

THERAPEUTIC EFFICACY OF ANTI-OXIDANTS IN RECURRENT CASES
OF MASTITIS IN BUFFALOESS. Tiwari¹ and M.P. Gupta²

ABSTRACT

Eighteen buffaloes selected from a private dairy farm of the Ludhiana region were divided into three groups of six animals each. The first group served as healthy control, the second and third as treatment groups. Treatment was given on the 7th day of observation with injRedoxin 20 ml I/M s.i.d for 5 days in Group 2 and with inj Vetade 10 ml/500 kg b.wt. s.i.d. for 4 days in Group 3. Vitamin C and vitamin E levels were assayed on the 0th, 7th, 14th and 21st days of observation. In Group 1, the levels of vitamin C, vitamin E and selenium were 1.0795 ± 0.019 to 1.088 ± 0.025 mg/dl, 3.983 ± 0.064 to 3.946 ± 0.133 $\mu\text{mol/l}$ and 0.611 ± 0.059 to 0.62 ± 0.072 $\mu\text{g/ml}$, respectively. Pre-treatment and post-treatment value values of vitamin C were 0.765 ± 0.128 to 0.924 ± 0.088 mg/dl and 1.0795 ± 0.019 to 1.088 ± 0.025 mg/dl, respectively. in Group 2, showing a significant difference, i.e. a 28% increase in the level after treatment. Pre-treatment and posttreatment values of vitamin E ranged from 2.58 ± 0.24 to 3.006 ± 0.434 $\mu\text{mol/l}$ and 3.983 ± 0.064 to 3.946 ± 0.133 $\mu\text{mol/l}$, respectively, in Group 3, showing a significant difference, i.e. a 41% increase in the level. Pre-treatment and post-treatment values of selenium ranged from $0.19 \pm$

0.062 to 0.245 ± 0.068 $\mu\text{g/ml}$ and 0.690 ± 0.025 to 0.695 ± 0.031 $\mu\text{g/ml}$, respectively, showing a 2.18 % increase in the level. Eight animals out of the 12 were cured suggesting that antioxidant containing drugs can act as an adjunct therapy in the treatment of mastitis.

Keywords: mastitis, redoxin, vetade, vitamin C, vitamin E, selenium

INTRODUCTION

Buffaloes are the preferred dairy animal in rural India, contributing over 50% of the total milk production in the country. Unhygienic and unscientific milking practices and hot-humid environmental conditions predispose them to mastitis. The loss of US\$ 8.80 per buffalo per lactation due to mastitis speaks of its tremendous economic losses due to reduced milk production without considering the cost of disease management (Thirunavukarsu and Prabakaran, 2000). In India, total annual losses due to mastitis (clinical and subclinical) in buffaloes have been estimated at US\$526million (Dua, 2001). Antibiotic therapy is not only costly, but also poses residue problems in milk adversely affecting human health (Hoeben

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et al., 1997). The cure rate with antibiotics is approximately 60% under field conditions. When β lactamase producing organisms are implicated, the cure rate becomes poorer still (Radostits *et al.*, 2000). Treatment failure with available allopathic drugs in fibrosed quarters also poses challenges to veterinarians. Therefore, the search for cost effective complementary and alternative treatment approaches for management of mastitis is being pursued throughout world.

Deficiency of many vitamins and micronutrients particularly vitamin A, vitamin D, vitamin E, selenium, copper in the diet leads to increased incidence of mastitis with infection of longer duration and more severe clinical signs. For lactating dairy animals, nutrient supplementation for trace minerals and vitamins goes beyond correcting for deficiencies; it is aimed rather at minimizing stress and optimizing production efficiency (McDowell, 2002).

Free radicals can be extremely damaging to biological systems (Padh, 1991). Also, phagocytic granulocytes undergo respiratory bursts to produce oxygen radicals to destroy intracellular pathogens. However, these oxidative products can, in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells (Chew, 1995). Therefore, antioxidants are very important to immune defense and health of animals.

Tissue defense mechanisms against free radical damage generally include vitamin A, vitamin C, vitamin E and some trace minerals like selenium. These are also critical in protecting the internal cellular constituents from oxidative damage. Both *in vitro* and *in vivo* studies show that these nutrients generally enhance different aspects of cellular and non-cellular immunity.

So, the antioxidant function could at least in part enhance immunity by maintaining the functional and structural integrity of important immune cells. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to diseases, thereby leading to increase animal morbidity and mortality. Antioxidant supplementation could decrease the duration, incidence and severity of clinical mastitis (Erskine *et al.*, 1989; Smith *et al.*, 1984) and was associated in lower prevalence of intra-mammary infections caused by contagious pathogens (Erskine *et al.*, 1987).

Keeping the above facts in view, this study was planned to evaluate the therapeutic efficacy of anti-oxidants like vitamin C, vitamin A, vitamin E, and vitamin D₃ in recurrent cases of mastitis in buffaloes.

MATERIALS AND METHODS

Eighteen buffaloes selected from a private dairy farm of the Ludhiana region were divided into three groups of six animals each. Group 1 served as a healthy control, Groups 2 and 3 as treated groups. Treatment was given on the 7th day of observation with inj Redoxin* 20 ml I/M s.i.d for 5 days in Group 2 and with inj Vetade** 10 ml/500 kg b.wt. s.i.d. for 4 days in Group 3. Pre-treatment and post-treatment blood sampling was done on the 0th, 7th, 14th and 21st day of observation.

*Inj. Redoxin containing vit. C. Each 5 ml contains 500 mg of ascorbic acid. Ramson remedies, 186, Industrial focal point, Amritsar.A.O. 2, G Floor, Baktawar Malabar Hill, Mumbai-6. Dose - 20 ml i/m s.i.d. for 5 days.

**Vit. ADE₃ injection. Each ml contains vit. A - 250000 IU, Vit.D₃ - 25000 IU and Vit. E -

100 IU. Marketed by Sarabai Chemicals, Animal Health Ltd. Administration Building, Gorwa Road, Vadodhara-390023, India. Dose - 10 ml/500 kg b.wt. s.i.d. for 4 days.

Collection of whole blood

30 ml of blood was collected in heparinized vials (1:10,000) from each animal suffering from clinical/subclinical mastitis. Twenty milliliters of blood was centrifuged at 3000 rpm for 10 minutes to collect plasma for estimation of vitamin C (estimated within 24 h) and vitamin E (estimated within 48 h) and 10 ml of blood was kept in a deep freeze for estimation of selenium.

Estimation of vitamin C

Vitamin C was estimated by the 2,4-dinitrophenyl hydrazine (DNPH) method as described by Baker and Frank (1968). This method is based upon principle that coupling of 2, 4-dinitrophenyl hydrazine to the keto groups of carbon 2 and 3 of diketogluconic acid yields an osazone called bis-2, 4-dinitrophenyl hydrazone. In strong acid, this osazone rearranges to a stable reddish brown product, which is measured photometrically. In this method, 2, 6-dichlorophenolindophenol oxidized ascorbate to dehydroascorbate which in strong acidic medium is hydrolyzed to diketogluconic acid so that hydrozone formation could take place.

Procedure

A sample of 0.2 ml of plasma was pipette into a centrifuge tube and 6.0 ml of 5 percent TCA was added. The contents were mixed thoroughly with a vortex mixer and centrifuged, and 2.0 ml of

filtrate was pipette into each of sample control tube. Next, 2 ml of ascorbate standard was added to each standard and standard control tube. Then, one drop of indophenol reagent was added to all the test tubes and mixed well followed by addition of 0.5 ml of DT mix to sample and standard tubes. All the test tubes were incubated for one hour in a water bath at 60°C and cooled in ice water. DT mix (0.5 ml) was pipetted into each sample control tube and standard control tube. While all the tubes remained in ice bath, 2.5 ml of 85% H₂SO₄ was slowly pipetted into all tubes and mixed well. The absorbance of all tubes was read at 505 nm against distilled water.

Estimation vitamin E

Vitamin E was estimated by the method of Kayden *et al.* (1973) and was based on the principle that vitamin E reduced ferric ions to ferrous ions quantitatively, and these combined with bathophenanthroline to form an orange coloured complex. After adding phosphoric acid to stabilize complex, the colour was read at 536 nm.

Procedure

Volumes of 0.6 ml of plasma, of standard vitamin E and of glass distilled water were pipette into three glass stopped centrifuge tubes marked test, standard and blank, respectively. Then, 0.6 ml of absolute alcohol was added to test and to blank and 0.6 ml of distilled water to standard. The tubes were then stoppered and mixed thoroughly on a vortex mixer. To each tube, 0.6 ml of purified xylene was added and mixed for two minutes on a vortex mixer. Then, the tubes were centrifuged at

$$\text{Vitamin C (mg/dl)} = \frac{\text{A of sample} - \text{A of sample control}}{\text{A of standard} - \text{A of standard control}} \times 2$$

800 g for 5 minutes.

From each centrifuged tube, 0.4 ml of xylene extract was carefully pipetted into fresh appropriate level glass stoppered test tubes containing 0.2 ml of bathophenanthroline reagent and mixed. Then, 0.2 ml ferric chloride reagent was added followed by 0.2 ml of o-phosphoric acid. The contents were mixed thoroughly on a vortex mixer after every addition of reagent. The absorbance of test (A_T) and standard (A_S) was read at 536 nm against blank.

Plasma vitamin E was estimated according to following equation:

$$\text{Vitamin E } (\mu\text{mol/L}) = \frac{A_T}{A_S} \times \text{concentration of standard } (\mu\text{mol/L})$$

solution was diluted with distilled water make the volume to 1 liter. The resulting solution contained 1000 ppm selenium. In case of selenourea, on the basis of molecular weight, 1.558 g was added to 1 liter distilled water so as to obtain 1000 ppm selenium from selenourea. Both the selenium sources were then serially diluted to get various dilutions of selenium: 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 40.0 and 100 $\mu\text{g se/ml}$. These dilutions were used to get a standard graph.

One ml of each of the above dilutions were placed in a 250 ml conical flask and 5 ml of digestion mixture was added. Condensers (coolers) filled with cold water were kept in the mouth of conical flasks so as to avoid evaporation losses. The flasks were heated at around 160-180°C on a hot plate until dense white fumes started to appear. This took around 5-7 minutes. The flasks were then removed from the hot plate and cooled for 20-25 minutes at room temperature. The contents of the flask were rinsed with 20 ml distilled water, and the bottoms of the condensers were also rinsed. The

Estimation of selenium

The selenium levels in whole blood, hair and tissues were analyzed by the spectrophotometric method described by Cummins *et al.* (1965) with slight modifications.

Procedure

The standard curve for selenium was prepared by using both sodium selenite and selenourea as the source of selenium. To 80 ml of 40 percent hydrobromic acid around 60 ml distilled water was added to which 2.190 g sodium selenite was mixed. A yellow solution was formed and this

contents of the flask were thus dissolved and then placed into a separate 50 ml conical flask. To each flask, 2-3 drops of metacresol purple indicator was added and the solution turned pink. This solution was then brought to pH 7 by adding saturated sodium hydroxide at which the colour became purple. Around 5 ml of saturated NaOH was required in each flask. Now the sample solution was brought to acid pH (2-3) with the help of 90 percent formic acid. To bring the pH to 2.5-2.6 about 1 ml of formic acid was required, resulting in a yellow colored solution. To reduce the oxidative reagents, 4 ml of 40 percent hydroxylamine was added to each sample followed by the addition of 4 ml 0.2ml EDTA. The solution started turning greenish after the addition of hydroxylamine EDTA, which was added to mark any interfering ions. Again, the pH was checked to ascertain if it was between 2-3. If it was lower than 2, it was raised with few drops of liquor ammonia, and if higher, formic acid was used to lower it. For complexation of selenium, 2 ml of 0.5 percent diaminobenzidine solution was

added to each flask as the complex formation takes place only in a pH of 2-3.

The flasks were then incubated in a water bath at 60°C for exactly 20 minutes, after which they were removed and again the pH was adjusted. The pH was brought to 7.0 to 7.5 with the help of liquor ammonia. Around 3-4 ml liquor ammonia was required each time. The solution was then placed in a 125 ml separating funnel, and again the flask was rinsed with few ml of distilled water. Four milliliters of toluene was added for selenium extraction. The separatory funnel was vigorously shaken for one minute and then allowed to stand for 2-3 minutes to allow separation of the toluene layer. The lower liquid was discarded, and the toluene was collected in a centrifuge tube. The tubes were centrifuged for few minutes to remove any traces of water. The yellowish coloured toluene layer thus obtained was read for absorbance at 420 nm against clear toluene blank. The absorbance values of different concentrations were obtained and after several replications a standard graph of concentration of selenium vs. absorbance (00) was plotted for both sources of selenium: sodium selenite and selenourea.

Every time a reagent blank was run, as the water in the solutions may also have had selenium, the reading of absorbance of the reagent blank was subtracted from the sample absorbance values to get the actual reading of samples.

Statistical analysis

Statistical analysis was done by using completely randomized design (CRD) and randomized block design (RBD) as per the method of Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

Observations of the animals of Group 1

History and symptoms

The animals in this group had never suffered from mastitis. They were of age group 3-5 years. The mammary glands of these animals revealed normal glandular parenchyma. The animals were mostly recently calved. Colour, consistency, opacity and thickness of milk were normal. There was an absence of any flakes or blood in milk.

Vitamin C

Mean vitamin C level in plasma ranged from 1.0795 ± 0.019 to 1.088 ± 0.025 mg/dl (Table 1). There was no significant variation in vitamin C level during the observation period (Figure 1). Chaiyotwittayakun *et al.* (2002) who states that mean ascorbic acid concentration rapidly increased after the second infusion of vitamin C injection i.e. 650 $\mu\text{mol/l}$ (11.44 mg/dl) and rapidly dropped afterwards to below 100 $\mu\text{mol/l}$ (1.76 mg/dl).

Vitamin E

The mean value of vitamin E in plasma ranged from 3.983 ± 0.064 to 3.946 ± 0.133 $\mu\text{mol/l}$ (Table 1). There was no significant variation in vitamin E level during the whole observation period (Figure 2). Ndiweni *et al.* (1991) found that mean plasma vitamin E level above 4 $\mu\text{g/ml}$ i.e. 8.46 $\mu\text{mol/l}$ is regarded as adequate for cattle.

Plasma concentration of greater than 3.5-4 $\mu\text{g/ml}$ of α -tocopherol are considered adequate as evidenced by the relationship between intracellular killing of bacteria by neutrophils and plasma vitamin E concentration (Hogan *et al.*, 1993a).

Vitamin E injected would mitigate the normal peripartum drop in serum α -tocopherol, reduce the impairment of neutrophil function

and thereby decrease incidence of early lactation clinical mastitis (LeBlanc *et al.*, 2002).

Selenium

The mean value of selenium ranged from 0.611 ± 0.059 to 0.62 ± 0.072 $\mu\text{g/ml}$ (table 1). There was no significant variation in selenium levels during whole observation period (Figure 3). Milk neutrophils from cows fed selenium-deficient diets may also be compromised in their capacity to evoke effective responses to microorganisms known to be mastitis pathogens. Neutrophils obtained from cows fed the Se-deficient diet had approximately one-third the microbicidal activity of those from cows fed the Se-supplemented diet. The extra cellular H_2O_2 concentration was significantly higher in neutrophils harvested from milk of cows fed the Se-deficient diet. This is due to result of reduced catabolism of H_2O_2 rather than to greater production. Although the lipid structure of the phagolysosome protects the neutrophil from low amounts of H_2O_2 , increased amounts of lipid peroxide is a potential consequence of Se deficiency and can result in damage to cellular and sub-cellular membranes. Catalase and vitamin E are protective agents that may serve to sequester H_2O_2 and lipid radicals produced by neutrophils. The bovine neutrophil may be particularly susceptible to increased concentration of H_2O_2 because of the low amounts of cellular catalase. The higher concentration of H_2O_2 production in Se-deficient neutrophils has been associated with a decrease in microtubule assembly, a decrease in degranulation and damage to the myeloperoxidase systems. Loss of any of these functions would result in less efficient neutrophil bactericidal activity. The peroxides generated also lead to damage to epithelial tissue in which neutrophils have accumulated. In

the mammary gland, this damage could lead to irreversible loss of secretory tissue and reduction of milk yield from that gland.

Selenium deficiency in the neutrophil may involve the association of Se with the lipoxygenase pathway of eicosanoid metabolism. High concentrations of hydroperoxides are inhibitory to cyclo-oxygenase and 5-lipoxygenase and Se may protect the cyclooxygenase enzyme. A decrease in leukotriene B_4 , a product of lipoxygenase pathway occur in neutrophils obtained from Se-deficient cows. Leukotriene B_4 enhances chemotaxis, recognition and degranulation of neutrophils. A decrease in leukotriene B_4 as a result of Se deficiency may result in a partial loss of these functions. A decrease in degranulation may result in a decreased killing capacity of neutrophils.

Observation of animals of Group 2

History and symptoms

The animals were in their 3rd to 5th lactation and recently calved. More hind quarters were infected than fore quarters (60% vs. 40%). This might be due to more exposure of hind quarters to floor urine and dung and higher milk content in hind quarters than in fore quarters. This finding is similar to finding of Rao and Naidu (1969) and Kapur and Singh (1978). Incidence was highest during first three months of lactation i.e. 60%. Similar observations have been recorded by Kapur and Singh (1978). The presence of pain response (purposeful lifting and kicking of the adjacent hind limb) during palpation of the mammary gland was noted (Maunsell *et al.*, 1998). There was a presence of flakes in the milk of most of the animals. Out of six animals, blood was present in the milk of two animals. The consistency of milk was watery in three animals out of the six.

Vitamin C

Pre-treatment and post-treatment value of vitamin C ranged from 0.765 ± 0.128 to 0.924 ± 0.088 mg/dl and 1.0795 ± 0.019 to 1.088 ± 0.025 mg/dl, respectively (Table 2), i.e. a 28% increase in level after treatment. There was a significant difference (5%) between pre-treatment and post treatment levels of vitamin C in the animals of this group (Figure 2). When compared with vit C level of animals of Group 1, there was a significant difference (5%). Four out of the six animals were cured symptomatically, but culturally they were positive for mastitis. Two animals did not recover and antibiotics based on culture sensitivity testing were prescribed for them. In one animal, mammary gland became fibrosed. On the basis of the case recovery rate of 80%, it can be concluded that injredoxin can be used as an adjunct therapy in treatment of recurrent cases of mastitis.

Ascorbate functions as antioxidants by neutralizing the peroxidase and other free radicals responsible for tissue injury thus protecting cells of body. Beside its anti-oxidant properties, ascorbate also has a pre-oxidant role to activate myeloperoxidase inside the neutrophils and thus enhancing the immune response.

Further ascorbate, a cofactor in the hydroxylation reaction of proline and lysine, is also involved in the maintenance of natural barriers. Thus a depression in udder defense due to high production and lower ascorbic acid concentration may initiate multiplication of mastitogens (Singh and Pachauri, 2003).

Observations of the animals of Group 3

History and symptoms

The animals in this group were in their first to third lactation, four to sixth month of calving and recently calved. All animals had previously been

treated with lemox (ampicillin and cloxacillin) but there was reoccurrence of mastitis. The affected quarters were swollen, hot, oedematous and painful to touch in starting. Then, after treatment, four animals out of six were cured and returned to normal condition. The inflammation subsided and the animal became clinically normal. One animal out of the six was not cured. In one animal, the mammary gland became fibrosed.

Vitamin E

Pre-treatment and post-treatment value values of vitamin E ranged from 2.58 ± 0.24 to 3.006 ± 0.434 $\mu\text{mol/l}$ and 3.983 ± 0.064 to 3.946 ± 0.133 $\mu\text{mol/l}$, respectively, showing a significant difference i.e. 41% increase in level (Table 3). While comparing it with vitamin E level of animals of Group 1, it showed significant difference (5%) (Figure 2). Four out of six animals were cured symptomatically, but culturally they were positive for mastitis. Two animals did not recover and antibiotics based on culture sensitivity testing were prescribed for them. The case recovery rate of 80% suggests that inj vetade can be used as an adjunct therapy in the treatment of mastitis.

Plasma concentrations of α -tocopherol are low during peripartum period (Goff and Stabel 1990; Weiss *et al.*, 1990a; Weiss *et al.*, 1994; Hogan *et al.*, 1997).

The periparturient period is associated with decreased activity of PMN and with increased susceptibility to mastitis. An increase in vitamin E levels after parturition could improve the animal immune responses and thus leads to lower incidence of mastitis (Ndiweniet *al.*, 1991).

Vitamin E is an integral component of all lipid membranes and serves to protect lipid membranes from attack by reactive oxygen species. Polyunsaturated fatty acids of membranes are

Table 1. Mean values of vitamin C and vitamin E of animals of Group 1.

Days of observation	Vitamin C (mg/dl)	Vitamin E (μ ml/L)	Selenium (μ g/ml)
0 day	1.0795 \pm 0.019	3.983 \pm 0.064	0.611 \pm 0.059
7 day	1.088 \pm 0.025	3.946 \pm 0.133	0.62 \pm 0.072
14 day	1.080 \pm 0.014	3.941 \pm 0.216	0.60 \pm 0.05
21 day	1.081 \pm 0.0134	4.958 \pm 0.194	0.61 \pm 0.05

Table 2. Mean values of vitamin C of Group 2.

Days of observation	Vitamin C (mg/dl)
0 day	0.924 \pm 0.088 ^x
7/0 day*	0.765 \pm 0.128 ^x
14/7 day	1.088 \pm 0.025 ^x
21/14 day	1.0795 \pm 0.019 ^x

* indicate day of treatment with injRedoxin.

^xindicates significant difference at 5% confidence interval.

Table 3. Mean values of vitamin E and Selenium of Group 3.

Days of observation	Vitamin E (μ mol/L)	Selenium (μ g/ml)
0 day	3.445 \pm 0.1899 ^x	0.245 \pm 0.068 ^x
7/0 day*	3.303 \pm 0.1576 ^x	0.19 \pm 0.062 ^x
14/7 day	6.73 \pm 0.1474 ^x	0.695 \pm 0.031 ^x
21/14 day	4.265 \pm 0.1157 ^x	0.690 \pm 0.025 ^x

* indicate day of treatment with injVetade.

^x indicates significant difference at 5% confidence interval.

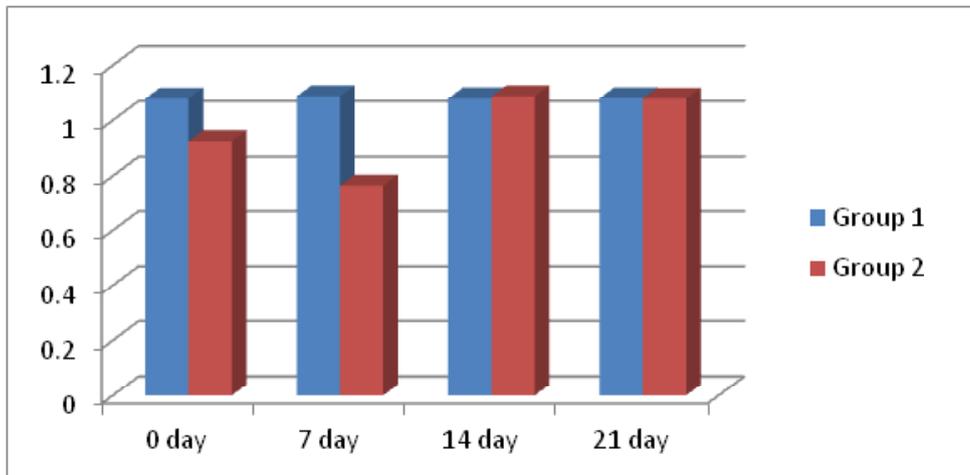


Figure 1. Comparison of vitamin C levels of Groups 1 and 2.

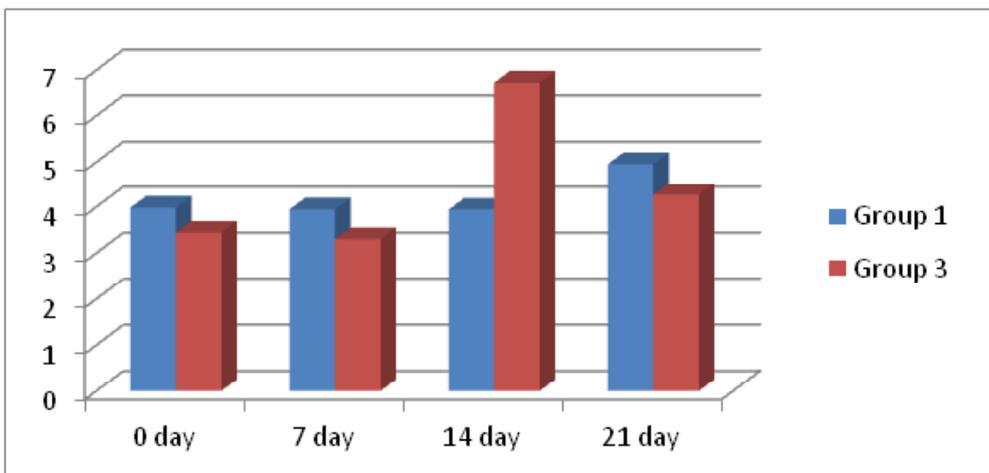


Figure 2. Comparison of vitamin E levels of Groups 1 and 3.

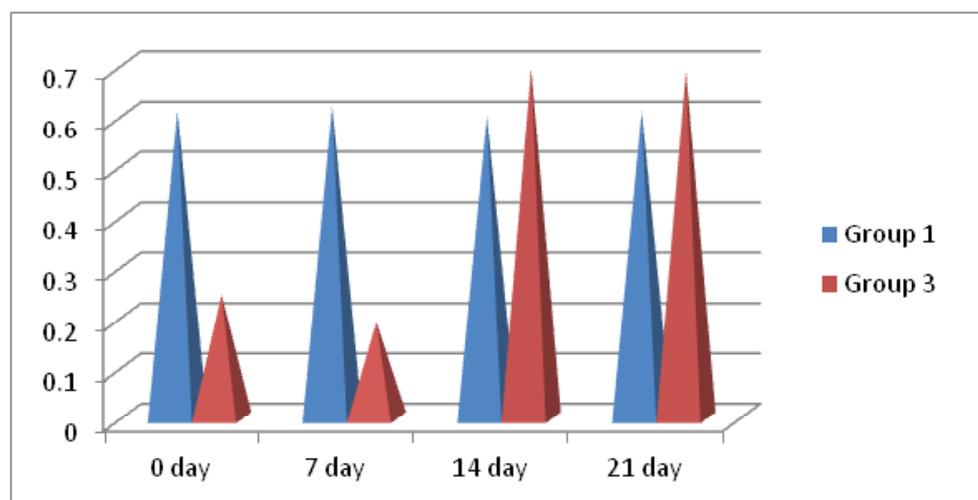


Figure 3. Comparison of selenium levels of Groups 1 and 3.

particularly vulnerable to attack by reactive oxygen species and ROS can initiate a chain reaction of lipid destruction that destroys the membrane of the cell. Vitamin E can quench peroxidation reactions in membranes.

The arachidonic acid metabolism is altered in animals deficient in vitamin E, selenium or both (Atroshiet *al.*, 1989; Aziz and Klesius, 1986). Vitamin E may function to control peroxidation of arachidonic acid or its unstable metabolites. The arachidonic acid metabolites are important for PMN function and the amplification of inflammatory response following pathogen invasion of tissues including the mammary gland (Aziz *et al.*, 1984). The speed with which PMN can be mobilized following pathogen invasion and the efficiency of intracellular kill are events of critical importance to protection of the mammary gland from infection (Smith *et al.*, 1997). Vitamin E and Se play essential roles in these events and dietary deficiencies of either leads to impaired PMN function and increased incidence of intramammary infection in dairy cows (Hogan *et al.*, 1993a).

Selenium

Pre-treatment and post-treatment value values of selenium ranged from 0.19 ± 0.062 to 0.245 ± 0.068 $\mu\text{g/ml}$ and 0.690 ± 0.025 to 0.695 ± 0.031 $\mu\text{g/ml}$, respectively, showing a 2.18% increase in level (Table 3). There was significant difference (5%) between pre- and post-treatment levels of selenium.

Mukherjee (2008) also found that treatment of mastitis in buffaloes with vitamin E and selenium deficiencies. Mammary gland infections and clinical mastitis were not affected by treatment, but milk production was increased by treatment; milk production was also increased by feeding high amounts of vitamin A (Weiss, 1998).

Milk neutrophils from Se-deficient cows had impaired bactericidal activity compared to Se-supplemented cows. This indicates an important role of Se in mammary resistance to infection (Grasso *et al.*, 1990).

A dose of 0.1 mg Se/kg of body weight at 21 days before calving had no effect on incidence of clinical mastitis. However cows supplemented with both vitamin E and Se had shorter duration of clinical signs than cows supplemented with either micronutrient alone (Smith *et al.*, 1997).

Vitamin D also is involved with immune function (Reinhardt and Hustmyer 1987). Increased lymphocyte proliferation was observed when Jersey cows were infused subcutaneously with 50 μg of 1, 25 -dihydroxy vitamin D/day for 7 days (Hustmyer *et al.*, 1994).

CONCLUSION

We can forecast the incidence of mastitis in a particular herd by estimating vitamin E and vitamin C levels in plasma of animals. Further, antioxidant treatment may have cured the epithelium of teat canal and mammary gland of mastitis affected animals. Integrity of epithelium has been maintained as no cases of mastitis were recorded after treatment.

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