shiny colonies surround by hallow zone on Baired parker. *Escherichia coli (E. coli)* appeared as green metallic sheen colonies on the Eosin Methelene Blue media. While *Streptococcus agalactiae (S. agalactiae)* appeared on Edward's media (B hemolysis)as colorless colonies with bluish hue and surrounded with complete zone of hemolysis. In the current study, the bacteriological isolation profile showed that about (159) bacterial isolates were obtained from the (100) cultured milk samples. The mixed infection 118/159 (74.21%) between *Staphylococcus spp*., *Streptococcus spp*. and *E.coli* was the

largest percentage of isolation, then isolation percentage, came to be for *Staphylococcus spp.* 21/159 (13.22%) then for *E. coli* 15/159 (9.43%) and Streptococcus *spp.* 5/159 (3.14%).

Regarding the level of different oxidative stress markers and enzymatic antioxidants activities in clinical mastitis, there was a significant($p < 0.05$) decrease in Super oxide dismutase (SOD), Catalase (CAT) and Total antioxidant capacity (TAC) compared to those of the control group. Whereas there was a significant ($p < 0.05$) increase in Malondialdehyde (MDA) and Glutathione reductase (GR) compared to control group.

Regarding different antioxidant trace elements levels in clinical mastitis, there was a significant ($p< 0.05$) decrease in Zn level compared to control group. Whereas there was a significant ($p< 0.05$) increase in Cu level compared to those of the control group.

Table 1: Clinical signs of cows affected with mastitis caused by Staphylococcal spp., E. coli, Streptococcal spp. and mixed infection.

Clinical variables	Staphylococcus spp.	Streptococcus spp.	E. coli	Mixed infection
	Number of animal affected(n=21)	Number of animal affected(n=5)	Number of animal affected(n=15)	Number of animal affected(n=59)
Evidences of fever with systemic illness				
Mild(39.5°c- 39.9°c)	16	4	2	38
Sever(40°c- 41.4°c)	5	1	13	21
No systemic illness	0	0	0	0
Udder abnormalities				
Cardinal signs of inflammation	21	5	15	59
Evidence of fibrosis or atrophy	0	0	0	0
physical characters of milk				
Pus and greenish discoloration	2	2	1	6
Yellowish discoloration	5	0	10	16
Milk clots	7	3	4	30
Milk tinged with blood	5	0	0	6
Whole bloody milk	2	0	0	1

Table 2: Different Oxidative Stress Markers, Enzymatic Antioxidants Activity and Trace elements level (mean values ± SD) in Clinically Healthy cows and in those with clinical mastitis disease.

Groups	SOD (U/mL)	CAT (U/L)	MDA (nmol/mL)	TAC (mM/L)	GR (U/L)	Zn (µg/dl)	Cu (µg/dl)
Control ***(n = 16)***	219.8± 50.8[a]	494.6±147.0[a]	15.1±2.1[a]	1.1±0.2[a]	934.5± 306.6[a]	267.4±64.4[a]	70.2±7.2[a]
E-coli ***(n=15)***	151.7±36.0[b]	406.8±122.8[a]	17.7±2.2[b]	0.9±0.1[b]	1214.9±448.0[b]	190.5±9.0[b]	77.2±2.3[b]
Staphylococcus ***(n=21)***	159.5±37.5[b]	349.8±126.8[b]	16.4±1.4[a]	0.9±0.1[b]	1298.4±335.2[b]	193.0±13.3[b]	76.8±16.8[b]
Streptococcus **(n = 5)**	164.5±39.0[b]	242.3±207.4[b]	17±0.8[b]	0.8±0.1[b]	1171.6±256.3[ab]	184.9±3.6[b]	77.0± 1.8[b]
Mixed **(n = 59)**	157.6±35.6[b]	294.8±147.9[c]	16.9±1.9[b]	0.9±0.1[b]	1153.8±274[ab]	190.6±11.4[b]	76.5± 2.7[b]

DISCUSSION

All clinically examined dairy cows, in the current study, displayed a significant increase in the clinical index score. These findings were in agreement with those reported by **Biffa *et al.* (2005) and** _ENREF_38**Bedane *et al.* (2012).** Such findings might be attributed to the inflammation of the mammary glands following their infection which represents the stage at which clinical mastitis occurs with appearance of gross abnormalities of the milk, variable degrees of clinical abnormalities of the udder and variable systemic effects. Moreover, the clinical signs of the disease was found to be varied in severity depending on the causative microorganisms and their virulence **(Radostits *et al.*, 2007)**.

In the present study SOD was significantly decreased compared to those of the control group. These results were similar to those reported by **Machado *et al.*, (2014)** who attributed such decrease to the low level of zinc in the blood of mastitic cows.

In the current study, there was a significant decrease in catalase activity which was similar to other finding observed by **Jhambh *et al.* (2013).** The decreased enzymatic antioxidant activities might be related to the increased consumption to counteract the effect of reactive oxygen species as catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide into hydroxyl radicals and water.

Lipid peroxidation was a non-enzymatic chain reactionleaded to creation of lipid peroxides and other intermediates, based on oxidation of mainly unsaturated fatty acids. These intermediates including malondialdehyde (MDA) might influence the properties of cell membranes **(Catalá, 2010)**. In the present study, malondialdehyde was significantly increased comparatively to the control group. These results were similar to those reported by**Jhambh *et al.*, (2013)**. During clinical mastitis, the enhanced concentrations of plasma MDA, could be due to excessive ROS production from the clinically inflamed mammary gland suggesting a compromise in antioxidant defense of the body **(Boulanger *et al.*, 2002)**.

In the current study, the antioxidant defense system was compromised in dairy cows with clinical mastitis, which was evidenced by decreased plasma TAC and increased MDA level, which might indirectly indicate increased whole activity of free radical reflecting the worse state of oxidative stress in such cases**(Celi, 2010).**

Glutathione reductase was significantly increased comparatively to the control group. The involvement of glutathione dependent enzymes (glutathione peroxidases and glutathione reductase) would be important for lipid peroxides neutralization during clinical mastitis. These results were similar to those previously reported by **(Halliwell and Chirico, 1993).** Also our findings may be owing to the enhancement of the coupled activities of the glutathione dependent enzymes, leading to intense regeneration of reduced glutathione (GSH) from oxidized glutathione(GSSH) obtained after reduction of peroxides into alcohols as previously stated by **Kizil *et al.* (2007).**

Trace elements as copper and zinc playedan important role in several biochemical processes as they are essential component in the antioxidant enzymes as SOD and CAT **(Yang and Li, 2014)**.In the current study zinc was significantly decreased comparatively to the control group. Such results were similar to the result previously reported by**Ranjan *et al.*, (2005)**. Its deficiency was associated with decreased leukocyte function and increased susceptibility to bacterial infection **(Hayajneh, 2014)** with a resultant of a state of oxidative stress **(Zago and Oteiza, 2001).** Moreover, the decreased serum zinc level was regarded as non-specific host defense mechanism against bacterial infection, which would reduce the availability of that divalent cation needed for bacterial growth as previously stated by **(Failla, 2003)**.

Copper is involved in the antioxidant system via its involvement in ceruloplasmin and the enzymes Cu–Zn SOD. Ceruloplasmin is a Cu transport protein that also exhibits oxidase activity. In addition it is an acute phase protein that increases during infection and may be important in scavenging superoxide radicals **(Broadley and Hoover, 1989)**. In the present study copper was significantly increased compared to control group, these results were similar to those recorded by **(Ranjan *et al.*, 2005)**. Ceruloplasmin contains more than 95% of the circulating copper in animals. Its level in blood has been reported to increase many fold during inflammation.Therefore, increase in blood copper level in the clinical cases of mastitis might be due to elevated level of ceruloplasmin in inflammatory udder conditions **(Conner *et al.*, 1986)**.

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الملخص العربي

العناصرالنزرة المضادة للأكسدة وحالة الاكسدة في الأبقارالحلوب المصابة بالتهاب الضرع

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مرض إلتهاب الضرع هو إلتهاب في الغدد الثديية الذي يسببه العديد من المسببات المرضية. تعتبر أنواع الأكسجين النشطة هي الناتج الطبيعي النهائى للأيض الخلوي. يحدث الإجهاد التأكسدي عندما يكون هناك إضطراب في التوازن ويؤدي ذلك إلي إلتهاب الضرع في الأبقار عالية الإنتاج. وقد أجريت هذه الدراسة لتقييم دور مضادات الأكسدة في دم الأبقار التي تعاني من إلتهاب الضرع.

لقد تم تجميع عينات الدم الوريدي عشوائيا من ١٦ بقرة لاتعاني من أمراض كمجموعة ضابطة و ١٠٠ بقرة حلوب مصابة بإلتهاب الضرع بناءا علي الفحص الطبي الشامل. وجد في الأبقارالمصابة بإلتهاب الضرع أن هناك زيادة ملوحظة في مؤشر الفحص الإكلينيكي مقارنة بالمجموعة الضابطة.

لوحظ أن هناك نقص نوعي في قيمة السوبر أكسيد ديسموتيز و الكتاليز و القدرة المضادة للأكسدة الكلية والزنك بينما كانت هناك زيادة ملحوظة في المالون داي الديهيد و الجلوتاثيون ريداكتاز و النحاس.

نستخلص من نتائج الدراسة الحالية أن هناك تأثير سلبي علي نظام الدفاع المضاد للأكسدة بالجسم في الأبقار المصابة بإلتهاب الضرع الإكلينيكي مؤدياً الي حالة من الإجهاد التأكسدي.