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Review Article

Role of Antioxidants in Udder Health: A Review

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Udder is a productive organ of the dairy animals; hence for better production it should be healthy. Because of their anatomical position are subject to outside influences and are prone to both inflammation or non inflammatory conditions (Tripathi, 2000). Mastitis is an inflammation of caused by microorganisms, usually bacteria, that invade the udder, multiply and produce toxins that are harmful to the mammary gland. Mastitis is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al*., 2000). It is complex, there is no simple solution to control it. Mastitis continues as a problem in many dairy herds despite proper application of proven control methods of teat dipping and total dry cow therapy. Traditionally, the mastitis control programmes are focused at use of chemical disinfectants, antiseptic teat dips and antibiotic therapy. Any control procedure employed that tries to improve the dairy animal ability to fight off environmental pathogens is reactionary and implies that the cow needs to respond to an infection. It is much more logical to place the majority of our control efforts by enhancing the immune status of animal. This goal could be achieved by the use of antioxidants such as Vitamin E, Vitamin C, Selenium, Copper and Zinc etc. But we will discuss especially on Vitamin-E and Selenium. The mastitic cows have lower Vitamin E and Selenium concentration (Nauriyal and Pachauri, 1997).

The micronutrients being important for proper immune cell function and protection, deficiencies of these micronutrients can have serious consequences on mammary gland health and thus supplementation could provide great benefit to the control of bovine mastitis (Singh *et al*., 2003). α-tocopherol was discovered and isolated by Evans *et al*. (1936). Compounds having vitamin E activity are known chemically as tocopherols but biologically most active form is α-tocopherol. Fresh, green forage is an excellent source of Vitamin E. The first clinical study on the effects of Vitamin E and Selenium on mastitis was conducted by Smith *et al*. (1984). Vitamin E is an essential antioxidative component of cell membranes that prevents per oxidative damage to the cell membranes of sub cellular organelles by free radicals as natural biological antioxidant.

Selenium has biological function related to Vitamin E in that selenium is an essential component of glutathione peroxidase (GSHPx), an enzyme involved in detoxification of hydrogen peroxide and lipid hydroperoxides (Rotruck *et al*., 1973). First of all Selenium was identified as toxic factor. It was discovered as an essential nutrient in 1957, though it was not discovered what role it played in the body until 1973. Fish meal (1.4-2.4 mg/kg) and sunflower meal (2.3 mg/kg) are good source of Selenium. It is well documented that vitamin E and Selenium play important roles in maintaining herd health of dairy cattle (Muth *et al*., 1958; Smith *et al*., 1972, 1984). The antioxidative role become very important during the immune response when neutrophils produce large quantities of super oxides and hydrogen peroxide from molecular oxygen to destroy ingested foreign organisms (Ross, 1977). The lymphocyte seems to be especially more susceptible to oxidative damage because their membranes have a relatively high frees fatty acid content.

Synergism of Vitamin E and Selenium

Vitamin E and Selenium are synergistic. In recent years, Vitamin E and Selenium have been frequently discussed together because of their close relationship. Both Vitamin E and Selenium protect cells from the detrimental effect of oxidation but they do so in different ways. Vitamin E fat soluble antioxidant which is found in cell membrane prevents the cell formation of harmful free radicals. Because of this localization within cell membrane, Vitamin E can not protect the cymosely from free radicals. Selenium is found in cytosol and its functions throughout the cell to destroy peroxides and another harmful compound. This localization also explains the relationship between Selenium and Vitamin E. Vitamin E and Selenium appear to enhance host defenses against infections by improving phagocytic cell function. Selenium spares Vitamin E or reduces the requirements for Vitamin E by preserves the integrity of pancreas which allows normal fat digestion and thus normal Vitamin E absorption, reduces the amount of Vitamin E required to maintain integrity of membranes via GSHPx and aids in some unknown ways in the retention of Vitamin E in blood plasma. Likewise, Vitamin E appears to reduce the requirements for Selenium by maintains body selenium in active form and prevent its loss from the body and, prevents the destruction of membrane lipids within the membrane thereby inhibiting the production of hydroperoxides and reducing the amount of selenium dependent enzyme (GSHPx) needed to destroy peroxides formed in the cells.

Why alternative approach…….?

Mastitis is a complex disease; there is no simple solution to control it. Well over 100 different microorganisms can cause mastitis. Even also no single vaccine is successful to control mastitis due to its multietiolgical nature of disease. However, antibiotics were introduced in mastitis therapy from 50 year back for the control of mastitis. But the problem in dairy animals remain as same as it was prior to antibiotic era. The antibiotic treatment may help in minimizing the losses but may lead drug resistance. The efficacy of antibiotics in treatment of clinical coliform mastitis is questionable, and antibiotic treatment for *S. aureus* induced mastitis is not economically sound because of low bacterial cure rate (Kirk *et al.,* 1994). Further factors such as pharmacokinetic problems, and phagocytosis depressing effect of certain antibiotics (Dulin *et al.,* 1988 and Hoeben *et al.,* 1997, 2000) and residue in milk limit the success of antibiotic therapy in mastitis. Therefore, attention is being paid to find alternative approaches to control mastitis. These approaches are directed to enhance udder defense and antibacterial system in milk by using immunoregulatory micronutrients (Vitamin E and Selenium), vaccines and cytokines.

How it affects dairy industry…….?

Mastitis is a very costliest disease to dairy farmers. It is a most important deadly disease of dairy animals and causes heavy economic losses to dairy industry due to reduced milk yield (up to 70 %), milk discard after treatment (9 %), cost of veterinary services (7%) and premature culling (14 %) (Bhikane and Kawitkar, 2000). The first report on mastitis caused losses in India was about Rs.52.9 crore annually (Dandha and Sethi, 1962). These losses increased tremendously i.e. it was about Rs.6053.21 crore annually in the year 2001. Out of this, loss of Rs. 4365.32 crore (70 % - 80 % loss) has been attributed to sub-clinical version of udder infections (Dua, 2001). Apart from its economic importance it also carries public health significance (Vasavda, 1988). Moreover, presence of antibiotic residues in the milk is undesirable due to its public health concern. Today it stands second to FMD as a most challenging disease in high yielding dairy animals in India (Varshney and Mukherjee, 2002).

Free radicals

Free radicals are chemicals possessing an unpaired electron, which are usually very reactive. Antioxidants are molecules they can easily and harmlessly give up an electron. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally free radicals attack the nearest stable molecule “stealing” its electron (Singh *et al*., 2004). They can attack enzymes, fat and proteins disrupting normal cell activities or cell membranes, producing a chain reaction of destruction. The major free radicals found in

biological systems are superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and fatty acid radical. The other biologically important free radicals are trichloromethyl (CCl_3) formed during metabolism of carbon tetrachloride in the liver and contributes to carbon tetrachloride toxicity; thiyl (RS), peroxyl (RO_2) and alkoxyl (RO). Hydrogen peroxide found primarily in the cytosol of the cells and fatty acid radicals are found in cell membranes. Superoxide and hydroxyl radicals can be found in both cell components. During normal metabolism, electrons are transferred in the process of oxidative phosphorylation in a chain of enzymes in the mitochondria to yield ATP. But all electrons do not reach to cytochrome-c oxidase and about 2-5% is leaked during electron transfer chain. These electrons reduce oxygen and generate O_2^- an initial ROS. The free radicals that are formed in excess and could not be scavenged by the antioxidative defenses induce a series of biochemical reactions starting with participation of O_2^- in Fenton type reactions. This reaction is characterized by reduction of Fe^{3+} and production of extremely reactive hydroxyl radicals that attack macromolecules and initiate peroxidative reaction (Swarup, 2001).

Effect of free radicals

Free radicals cause severe oxidative damage. Tissue injury due to free radicals contributes to the development of disease conditions, mastitis being one of them. Damages at cellular level are produced by free radicals in various organs in which they are produced. They causes damages due to formation of lipid peroxides, breakdown of cellular substances, accumulation of inert substances such as lipofuschin and/or alteration of membrane characteristics like fibrosis of vessel walls and destruction of microbes. The damage occurs from free radicals are due to the fact that whenever an atom is split, energy is released. A severe oxidative stress, however, induces cascade of biochemical reactions leading to injury at cellular site including DNA strand breakage, increase in intracellular free calcium, and damage to membrane proteins and ionic transport, and peroxidation of lipids. Hydrogen peroxide radicals can degrade heme proteins such as hemoglobin and myoglobin (Halliwell, 1994).

Way of action of antioxidants

An antioxidant is a substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Halliwel, *et al.,* 1992). Chemically antioxidants work by one of the following mechanisms:

- They donate electrons.
- They donate hydrogen.
- They scavenge oxygen.
- They are scavenging free radicals.

How body protects against free radicals .....?

Even under healthy conditions, potentially toxic free radicals are produced in the body. But antioxidant defenses in the body effectively neutralize them. Normally the body is protected against oxidative stress and free radical injury by a host of defense system. But when free radicals/ ROS are produced faster than scavenging capability of antioxidant defenses in the body, the condition of oxidative stress arises. So, why are antioxidants important?

Because free radicals are extremely toxic to cells, the body has developed a sophisticated antioxidant system (Table 1). Superoxide dimutase (an enzyme that contain Cu and Zn) converts superoxide to hydrogen peroxide. Hydrogen peroxide is converted to water by the enzyme GSHPx (contain selenium). These two enzymes effectively control most free radicals within the cytosol. Superoxide and hydroxyl radicals can migrate into cell membrane where they attack fatty acids (especially polyunsaturated fatty acids) and produce fatty acid radicals (a process called initiation). Fatty acid radicals then react with other fatty acids producing a chain reaction. Vitamin E reacts with fatty acid radicals and stops the chain reaction. It explains importance of Vitamin E as antioxidant.

There are also some macromolecules such as transferring, ceruloplasmin and albumin involved in the body defense mechanism. Vitamin E and selenium are important for optimal immune functions associated with T and B lymphocytes (Bendich, 1990).

Table 1. Antioxidant system of mammalian cell

Component and location in cell	Nutrients involved	Function
Superoxide dimutase (Cytosol)	Cu, Zn & Mn	An enzyme that converts superoxide to hydrogen peroxide.
Glutathione peroxidase (Cytosol)	Se	An enzyme that converts hydrogen peroxide to water.
Catalase (Cytosol)	Fe	An enzyme (primarily found in liver) that converts hydrogen peroxide to water.
α-tocopherol (Membrane)	Vitamin E	Breaks fatty acid peroxidation chain reactions.
β-carotene (Membrane)	Carotene	Prevents initiation of fatty acid peroxidation chain reaction.

How Vitamin E and Selenium helps in Mastitis Control…….?

Effective management of mastitis is a major challenge to dairy farmers and modern veterinarians. One of the most important approaches to control mastitis and to enhance therapeutic efficacy of antimicrobial treatment is to enhance udder defense against pathogens. Antioxidants such as Vitamin E and Selenium have been tried with encouraging results in this direction. Vitamin E, is the most important free radical scavenger within cell membrane (Rice and Kennedy, 1986). Polyunsaturated fatty acids (PUFA) of membranes are particularly vulnerable to attack by free radicals, and the free radicals can initiate a chain reaction of lipid destruction that destroys the cell membrane. An important PUFA in cellular membranes is arachidonic acid (AA). AA can be metabolized to leukotriens, prostaglandins etc. by the cyclooxygenase enzyme complex (Kuehi and Egan, 1980). Leukotriens act as chemotactic agent for PMN, eosinophils and monocytes. The main function of vitamin E in udder defense is linked to maximize polymorphonuclear (PMN) cells activity as it protects polyunsaturated fatty acids in the PMN membrane from destruction by free oxygen radicals that are produced during phagocytosis (Boxer, 1986). The prostaglandins are known to influence the function of smooth muscel tissue (Monicada and Vane, 1979). Smooth muscle, which surrounds the streak canal of the bovine teat, helps to keep the canal tightly closed. So, altered, prostaglandin synthesis results failure of complete tight closure and consequently the pathogens may enter into the gland more easily (Sharma *et al.,* 2003). This explains as to how Vitamin E deficient dairy cows become prone to mastitis (Smith et al., 1988). Vitamin E inhibits peroxidation of AA by scavenging peroxyl radicals thus slowing the chain reaction (Fig 1). This free Radical scavenging Vitamin is an important component of udder defense and has been proved effective in augmenting therapeutic efficacy of antimicrobial treatment in mastitis. Primary source of α-tocopherol (Vitamin E) in the normal milk is the fat globule membrane. But during inflammation, neutrophils are rich source of vitamin E and contain about 17.6mg α-tocopherol/million cells. It is reported that neutrophil can account for 10% of milk a-tocopherol in non-infected and nearly 25% in milk from mastitic udder. Therefore, the increase in somatic cell count (SCC) is associated with increase in α-tocopherol content in milk (Barret *et al.,* 1997).

Selenium is an essential component of an enzyme glutathione peroxidase (GSHPx). GSHPx is a major intracellular antioxidant that catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to nontoxic compounds. If an animal is selenium deficient, GSHPx will not be present in adequate amounts, which allows free

Fig.1. Illustration of role of Vitamin E in the control of mastitis by alteration of arachdonic acid metabolism.

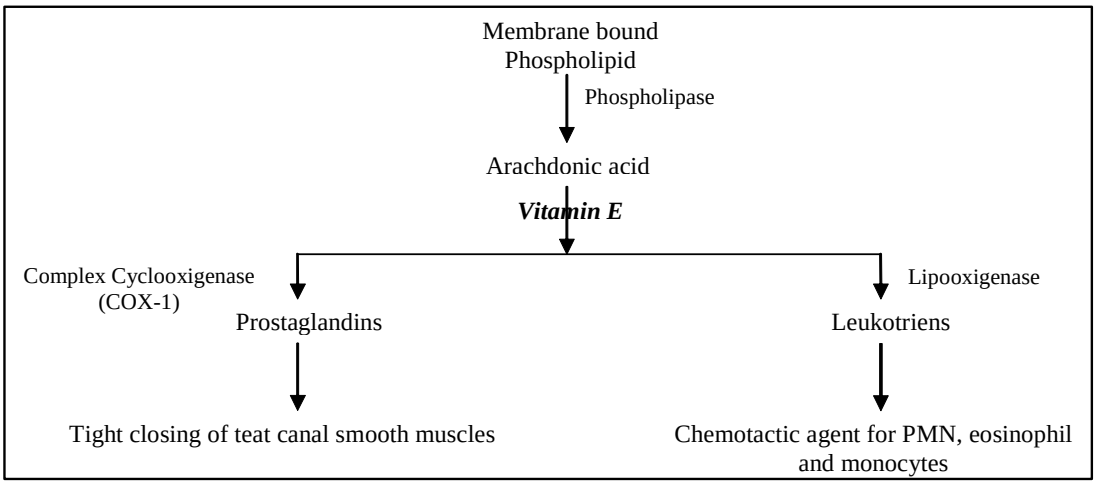

Fig.2. Illustration of action of selenium in the control of free radical production and killing of bacteria.

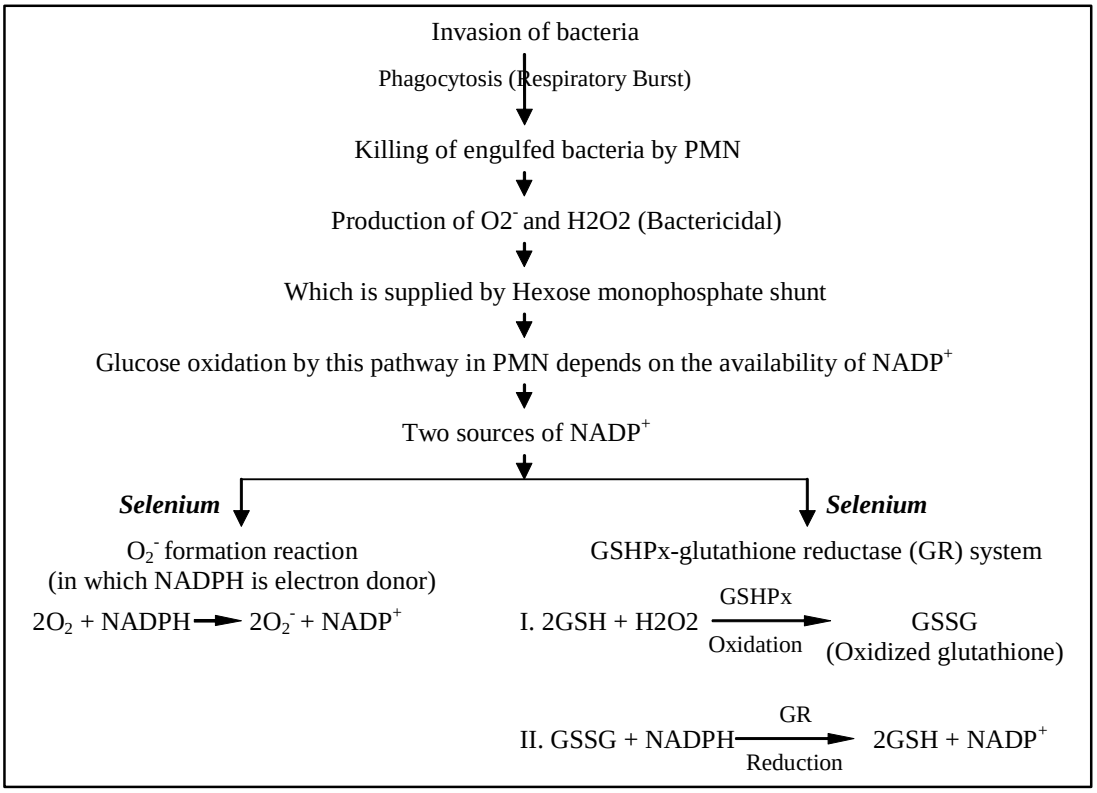

Fig.3. Illustration of interaction among selenium, vitamin E and sulphur amino acids.

radicals to interact with cell membranes. When selenium is deficient, additional Vitamin E is needed to protect cell membranes. If a cow is deficient in Vitamin E, more free radicals are produced in the cell membranes, some of which interact with compounds in the cytosol, which increase the need for selenium.

In mastitis neutrophils are the predominant cell type found in mammary tissues and in mammary secretions during early inflammation and can constitute >90% of total mammary gland leukocytes (Sordillo *et al*., 1987). When a pathogen invades the mammary gland of a cow, a cascade of events occurs. First, neutrophils move from the blood to mammary gland in response to variety of inflammatory mediators (Person *et al*., 1993). When the mastitogens enter the mammary gland, they are engulfed and killed by these neutrophils, through the process of *Respiratory Burst* (Singh *et al*., 2003) (Fig.2). This process produces a high concentration of free radicals help to kill the bacteria i.e. without sufficient free radicals, neutrophils can not function properly. However, the level of free radicals must be controlled, otherwise they will damage or kill the neutrophils before the neutrophils can kill the bacteria. Neutrophils contain large concentrations of a-tocopherol to protect it from free radicals. This is where Vitamin E important. It appears, Vitamin E in cellular and sub cellular membranes is the first line of defence against peroxidation of phospholipids, but even with adequate Vitamin E, some peroxides are formed.

Selenium as part of GSHPx is a second line of defence that destroys these peroxides before they have an opportunity to cause damage to membranes. Therefore, Selenium, Vitamin E and sulphur containing amino acids, through different biochemical mechanisms (Fig.3) such as prevention of hydroperoxide formation by Vitamin E, role of sulphur containing amino acids as precursor of glutathione peroxidase (GSHPx) and selenium as an integral component of GSHPx.

Antioxidants can however scavenge free radicals and minimize the formation of these free radicals/ROS, but not 100% effective. When ROS are not effectively and safely removed, oxidative stress may impair health in dairy animals both directly (peroxidative damage to important lipids and

macromolecules) and indirectly (changes induced in cellular membranes and components).

Supplementation of Vitamin E and Selenium

The antioxidants viz. Vitamin E and Selenium have extensively used in bovine mastitis therapy (Nauriyal, 1996). More recently, a role for Vitamin E and Selenium in mammary gland health has been defined (Hogan *et al*., 1993; Weiss *et al*., 1990, 1997; Erskine *et al*., 1987, 1990, 1989; Smith *et al*., 1984, 1997). Deficiencies of either Vitamin E or Selenium have been associated with increased incidence and severity of intramammary infection (IMI), increased clinical mastitis cases and higher somatic cell count in milk.

Plasma Vitamin E concentrations in dairy cows are normally lowest when rates of intramammary infection are highest and when neutrophil functions are depressed during the periparturient period (Smith *et al*., 1985b, c; Kehrli *et al*., 1989; Weiss *et al*., 1990). Low plasma concentration of α-tocopherol at parturition is considered as a significant risk factor for intrammamary infection and mastitis during first week of lactation (Samanta *et al*., 2005). The plasma a-tocopherol decreases during the periparturient period is may be due to a combination of reduced dry matter intake in dry cows, decrease transport capacity for the vitamin in plasma and loss of Vitamin E into milk of lactating cows. Cows are immunosuppresed during the time when plasma concentrations of Vitamin E are low (especially from 7days prepartum to 1 or 2 wk postpartum). Because of the beneficial effects of Vitamin E on neutrophil function (Table 2). About 30 to 50% of all clinical mastitis occurs during the first month of lactation. Dietary supplementation of cows with Vitamin E and selenium results in a more rapid PMN influx into milk following intramammary bacterial challenge and increased intracellular kill of ingested bacteria by PMN. The killing ability of blood neutrophils has been correlated with the concentration of a-tocopherol in neutrophils (Weiss *et al*., 1994).

Current NRC (1989) recommendation for total not supplemental, Vitamin E for dry and lactating cows is 15 IU/kg of DM. based on typical DMI, that amount is equivalent to about 300 and 150 IU/day for dry and lactating cows, respectively.

The first clinical study on the effect of Vitamin E and Selenium on mastitis was conducted by Smith *et al.* (1984). In that study, dry cows were fed high forage diets based on hay and silage that provided 0 or 740 IU/day of supplemental Vitamin E and were fed no supplemental Selenium or injected with 0.1 mg of selenium/kg of body weight 21 days

Table.2. Effect of Vitamin E supplementation on neutrophil function in dairy cows.

Type of cows	Supplementation	Response	Reference
Lactating cows, 30 days in lactation	1000 IU/day of dietary vitamin E during the dry period and 500 IU/day during the first 30 days of lactation	• Phagocytosis not affected • Ability to kill *S. aureus* and *E. coli* was improved	Hogan *et al*., 1990
Fresh Cows (<3 days in lactation)	3000 IU of vitamin E injected at 10 and 5 days before anticipated calving	• Phagocytosis not affected • Ability to kill *E. coli* was improved	Hogan *et al*., 1992
Dairy cows from 4 wk to 5wk postpartum	3000 IU/day of dietary vitamin E from 4 wk pre to 8 wk post partum + 3000 IU of vitamin E injected 1 wk prepartum	• Neutrophil chemotaxis was improved	Politis *et al*., 1996
Fresh cows (<7 days in milk)	3000 IU/day of dietary vitamin E from 4 wk pre to 8 wk post partum + 3000 IU of vitamin E injected 1 wk prepartum	• Overall neutrophil function was improved	Politis *et al*., 1995

before anticipated calving. Diets fed to lactating cows were not supplemented with Vitamin E and Selenium. Supplemental Vitamin E with or without a Selenium injection reduced the incidence of clinical mastitis during the subsequent lactation by 37% compared with cows not fed supplemental Vitamin E or injected with Selenium. Selenium without supplemental Vitamin E reduced the incidence of clinical mastitis by 12% compared with unsupplemented cows, Vitamin E without selenium injections reduced the duration of clinical mastitis by 44% and by 62% when Selenium was injected.

Politis *et al*. (1995, 1996) fed cows 3,000 IU/day suplemental Vitamin E during the last 28 day prepartum and the first 56 day postpartum (base ration supplied 300 IU/day Vitamin E). Cows in the supplemental group also received a single injection of 5,000 IU Vitamin E ~1 wk before calving. Selenium status of cows was more than adequate at 88 ng/ml plasma. Somatic cell count (log 10) was significantly lower in the Vitamin E supplemented cows (2.1 vs2.5), and several measures of leukocyte function were improved by supplementing Vitamin E at these levels. It is concluded that "Vitamin E prevented suppression of leukocyte function during the early postpartum period". Their findings on Vitamin E and leukocyte function confirmed by the earlier reports of N diweni and Finch (1996) and Hogan *et al*. (1993).

Baldi *et al.* (2000) also reported that significantly lower somatic cell count (log 10) in cows fed 2,000 IU/day supplemental Vitamin E *vs* 1,000 IU/day from 14 day prepartum through 7 day postpartum in rations with 0.3 ppm added selenium. Plasma and milk Vitamin E were increased in cows fed 2000 *vs* 1000 IU/day. Taken together, these studies sub stantiate the cooperative roles of Vitamin E and selenium in maintaining mammary immune function and suggest that 2,000 and 4,000 IU Vitamin E/day during the prepartum period can be beneficial to dairy cows in terms of udder health and milk quality.

Smith *et al*. (1985a) also concluded that diets of heifers were either unsupplemented or supplemented with Selenium (0.3 ppm) and Vitamin E (1000 IU/day total intake) from 60 days prepartum and continuing throughout lactation. Supplemented heifers had significantly fewer quarters infected at calving, reduced prevalence of infection throughout lactation, fewer cases of clinical mastitis, infections of shorter duration and lower milk SCC compare with unsupplemented heifers.

Weiss *et al*. (1997) conducted a trial to examine effect of feeding of various amounts of Vitamin E during the dry period on the prevalence of clinical mastitis during the first week of lactation when all cows were fed 0.1 mg/kg of diet DM of Selenium. In that study cows at dry-off (approximately 60 days before calving) were fed diets with 0.1 mg/kg of Selenium and 100 or 1000 IU/day of supplemental Vitamin E (all-*rac*- α-tocopherol acetate). At 14 days before anticipated calving, cows that were fed 1000 IU/day of supplemental Vitamin E either continued to receive 1000 IU/day or were fed 4000 IU/day of supplemental Vitamin E. Total intramammary infections during the first week of lactation were not different among cows fed 100 or 1000 IU/day throughout the dry period (30 and

Table 3: Effect of supplemental Vitamin E during the dry period on mammary gland health of dairy cows during the first week postpartum when diets contained 0.1 mg/kg of Selenium (Weiss *et al*., 1997)

Group	Supplementation		*Intrammary infection rate (%)	*Clinical mastitis (%)
	Prepartum	Postpartum		
A	100 IU/day for 8 wk	100 IU/day	31.8	25.0
B	1000 IU/day for 8 wk	500 IU/day	32.1	16.7
C	100 IU/day for 6 wk and 4000 IU/day for 2 wk	2000 IU/day	11.8	2.6

28% of lactating quarters, respectively) but cows fed 4000 IU/day of supplemental Vitamin E during the 14 days prepartum period had fewer intramammary gland infections (13% of lactating quarters) (Table 3). The incidence of clinical mastitis was 24, 17 and 3% for cows fed 100, 1000 and 4000 IU/day of supplemental Vitamin E, respectively.

Correlation of Vitamin E and Selenium level in blood with status of mastitis:-

Vitamin E status has a positive relationship with udder health in dairy cows (Smith, 2000 and Weiss, 1998). Plasma concentrations of a-tocopherol are significantly lower during the prepartum period than during lactation and gestation. The concentration of a-tocopherol in plasma is highly correlated with plasma concentrations of cholesterol (Weiss *et al*., 1992, 1994); cholesterol concentrations are indicative of blood lipid concentrations. Feeding fat to dairy cows increases plasma a-tocopherol concentrations in dairy cows (Atwal *et al*., 1990). Based on neurophil functions (Weiss *et al*., 1994), the suggested minimal plasma concentration of α-tocopherol was 3 to 3.5 mg/liter. Low plasma concentrations of α-tocopherol were found to be a significant risk factor for clinical mastitis. Cows with plasma concentrations of α-tocopherol less than 3 mg/liter were 9.4 times more likely to have clinical mastitis than cows with concentrations greater than 3 mg/liter (Weiss *et al*., 1997). Cows with plasma concentrations of α-tocopherol less than 2.5 mg/liter were 2.8 times more likely to have an intramammary infection then were cows with plasma α-tocopherol concentrations greater than 2.5 mg/liter (Weiss *et al*., 1997).

Concentration of Selenium in whole blood or plasma is a reliable indicator of selenium status (Table 4). For the normal cows, about one-third of the selenium in whole blood is in the plasma and two-thirds is in the red cells. Selenium is incorporated into red cells only when the cell is made; therefore, Selenium content of the red cell reflects Selenium intake 1 to 3 months previously. The Selenium in plasma mainly represents a transport pool and reflects the current status. By parenteral route generally recommended dose is 0.1 to 0.15 mg/kg b. wt. S/C. In most situations, feeding 03 ppm provides adequate Selenium, but occasionally that amount is not adequate.

Table 4: Recommended concentrations of selenium in plasma (or serum) and whole blood of dairy cows.

Classification	Plasma/ Serum (mg/ml)	Whole blood (mg/ml)
Adequate	>0.075	>0.20
Marginal	0.05 to 0.075	0.14 to 0.20
Deficient	<0.05	<0.14

- Deficient means the animal is at risk for deficiency syndrome
- Adequate means there is little possibility of benefit from additional supplementation.

References

Atwal, A.S., Hidiroglou, M., Kramer, J.K.G. and Binns, M.R. **(1990)**. Effects of feeding of α-tocopherol and calcium salts of fatty acid on vitamin E and fatty acid composition of cow's milk. *J. Dairy Sci*. **73**:2832.

Baldi, A., Savoini, G. Pinotti, L. Monfardini, E. Cheli, F. and Dellorto, V. **(2000)**. Effects of vitamin E and different energy sources on vitamin E status, milk quality and reproduction in transition cows. *J. Vet. Med. Assoc.* **47**:599.

Barret, J.J., Hogan, J.S., Weiss, W.P., Smith, K.L. and Sordillo, L.M. **(1997)**. Concentration of α-tocopherol after intramammary infusion of *E. coli* or lipopolysaccharide. *J. Dairy Sci*., **80**:2826-2832.

Bendich, A. **(1990)**. Antioxidant micronutrients and immune responses. *Ann. New York Acad. Sci*. **587**:168.

Bhikane, A.V. and Kawitkar, S.B. **(2000)**. Hand book for Veterinary Clinician.Venkatesh Books. Udgir, India.

Boxer, L.A. **(1986)**. Regulation of phagocytic function by α-tocopherol. *Proc. Nutri. Soc*. **45**:333-338.

Dhanda, M.R. and Sethi, M.S. **(1962)**. Investigation of mastitis in India. ICAR Res. Series No. 35. New Delhi, India.

Dua, K. **(2001)**. Incidence, etiology and estimated economic losses due to mastitis in Punjab and in India- An update. *Indian Dairyman,* **53**: 41-48.

Dulin, A.M., Paape, M.J. and Weinland B.T. **(1988)**. American *J. Vet. Res.* **49**: 172-177.

Erskine, R.J., Eberhart, J. and Hutchinson, L.J. **(1987)**. Blood selenium concentration and glutathione peroxidase activities in dairy herds with high and low somatic cell counts. *J. Am. Vet. Med. Assoc.* **190**:1417.

Erskine, R.J., Eberhart, J. and Scholz, R.W. **(1990)**. Experimentally induced *Staphylococcus aureus* mastitis in selenium deficient and selenium supplemented dairy cows. *Am J. Vet. Res.* **51**:1107.

Erskine, R.J., Eberhart, R.J., Grasso, P.J. and Scholz, R.W. **(1989)**. Induction of *E. coli* mastitis in cows fed selenium deficient and selenium supplemented diets. *Am. J. Vet. Res.* **50**:2093.

Evans, H.M., Emerson, O.H. and Emerson, G.A. **(1936)**. *J. Biol. Chem.* **113**: 319.

Halliwel, B. **(1994)**. Free radicals and antioxidants in management of diseases of large ruminants. A personal view. *Nutri. Rev.* **52**:253-265.

Halliwel, B., Gutteridge, J.M.C. and Cross, C.E. **(1992)**. Free radicals, antioxidants and human disease: where are we how? *J. Lab. Clinical Med.* **119** (6):598-620.

Hoeben, D., Burnewitch, C. and Hamann, J. **(2000).** *J. Dairy Res.* **67:** 485-502.

Hoeben, D., Dosogni, H. and Burnevich, C. **(1997).** *J. Dairy Sci.* **81:** 403-410.

Hogan, J.S., Smith, K.L.,Weiss, W.P., Todhunter, D.A. and Shocky, W.L. **(1990)**. Relationship among vitamin E, selenium and bovine blood neutrophils. *J. Dairy Sci.*, **73**: 2372-2578.

Hogan, J.S., Weiss, W.P. and Smith, K.L. **(1993)**. Role of vitamin E and selenium in host defense against mastitis. *J. Dairy Sci.* **76**:2795.

Hogan, J.S., Weiss, W.P., Todhunter, D.A., Smith, K.L. and Schoenberger, P.S. **(1992)**. Bovine blood neutrophil response to parenteral vitamin *E. J. Dairy Sci.,* **75**:399-405.

Kehrli, M.E., Nonnecke, B.J. and Roth, J.A. **(1989)**. Alteration in bovine neutrophil function during the periparturient period. *Am. J. Vet. Res.* **50**:207.

Kirk, J.H., Degraves, F and Tyler, J. **(1994).** Recent progress in treatment and control of mastitis in cattle. *J. Am. Vet. Med. Assoc.* **204:** 1152-1158.

Kuehi, A.K. Jr. and Egan, R.W. **(1980).** *Science.* **210:** 978-984.

Monicada, S. and Vane, J.R. **(1979)**. In: The Prostaglandin System: Endoperoxides, prostacyclin, and Thromboxanes. Eds. F. Berti, G.F. Velo. Plenum Press, New York. pp. 203-221.

Muth, O.H., Oldfield, J.E, Remmert, R.F. and Schubert, J.R. **(1958)**. Effects of selenium and vitamin E on white muscle disease. *Science* **128**:1090.

National Research Council **(1987)**. Vitamin tolerance of animals. Natl. Acad. Sci., Washington, DC.

National Research Council **(1989)**. Nutrient requirement of dairy cattle. 6th rev. Ed. Natl. Acad. Sci., Washington, DC.

Nauriyal, D.S. **(1996)**. Profile studies in bovine mastitis with special reference to therapeutic consideration of Vitamin E and selenium. Thesis Ph.D., G.B. Pant Univ. of Agri and Tech., Pantnagar, India.

Nauriyal, D.S. and Pachauri, S.P. **(1997)**. Udder health monitoring based on Vitamin E and selenium status of dairy cows. *In Proc. of Natl. Symp. Of Indian Soc. for Vet. Med., held at Bangalore,* Feb.20-22.

Ndiweni, N., and Finch, J. M. **(1996)**. Effects of *in vitro* supplementation with alpha tocopherol and selenium on bovine neutrophil functions: implications for resistance to mastitis. *Vet. Immunol. Immunopathol.* **51**:67.

Person, K., Larsson, I and Hallen, S.C. **(1993)**. Effects of certain inflammatory mediators on bovine neutrophil migration *invivo* and *invitro. Vet. Immunol. Immunopathol.* **37**:99.

Politis, I., Hidiroglou, M., Batra, T. R., Gilmore, J. A., Gorewit, R. C. and Scherf, H. **(1995)**. Effects of Vitamin E on immune function of dairy cows. *Am. J. Vet. Res.* **56**:179-184.

Politis, I., Hidiroglou, N., White, J. H., Gilmore, J. A., Williams, S. N., Scherf, H. and Frigg, M. **(1996)**. Effects of Vitamin E on mammary and blood leukocyte function with emphasis on chemotaxis in periparturient dairy cows. *Am. J. Vet. Res.* **57**:468-471.

Radostits, O.M., Blood, D.C., Gay, C.C., Blood, D.C. and Hinchkliff, K.W. **(2000)**. Veterinary Medicine. 9th Ed. ELBS-Bailliere Tindal, London, p.563-618.

Rice, D. and Kennedy, S. **(1986)**. In: Proc. Roche Symposium, London, England. pp.1-23.

Ross, D. **(1977)**. Oxidative killing of microorganisms by phagocytic cells. *Trends. Biochem. Sci.* **2**:61.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. **(1973)**. Selenium: Biochemical component of glutathione peroxidase. *Science* **179**:585.

Samanta, A.K., Sharma, R.K., Ramachndran, M., Mandal, G.P. and Rastogi, A. **(2005)**. Influence of Vitamin E on health and fertility of dairy cows. *In* compedium of XXI Annl. Conv. and Natnl. Symp. of ISSAR Nov. 23rd – 25th , F.V.Sc. & A.H., R.S. Pura, Jammu. p.214.

Sharma, Neelesh, Maiti, S.K. and Roy, S. **(2003)**. Role of Vitamin E in the control of mastitis in dairy cows. *Vet. Pract.* **4**:140-143,

Singh, A.K., Singh, P.K., Singhal, L.K. and Agrawal, D.K. **(2004)**. Pathogenic effect of free radicals and their prevention through coepathy. *The Indian Cow.* **4**:27-34.

Singh, S.V., Singh, H.N. and Pachauri, S.P. **(2003)**. Antioxidants: A new approaches in prevention of bovine mastitis. *Compendium of Round Table Conf. on Mastitis, Palampur.* April 14-15 pp.47-51.

Smith, H.A., Jones, T.C. and Hunt, R.D. **(1972)**. Veterinary pathology, 4th ed. Lea and Febiger, Philadelphia, PA.

Smith, K. L. **(2000)**. Role of Vitamin E in optimizing mammary gland health and productivity of dairy cows. p. 7, Roche Pre Conference Symposium, Southwest Nutrition Conf., Phoenix, AZ.

Smith, K.L., Conrad, H.R., Amiet, B.A. and Todhunter, D.A. **(1985a)**. Incidence of environmental mastitis as influenced by Vitamin E and selenium. *Kiel. Mil chwirtsch. Forschungsber.* **37**:482.

Smith, K.L., Harrison, Hancock, D.D., Todhunter, D.A. and Conrad, H.R. **(1984)**. Effect of Vitamin E and selenium supplementation on incidence of sub clinical mastitis and duration of clinical symptoms. *J. Dairy Sci.,* **67**:1293.

Smith, K.L., Hogan, J.S. and Weiss, W.P. **(1997)**. Dietary Vitamin E and selenium affect mastitis and milk quality. *J. Anim. Sci.* **75**:1659.

Smith, K.L., Todhunter, D.A. and Schoenberger, P.S. **(1985b)**. Environmental mastitis: cause, prevalence, prevention. *J. Dairy Sci.* **68**:1531.

Smith, K.L., Todhunter, D.A. and Schoenberger, P.S. **(1985c)**. environmental pathogens and intramammary infection during the dry period. *J. Dairy Sci.* **68**:402.

Sordillo, L.M., Nickerson, S.C., Akers, R.M. and Oliver, S.P. **(1987)**. Pathology of *Staphylococcus aureus* mastitis during lactogenesis: relationship with bovine mammary structure and function. *J. Dairy Sci.* **72**:228.

Swarup, D. **(2001)**. Potential uses of antioxidants in management of diseases of large ruminants. *In* Proc. XIX Annl. Con. Indian Soc. Vet. Med. On "current trends on diagnosis, therapeutic approach and development of vaccines against diseases of livestock and poultry. April 9-11. pp 5-9.

Tripathi, B.N. **(2000)**. Diseases of mammary glands of goats and sheep. Vet. Bull., **70**: 1142.

Vaeshney, J.P. and Mukherjee, R. **(2002)**. Recent advances in management of bovine mastitis. *Intas Polivet* **3**: 62-65.

Vasavda, P.C. **(1988)**. Public health aspects of raw goat milk. *Dairy Goat J.* **64**: 226-227.

Weiss, W. P. **(1998)**. Requirements of fat soluble Vitamins for dairy cows: A review. *J. Dairy Sci*. **81**:2493.

Weiss, W.P., Hogan, J.S. and Smith, K.L. **(1994)**. Use of α-tocopherol concentrations in blood components to assess Vitamin E status of dairy cows. *Agri. Practice*. **15**:5.

Weiss, W.P., Hogan, J.S., Smith, K.L. and Smith, K.L. **(1990)**. Relationship among selenium, Vitamin E and mammary gland health in commercial dairy herd. *J. Dairy Sci*. **73**:381.

Weiss, W.P., Hogan, J.S., Smith, K.L., Todhunter, D.A. and Williams, S.N. **(1992)**. Effect of supplementing periparturient cows with Vitamin E on distribution of α-tocopherol and calcium salts of fatty acids on Vitamin E and fatty acid composition of cow's milk. *J. Dairy Sci.* **73**:2832.

Weiss, W.P., Hogan, J.S., Todhunter, D.A. and Smith, K.L **(1997)**. Effect of Vitamin E supplementation in diets with a low concentration of Selenium on mammary gland health of dairy cows. *J. Dairy Sci*. **80**:1728.