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Amelioration of Oxidative Stress Through Supplementation of Self- formulated Anti-oxidant Mixture for Early Recovery and Prophylaxis of Bovine Mastitis in Kashmir Valley

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ABSTRACT

Background: During lactation, mammary epithelial cells exhibit a high metabolic rate and thus produce large amounts of ROS and lipid peroxides *in vivo* (Jin *et al.,* 2014; Ganguly *et al*., 2016). A surplus of ROS and the absence of optimal amounts of antioxidants (which neutralize these free radicals or ROS) results in oxidative stress (Lykkesfeldt and Svendsen, 2007). A clinical study was undertaken on Bovine Mastitis in Kashmir valley to study the relation between oxidative stress and clinical mastitis. An attempt was also made to see the effect of supplementation of self-formulated anti-oxidant trace mineral mixture on recovery and prophylaxis of Bovine mastitis through amelioration of oxidative stress.

Methods: The oxidative stress was assessed through estimation of superoxide dismutase (SOD), catalase, reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO). In addition, blood trace mineral status for copper (Cu), zinc (Zn), manganese (Mn) and selenium (Se) were also assessed in mastitic animals and compared with normal healthy lactating animals. The utility of anti-oxidants in clinical management of mastitis was measured through response to treatment with trace minerals like Cu, Zn, Mn and Se in addition to conventional antibiotic therapy. Two groups of mastitic animals received two therapeutic regimens. Group I animals received antibiotics and self formulated anti-oxidant mixture at therapeutic doses while as Group II animals received only antibiotics (at same dose rate and frequency). Clinical recovery was assessed on the basis of CMT point score, milk somatic cell count and milk biochemistry. For prophylactic study, forty recently parturated lactating animals having susceptibility to occurrence of mastitis were divided into two groups of twenty animals each. One group of animals was supplemented with self-formulated anti-oxidant mixture at prophylactic doses for a period of thirty days so as to see the effect of supplementation on oxidative stress parameters and occurrence of clinical mastitis.

Result: A significant decrease was found in the values of SOD, catalase, GSH and Cu, Zn, Mn and Se but a significant increase was found in the values of MDA and NO in clinical cases of mastitis as compared to healthy control group. Therapeutic regimen I proved efficacious than the therapeutic regimen II in treatment of clinical mastitis with higher recovery rates and lesser number of mean days required for recovery in group I than group II animals. The efficacy of prophylactic treatment was monitored by occurrence of mastitis during the course of therapy and one month after therapy. Group I animals did not suffer from clinical mastitis and showed considerable improvement in oxidative stress parameters, milk SCC and blood trace mineral status as compared to group II.

Key words: Antioxidants, Bovine mastitis, Catalase, GSH, MDA, Nitric oxide, Oxidative stress, SOD, Trace minerals.

INTRODUCTION

Reactive oxygen species (ROS) are natural end products of the intensive cellular metabolism. When the disturbance of homeostasis occurs, oxidative processes lead to oxidative stress causing inflammation of the mammary gland (mastitis) in high-yielding dairy cows. Mastitis is characterized by a range of physical and chemical changes of the milk and pathological changes in the udder tissues (Constable *et al*., 2017). The importance of mastitis is emphasised by the fact that it produces huge losses in cattle industry (Salman, 2009; Maćešić *et al*., 2016). The overall prevalence of bovine mastitis has been recorded to be 14.86% in Kashmir valley which highlights the economic importance of the disease (Ifat *et al*., 2017). Since mastitis is caused by different microorganisms, the prevention, treatment and control of the disease is hardly feasible (Russell, 2011; Deb *et al*., 2013). So far, conventional antibiotic therapy is the only proven method for the prevention and control of mastitis,

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but several problems arise from the use of antibiotics like developing resistance to antibiotic, questionable drug efficacy and presence of antibiotic residues in the milk (Petrovski and Eats, 2014). These facts highlight the need for completely newer moieties for treatment of mastitis.

During lactation, mammary epithelial cells exhibit a high metabolic rate and thus produce large amounts of ROS and lipid peroxides *in vivo* (Jin *et al.,* 2014; Ganguly *et al*., 2016). A surplus of ROS and the absence of optimal amounts of antioxidants (which neutralize these free radicals or ROS) results in oxidative stress (Lykkesfeldt and Svendsen, 2007). Oxidative stress in turn affects the cellular metabolism leading to deteriorated health in animals (Celi, 2011). It is the primary factor that leads to immune dysfunction and impairs the inflammatory response which in turn is reflected particularly as the inflammation of the udder (Abuelo *et al*., 2013). The suppression of immune resistance due to oxidative stress can lead to the establishment of different microbial pathogens in mammary gland leading to mastitis (Mir *et al*., 2017). Mastitis could induce the increase of free radicals formation in milk and leading to oxidative stress (Gu *et al*., 2009). Both CM and SCM are associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk (Atakisi *et al*., 2010; Patnaik *et al*., 2014). It has been reported that significant decrease in blood superoxide dismutase (SOD) and catalase activities, reduced glutathione (GSH) concentration and an increase in erythrocytic lipid peroxides was observed in cows with clinical mastitis (Jhambh *et al*., 2013).

Trace minerals are critical for proper immune response and play an important role in udder health (Ganda *et al*., 2016). Trace minerals with an antioxidant function include selenium (Se), copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe). While some nutrients have a role in directly quenching free radicals, these trace minerals have an indirect role in which they are required as components of a variety of antioxidant enzymes (Marta Wolonciej *et al*., 2016). For example, enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase are considered to be an important defense system against free radical accumulation. The determination of products of peroxidative damage to macromolecules and antioxidant substances like reduced glutathione and enzymes (*e.g*. SOD and Catalase) are useful markers for the oxidative stress and antioxidant status respectively (Sharma *et al.,* 2011b). Membrane phospholipids are prone to oxidation and malondialdehyde (MDA) is generated as a consequence of lipid peroxidation and as such is assayed as a biomarker of oxidative stress (Abuelo *et al*., 2014). Nitric oxide (NO) is one of the most important reactive nitrogen intermediates produced in a significant amount by epithelial cells and macrophages of mammary gland during mastitis (Bouchard *et al*., 1999). This excessive release of NO results in oxidative damage to mammary gland and thus estimation of NO is also assayed as a biomarker of oxidative stress (Komine *et al*., 2004; Atakisi *et al*., 2010). So, joint evaluation of oxidative stress biomarkers and anti-oxidants more accurately indicates the oxidative status of the animals (Cigliano *et al*., 2014; Costantini and Verhulst, 2009). Supplementation of antioxidant trace elements stabilize the highly reactive free radicals generated as a result of oxidative stress during mastitis, reduce the inflammatory response and maintain the structural and functional integrity of cells (Kushwaha and Mohan, 2019). The present study was therefore undertaken to evaluate the possible oxidative stress and alterations in anti-oxidant and trace mineral profile during clinical mastitis and to investigate the possible role of antioxidants in treatment and prophylaxis of this major economic disease of dairy animals.

MATERIALS AND METHODS

A total of 36 multiparous cows in the age group of 4-8 years with BCS 3.0 were included in the therapeutic study. The study was undertaken on clinical cases of bovine mastitis presented to Teaching Veterinary Clinical Complex (TVCC), Faculty of Veterinary Sciences and Animal Husbandry (F.V.Sc and A.H), Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) and local animal husbandry dispensaries in Ganderbal, Manasbal, Shuhama, Gulab bagh and Shalimar areas for treatment. 24 cases of clinical mastitis were studied and all the animals included were clinical cases. 12 apparently healthy lactating animals served as control group. The cases of clinical mastitis were diagnosed on the basis of clinical examination, case history and clinical findings. The severity of the cases was assessed on the basis of CMT score and SCC of milk samples. Cases with CMT score of 2 or 3 with somatic cell counts more than 4×10^5/mL of milk were considered positive for mastitis. For therapeutic trial, the clinical cases of mastitis were divided into two groups of twelve animals each. Group I animals were given self formulated anti-oxidant trace mineral mixture at therapeutic doses as recommended by (NRC, 2001) whose formulation is given in Appendix-I. All the four ingredients were mixed making a total weight of about 5.005 gms and stored in air tight zip lock polyethene pouches. Total 84 pouches were made and 7 pouches were given to individual animal owners for providing contents of one pouch to the animal once daily for 7 days. Group II animals were not given any anti-oxidant trace mineral mixture. Both the groups of animals were given antibiotic enrofloxacin at the dose rate of 6 mg/kg per animal twice daily for 5 days. The choice of the antibiotic was based on antibiotic sensitivity test of milk which was performed in each case. Animals which were not found sensitive to enrofloxacin were excluded from the study.

For prophylactic studies forty recently parturated lactating animals maintained at Mountain Livestock Research Institute (MLRI), SKUAST-K, Manasbal under similar managemental conditions but having susceptibility to occurrence of mastitis (as evidenced by precedence to occurrence of mastitis in previous lactations) were studied. One group of animals (Group I) comprising of twenty animals received self-formulated anti-oxidant trace mineral mixture

Appendix- I: Therapeutic dose of mineral mixture.

Copper sulphate	: 1 gm
Zinc sulphate	: 2 gm
Manganese sulphate	: 2 gm
Selenium	: 5 mg
Total dose	: 5.005 g/animal/day for 7 days

Appendix- II: Prophylactic dose of mineral mixture.

Copper sulphate	: 500 mg
Zinc sulphate	: 500 mg
Manganese sulphate	: 400 mg
Selenium	: 2 mg
Total dose	: 1.042 g/animal/day for 30 days

for a period of thirty days while as the other group (Group II) did not receive any anti-oxidants. The anti-oxidant trace minerals were given at prophylactic doses (Appendix-II) as recommended by (NRC, 2001). The total dose per animal was 1.402 grams. Six hundred pouches were made and one pouch per animal per day was given to selected twenty animals (Group I) for 30 days.

Blood sampling

Blood samples were collected from healthy lactating animals to obtain baseline values of oxidative stress parameters and trace minerals. Approximately 10 mL of blood was collected in mineral free heparinised vials under aseptic conditions from the jugular vein of healthy as well as mastitic cows. Blood was immediately centrifuged at 3000 rpm for 20 minutes to separate plasma. 3 mL of plasma was stored at -80°C for estimation of oxidative stress parameters (MDA, GSH and NO) and trace minerals. The RBC's were washed thrice with phosphate buffer saline in the ratio of 1:1 on v/v basis and centrifuged at 2000 rpm for 10 minutes and 1% erythrocyte lysate was prepared in normal saline (100 µL washed RBC+9.9 mL normal saline) for estimation of rest of oxidative stress parameters (SOD and catalase). MDA, SOD, catalase and GSH were estimated using commercial kits supplied by Sigma Aldrich (USA) whereas nitric oxide was quantified using modified Greiss reaction as described by Miranda *et al.* (2001). To determine superoxide dismutase activity in erythrocytes, the lysate was diluted 50-fold with 0.01 mmol/L phosphate buffer, pH 7.0, and then SOD was measured by a kinetic method using a commercial kit by Sigma Aldrich (USA) and results were expressed in international units per milliliter. For estimation of catalase, 1% hemolysate was used. The procedures for estimation of catalase, reduced glutathione, malondialdehyde and nitric oxide were first standardised to obtain standard curves and regression equations after which the values of the test samples were estimated using regression equation. The standard curves for catalase, reduced glutathione, malondialdehyde and nitric oxide are given in Fig 1, 2, 3 and 4 respectively. The plasma samples were digested using distilled concentrated nitric acid (AR, 15 mL) and each digested sample was diluted to 10 mL using double glass distilled water for mineral estimation. The samples were analysed at Kashmir University, Hazratbal in the deparment of University Scientific Instrumentation Centre (USIC) using Polarised Zeeman Atomic Absorption Spectrophotometer (Z-2300, Hitachi).

Evaluation of treatment efficacy

The efficacy of therapeutic regimes in clinical cases of mastitis was evaluated on the basis of clinical recovery, mean days required for recovery, CMT score, milk SCC, blood oxidative stress parameters on day 0, day 5 and day 10 of treatment and trace mineral status on day 0 and day 10 of treatment in both the groups of animals. The efficacy

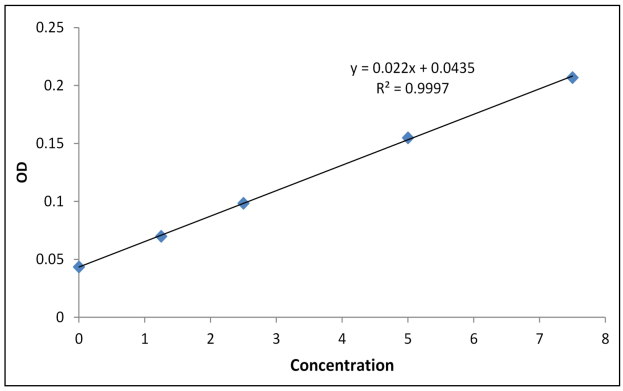

Fig 1: Std. curve of catalase.

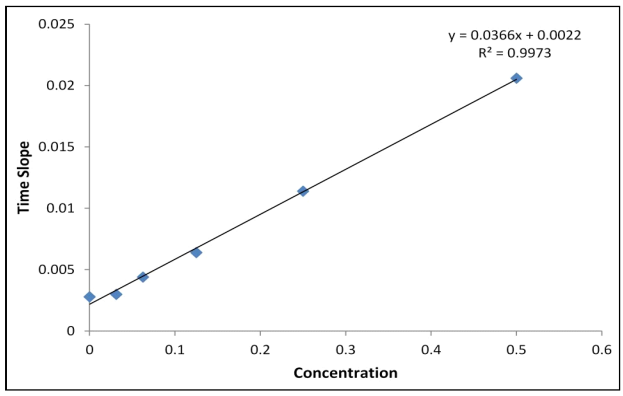

Fig 2: Std. curve for GSH.

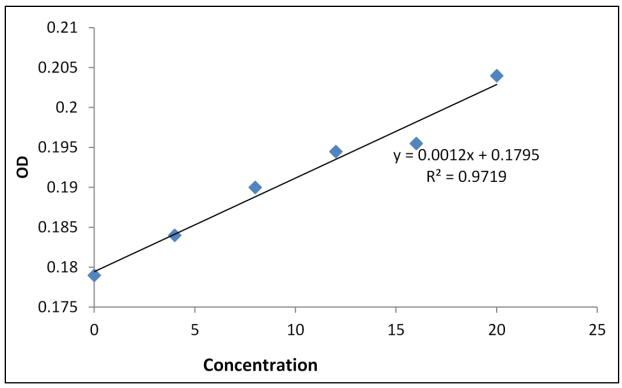

Fig 3: Std. curve for MDA.

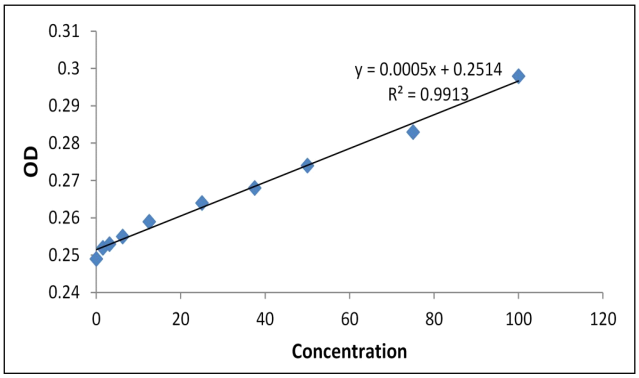

Fig 4: Standard curves of NO.

of prophylactic treatment was evaluated by occurrence of mastitis during the course of therapy and one month after therapy, estimation of SCC in milk and blood oxidative stress parameters on day 0, day 15 and day 30 of treatment in both the groups of animals. Mineral estimation for copper, zinc, manganese and Selenium was also done on day 0, day 15 and day 30 to determine the effect of anti-oxidant trace mineral supplementation on their blood values. After fifteen days of stopping the treatment, the udder health status of both the groups of animals was assessed through estimation of SCC of milk and CMT.

Statistical analysis

The data was analyzed using statistical tools (SPSS version 20). ANOVA followed by Duncan's muliple range test was used for multiple comparisons. Paired 't' test was used for pre and post treatment comparisons within each treatment group. Repeated measures ANOVA was used for pre and post treatment multiple comparisons. Statistical differences were determined at the 5% level of significance.

RESULTS AND DISCUSSION

A statistically significant decrease was found in the values of anti-oxidant enzymes like SOD, catalase and GSH and a statistically significant increase was found in the values of oxidative stress markers like MDA (µmoles/L) and NO (µmoles/L) in mastitic animals (mastitic group) than normal healthy animals (control group). A statistically significant decrease was found in the values of Cu, Zn, Mn and Se in mastitic group as compared to control group (Table 1). Therapeutic regimen I for Group I animals proved efficacious than the therapeutic regimen II for group II animals in treatment of clinical mastitis with recovery rates of 73.33 and 66.66% , respectively in the two groups. Group I animals showed early recovery with lesser number of mean days required for recovery (4±0.5 days) than group II animals (4.5±0.8 days).The early and efficacious clinical recovery in group I animals receiving therapeutic regimen I was also confirmed by the return of oxidative stress parameters, milk SCC and trace mineral status to normal or near normal values on day 10 of treatment than group II animals receiving therapeutic regimen II (Table 2, 3 and 4).

Prophylactic study

Group I animals receiving anti-oxidant minerals showed a considerable improvement in the oxidative stress parameters, trace mineral status and milk SCC as compared to group II on day 15 and day 30 post treatment. The animals in the group II showed consistently increasing oxidative stress parameters, lower levels of trace mineral status and increased milk SCC. Clinical mastitis was not detected in any of the animals in both the groups during the course of the therapy and one month after the study. However, the udder health status of group I animals was found better than group II as evidenced by significant decrease in milk SCC counts 15 days after stopping the treatment (Table 5 and 6).

Our findings of increased oxidative stress markers and decreased anti-oxidants in clinical mastitis are in agreement with (Ibrahim *et al.*, 2016) who reported that in dairy cows with acute clinical mastitis, there is a significant ($p<0.05$) decrease in the TAC (total antioxidant capacity), GSH and CAT as well as in the level of zinc and iron but there is a significant ($p<0.05$) increase in the level of MDA, NO and the OSI (oxidative stress index). Since the levels of trace minerals were also found to be decreased in clinical mastitis in the present study, their supplementation resulted in better and earlier recovery rates in mastitic animals. Our findings are in agreement with (Khushwaha and Mohan, 2019) who reported that deficiency of vitamins and minerals as antioxidants particularly vitamin E, vitamin D, selenium, zinc and copper leads to increased incidence of mastitis with infection of longer duration and more severe clinical signs and supplementation of antioxidant vitamins and trace elements stabilizes the highly reactive free radicals generated as a result of oxidative stress, reduces inflammatory response and maintains the structural and functional integrity of cells during mastitis. Yang and Li (2015) also reported that supplementation of mastitic dairy cows

Table 1: Comparison of blood oxidative stress parameters, blood trace mineral profile and milk SCC between healthy lactatating (control group) and mastitic animals (Mean±S.E).

Parameters	Control group (N=12)	Mastitic group (N=24)
SOD (U/mL)	151.47±1.69^a	100.67±1.39^b
Catalase (nmoles/min/mL)	36.08±1.16^a	20.24±.67^b
GSH (nmoles/mL)	59.09±1.57^a	38.66±1.08^b
MDA (µmoles/L)	30.37±1.10^a	54.69±1.45^b
NO (µmoles/L)	33.02±1.29^a	58.48±1.09^b
Copper (µmol/L)	8.25±.07^a	7.01±0.06^b
Zinc (µmol/L)	14.47±.31^a	11.71±0.16^b
Mn (µmol/L)	3.78±.11^a	2.94±0.04^b
Se (ng/mL)	38.47±1.67^a	31.29±1.14^b
SCC ($\times 10^5$/mL)	2.83±.05^a	9.94±0.22^b

Values within a row having superscript (a,b,) with atleast one common alphabet do not differ significantly at 5% level ($p<0.05$) from each other.

Table 2: Effect of treatment on blood oxidative stress parameters and milk SCC in animals with clinical mastitis (Mean±S.E).

Parameters	Days of treatment	Group I (Antibiotic+Antioxidant trace minerals)	Group II (Antibiotic only)
SOD (U/mL)	Day 0	100.55±1.47^{a1}	100.81±2.61^{a1}
	Day 5	118.00±1.52^{b1}	110.44±2.47^{b2}
	Day 10	140.00±2.19^{c1}	116.88±2.12^{b2}
Catalase	Day 0	20.69±.98^{a1}	19.67±.89^{a1}
(nmoles/min/mL)	Day 5	25.67±1.08^{b1}	22.25±.81^{b2}
	Day 10	31.23±1.00^{c1}	23.60±.71^{b2}
GSH	Day 0	38.88±1.53^{a1}	38.39±1.55^{a1}
(nmoles/mL)	Day 5	49.91±1.60^{b1}	43.29±1.35^{b2}
	Day 10	60.57±.98^{c1}	48.25±1.37^{c2}
MDA	Day 0	57.73±1.69^{a1}	50.91±2.08^{a2}
(µmoles/L)	Day 5	44.14±1.38^{b1}	44.98±2.26^{ab1}
	Day 10	30.52±.85^{c1}	40.37±2.23^{b2}
NO	Day 0	59.95±1.33^{a1}	56.65±1.72^{a1}
(µmoles/L)	Day 5	37.97±1.26^{b1}	46.78±1.66^{b2}
	Day 10	30.33±.78^{c1}	40.80±1.19^{c2}
SCC	Day 0	9.88±.31^{a1}	10.03±.33^{a1}
(×10^5/mL)	Day 5	6.00±.36^{b1}	7.63±.17^{b2}
	Day 10	3.19±.09^{c1}	4.43±.13^{c2}

Values within a row having superscript (1, 2, 3) with atleast one common numerical do not differ significantly at 5% level ($p<0.05$) from each other. Values within a columnhaving superscript (a,b,c) with at least one common alphabet do not differ significantly at 5% level ($p<0.05$) from each other.

Table 3: Effect of therapeutic regimens on trace mineral profile of animals with clinical mastitis (Mean±S.E).

Parameters	Days of treatment	Group I (Antibiotic+Antioxidant trace minerals)	Group II (Antibiotic only)
Copper (µmol/L)	Day 0	6.88±.06^{a1}	7.18±.10^{a1}
	Day 10	7.65±.06^{b1}	7.12±.13^{a2}
Zinc (µmol/L)	Day 0	11.86±.22^{a1}	11.52±.22^{a1}
	Day 10	13.85±.21^{b1}	11.13±.16^{b2}
Mn (µmol/L)	Day 0	2.94±.06^{a1}	2.94±.04^{a1}
	Day 10	3.30±.03^{b1}	2.93±.05^{a2}
Se (ng/mL)	Day 0	29.94±1.80^{a1}	32.98±1.16^{a2}
	Day 10	35.39±1.46^{b1}	31.67±1.08^{b2}

Values within a column having superscript (a,b) with atleast one common alphabet do not differ significantly at 5% level ($p<0.05$) from each other. Values within a row having superscript (1,2,3) with atleast one common numericaldo not differ significantly at 5% level ($p<0.05$) from each other.

Table 4: Therapeutic efficacy of treatment regimes in animals with clinical mastitis.

	Group II	Group III
Animals treated	12	12
Animals recovered	10	8
% recovery	83.33%	66.66%
Recovery time (Days±S.E)	4±0.5	4.5±0.8

with antioxidant vitamins as vitamin A, C, E and β-carotene, and antioxidant minerals as selenium, zinc and copper is very important to help the animal recover early.

In the prophylactic trial, Group I animals supplemented with anti-oxidant trace minerals at prophylactic doses showed a considerable improvement in the oxidative stress parameters, trace mineral status and milk SCC as well as udder health status as compared to group II indicating increased capability of udder to defend against the attack of pathogenic microorganisms responsible for causing mastitis and hence decreased susceptibility to the occurrence of mastitis in this group. Our findings of improving udder health status through feeding of anti-oxidant formulation are in agreement with Machado *et al.* (2013), who reported that dairy cows supplemented with a combination of different trace elements (Se, Cu, Zn and Mn) showed lower SCC levels (decreased incidence of mastitis) in comparison to the control cows. Sordillo and Mavangira (2014) also advocated controlling mastitis through appropriate antioxidant

Table 5: Impact of prophylactic anti-oxidant trace mineral therapy on blood oxidative stress parameters and milk SCC of healthy actating animals (Mean±S.E).

Parameters	Days of treatment	Group I (With anti-oxidants)	Group II (Without anti-oxidants)
SOD (U/mL)	Day 0	126.33±1.89^{a1}	125.09±1.74^{a1}
	Day 15	135.33±1.90^{a2}	126.65±1.57^{b1}
	Day 30	137.33±1.89^{a2}	127.49±1.57^{b1}
Catalase (nmoles/min/mL)	Day 0	28.65±.77^{a1}	27.98±1.35^{a1}
	Day 15	38.86±.22^{a2}	29.14±1.27^{b1}
	Day 30	40.76±.26^{a3}	29.81±1.30^{b1}
GSH (nmoles/mL)	Day 0	55.86±1.52^{a1}	57.75±1.20^{a1}
	Day 15	59.78±1.19^{a1}	58.81±1.14^{a1}
	Day 30	65.23±1.33^{a2}	59.70±1.15^{b1}
MDA (µmoles/L)	Day 0	34.09±.91^{a1}	36.74±1.31^{a1}
	Day 15	31.56±.78^{a2}	35.52±1.32^{b1}
	Day 30	23.89±.63^{a3}	34.80±1.36^{b1}
NO (µmoles/L)	Day 0	36.95±1.20^{a1}	36.63±1.21^{a1}
	Day 15	34.17±1.07^{a1}	35.66±1.20^{a1}
	Day 30	28.01±.89^{a2}	34.68±1.19^{b1}
SCC ($\times 10^5$/mL)	Day 0	2.81±0.07^{a1}	2.81±0.06^{a1}
	Day 15	2.65±0.06^{a1}	2.77±0.06^{a1,2}
	Day 30	2.48±0.03^{a2}	2.97±0.07^{b2}
	15 days after stopping therapy	2.72±0.09^{a1}	3.07±0.06^{b3}

Values within a row having superscript (a,b) with atleast one common alphabet do not differ significantly at 5% level ($p<0.05$) from each other. Values within a column having superscript (1,2,3) with atleast one common numerical do not differ significantly at 5% level ($p<0.05$) from each other.

Table 6: Impact of prophylactic anti-oxidant trace mineral therapy on trace mineral profile of healthy lactating animals (Mean±S.E).

Parameters	Days of treatment	Group I (With anti-oxidants)	Group II (Without anti-oxidants)
Copper (µmol/L)	Day 0	8.08±.08^{a1}	8.09±.05^{a1}
	Day 15	8.26±.06^{a1,2}	7.99±.05^{b1,2}
	Day 30	8.43±.05^{a2}	7.88±.04^{b2}
Zinc (µmol/L)	Day 0	13.87±.17^{a1}	13.83±.12^{a1}
	Day 15	14.13±.16^{a1,2}	13.67±.13^{b1}
	Day 30	14.56±.14^{a2}	13.54±.14^{b1}
Manganese (µmol/L)	Day 0	3.65±.08^{a1}	3.36±.11^{b1}
	Day 15	3.95±.08^{a2}	3.32±.10^{b1}
	Day 30	4.07±.07^{a2}	3.32±.09^{b1}
Se (ng/mL)	Day 0	38.81±1.56^{a1}	36.87±.89^{a1}
	Day 15	40.78±1.46^{a1,2}	36.75±.54^{b1}
	Day 30	43.58±.99^{a2}	36.47±.46^{b1}

Values within a row having superscript (a,b) with atleast one common alphabet do not differ significantly at 5% level ($p<0.05$) from each other. Values within a column having superscript (1,2,3) with atleast one common numerical do not differ significantly at 5% level ($p<0.05$) from each other.

supplementation in terms of trace mineral supplementation that could potentially boost the animals' health status and performance. Ganda *et al*. (2016) also reported that a single subcutaneous injection containing zinc, manganese, selenium and copper administered to dairy cows with elevated SCC reduces the incidence of chronic clinical mastitis, particularly in primiparous cows, and tends to increase subclinical mastitis cure in cows with 3 or more lactations.

CONCLUSION

Oxidative stress plays a significant role in the pathogenesis of mastitis as evident by increase in biomarkers of oxidative stress like MDA and NO and decreased anti-oxidant enzymes like SOD, Catalase and GSH in mastitic animals. The requirements of trace minerals like Cu, Zn, Mn and Se are increased in mastitis as evidenced by their decreased levels in clinical mastitis and improved clinical response on

their supplementation. Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly reduce oxidative stress and thereby aid recovery in bovine mastitis and play a significant role in prophylaxis of mastitis in lactating animals. Thus, standardization of oxidative status assessment in dairy cattle is vital as a first step remedy for establishing critical thresholds that could aid in defining appropriate protective in dairy cattle on the basis of antioxidant supplementation.

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