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Alternative Approach to Control Intramammary Infection in Dairy Cows: A Review

Neelesh Sharma
Division of Veterinary Clinical Medicine and Jurisprudence,
Faculty of Veterinary Sciences and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Sciences of Technology,
R.S. Pura, Jammu-181 102, Jammu and Kashmir, India

Abstract: Mastitis is a complex disease; there is no simple solution to control it. It is complex. Mastitis continues as a problem in many dairy herds despite proper application of proven control methods of teat dipping and total dry cow therapy. However, antibiotics were introduced in mastitis therapy from 50 year back for the control of intramammary infection. 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. 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. The mastitic cows have lower Vitamin E and Selenium concentration. 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. Both Vitamin E and Selenium protect cells from the detrimental effect of oxidation but they do so in different ways.

Key words: Mastitis, free radicals, antioxidant, vitamin E, selenium

INTRODUCTION

Mastitis is a parenchymal inflammation of the mammary gland, which is caused by microorganisms, usually bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland (Sharma *et al.*, 2006). It is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al.*, 2000). Because of their anatomical position are subject to out side influences and are prone to both inflammation or non inflammatory conditions (Tripathi, 2000). Mastitis continues as a problem in many dairy herds despite proper application of proven control methods of teat dipping and total dry cow therapy. Prevalence of mastitis varies from 10 to 50%. Sharma *et al.* (2004) reported 70.32% incidence of sub clinical mastitis in buffaloes, while Maiti *et al.* (2003) reported 70.37% incidence of sub clinical mastitis in cows. Traditionally, the mastitis control programmes are focused at use of chemical disinfectants, antiseptic or herbal teat dips (Maiti *et al.*, 2004) 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 this study will discuss particularly 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). 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. 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^{-1}) and sunflower meal (2.3 mg kg^{-1}) are good source of selenium. It is well documented that vitamin E and selenium play important roles in maintaining herd health of dairy cows (Muth *et al.*, 1958; Smith *et al.*, 1972, 1984).

VITAMIN E AND SELENIUM SYNERGISM

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. Compounds having vitamin E activity are known chemically as tocopherols but biologically most active form is α-tocopherol. α-tocopherol was discovered and isolated by Evans *et al.* (1936). Fresh, green forage is an excellent source of Vitamin E. Vitamin E is a fat soluble antioxidant which is found in cell membrane prevents the formation of harmful free radicals. Because of this localization within cell membrane, vitamin E can not protect the cytosol from free radicals. 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). Selenium is found in cytosol and its functions throughout the cell to destroy peroxides and other harmful compounds. 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 (Uppal *et al.*, 2005). 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; 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 (Sharma, 2003). 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 continues to be an economically vital disease all over the world (Hyrettin *et al.*, 2005). 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 annualy (Danda 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. 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+} ($Fe^{3+}+O_2{}^- \rightarrow Fe^{2+}+O_2$) 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 injuries due to free radicals contribute 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. The lymphocytes seems to be especially more susceptible to oxidative damage because their membranes have a relatively high free fatty acid content. 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 (Fig. 1). Hydrogen peroxide radicals can degrade heme proteins such as hemoglobin and mayoglobin (Halliwell, 1994).
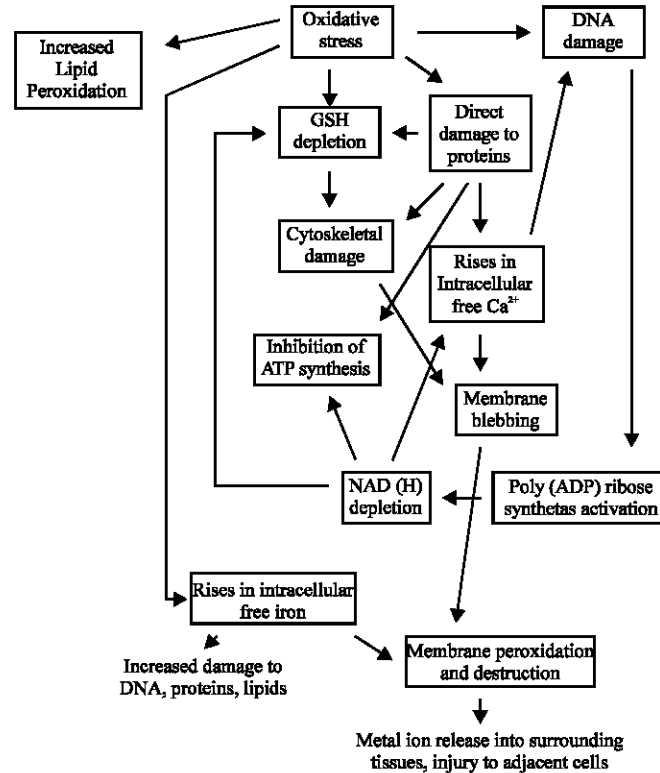
Fig. 1: Diagram to illustrate the complex multifactorial nature of oxidative damage to cells. Adopted from: Halliwell (1991)

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 (Sharma *et al.*, 2005). 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).

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.*, 1994).

Table 1: Antioxidant system of mammalian cell

Component and location in cell	Nutrients involved	Function
Superoxide dimutase (Cytosol)	Cu, Zn and 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.

The term oxidizable substrate includes almost everything found in living cells. Including proteins, lipids, carbohydrates and DNA (Halliwell, 1991). Chemically antioxidants work by one of the following mechanisms: They donate electrons or hydrogen or they scavenge oxygen or scavenging free radicals. Antioxidants protect body tissues from free radical damage either by scavenging ROS, by preventing their formation or by repairing damage.

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). Tocopherols, apart from being radical scavengers, react with singlet molecular oxygen (Kaiser *et al.*, 1990; Diplock *et al.*, 1989). 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 arachdonic 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. Vitamin E inhibits peroxidation of AA by scavenging peroxyl radicals thus slowing the chain reaction (Fig. 2). 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 17.6 mg α-tocopherol/million cells. It is reported that neutrophil can account for 10% of milk α-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 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 leukoytes (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. 3). This process produces a high concentration of free radicals help to kill the bacteria i.e.,
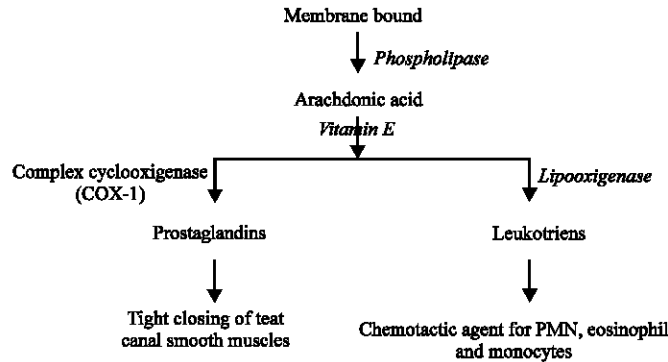
Membrane bound

↓ *Phospholipase*

Arachdonic acid

Vitamin E

Complex cyclooxigenase (COX-1) ──────────────────────── *Lipooxigenase*

Prostaglandins   **Leukotriens**

Tight closing of teat canal smooth muscles   **Chemotactic agent for PMN, eosinophil and monocytes**

Fig. 2: Diagram to illustrate the role of vitamin E in the control of mastitis by alteration of arachdonic acid metabolism

Invasion of bacteria

Phagocytosis (Respiratory burst)

Killing of engulfed bacteria by PMN

Production of O_2^- and H_2O_2 (Bactericidal)

Which is supplied by Hexose monophosphate shunt

Glucose oxidation by this pathway in PMN depends on the availability of $NADP^+$

Two sources of $NADP^+$

Selenium ──────────────────────── **Selenium**

O_2^- formation reaction (in which NADPH is electron)   **GSHPx-glutathione reductase (GR) system**

$2O_2 + NADPH \rightarrow 2O_2^- + NADP^+$ I. $2GSH + H_2O_2 \xrightarrow[\text{Oxidation}]{\text{GSHPx}}$ GSSG (Oxidized glutathione)

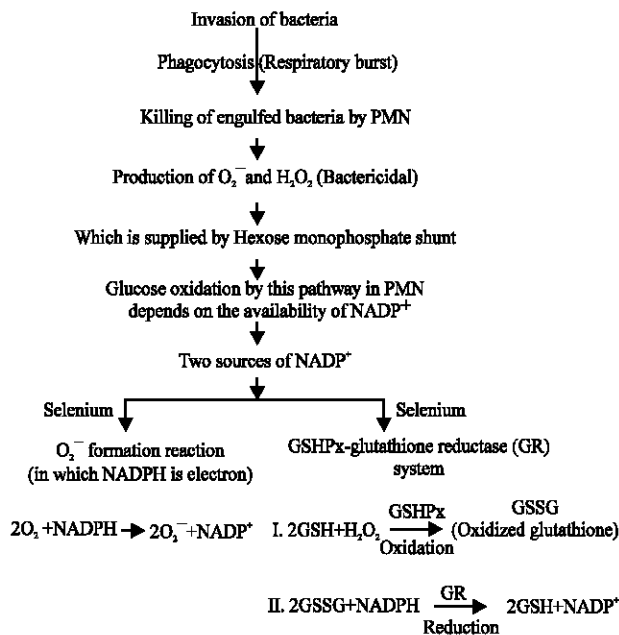II. $2GSSG + NADPH \xrightarrow[\text{Reduction}]{\text{GR}} 2GSH + NADP^+$

Fig. 3: Diagram to illustrate the action of selenium in the control of free radical production and killing of bacteria
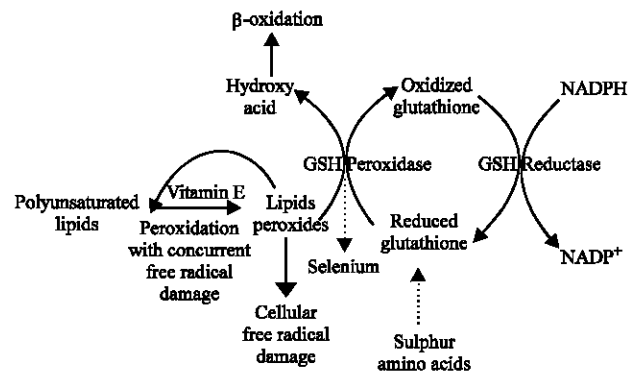
Fig. 4: Diagram to illustrate interaction among selenium, vitamin E and sulphur amino acids

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 α-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. 4) such as prevention of hydroperoxide formation by vitamin E, role of sulphur containing amino acids as a precursor of glutathione peroxidase (GSHPx) and selenium as an integral component of GSHPx. Antioxidants can however scavenges 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; 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 during first week of lactation (Samanta *et al.*, 2005). The plasma α-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 7 days prepartum to 1 or 2 week postpartum). Because of the beneficial effects of vitamin E on neutrophil function (Table 2).

Table 2: Effect of vitamin E supplementation on neutrophil function in dairy cows

Type of cows	Supplementation	Response	Reference
Lactating cows, first 30 days in lactation	1000 IU day^{-1} of dietary vitamin E during the dry period and 500 IU day^{-1} during the 30 days in 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 to 5week postpartum	3000 IU day^{-1} of dietary vitamin E from 4 week pre to 8 week post partum +3000 IU of vitamin E injected 1 week prepartum	Neutrophil chemotaxis was improved	Politis *et al.* (1996)
Fresh cows (<7 days in milk)	3000 IU day^{-1} of dietary vitamin E from 4 week pre to 8 week post partum +3000 IU of vitamin E injected 1 week prepartum	Overall neutrophil function was improved	Politis *et al.* (1995)

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 α-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^{-1} of DM. Based on typical DMI, that amount is equivalent to about 300 and 150 IU day^{-1} 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^{-1} of supplemental vitamin E and were fed no supplemental selenium or injected with 0.1 mg of selenium kg $^{-1}$ of body weight 21 days 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 62% when selenium was injected.

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^{-1} 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.

Politis *et al.* (1995, 1996) fed cows either 0 or 3,000 IU day^{-1} suplemental vitamin E during the last 28 days prepartum and the first 56 days postpartum (base ration supplied 300 IU day^{-1} vitamin E). Cows in the supplemental group also received a single injection of 5,000 IU vitamin E ~1 week before calving. Selenium status of cows was more than adequate at 88 ng mL^{-1} plasma. Somatic cell count (log 10) was significantly lower in the vitamin E supplemented cows (2.1 vs 2.5) 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 Ndiweni and Finch (1996) and Hogan *et al.* (1993).

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^{-1} 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^{-1} of selenium and 100 or 1000 IU day^{-1} of

supplemental vitamin E (all-*rac*- α-tocopherol acetate). At 14 days before anticipated calving, cows that were fed 1000 IU day^{-1} of supplemental vitamin E either continued to receive 1000 IU day^{-1} or were fed 4000 IU day^{-1} of supplemental vitamin E. Total intramammary infections during the first week of lactation were not different among cows fed 100 or 1000 IU day^{-1} throughout the dry period (30 and 28% of lactating quarters, respectively) but cows fed 4000 IU day^{-1} of supplemental vitamin E during the 14 days prepartum period had fewer intramammary gland infections (13% of lactating quarters). The incidence of clinical mastitis was 24, 17 and 3% for cows fed 100, 1000 and 4000 IU day^{-1} of supplemental vitamin E, respectively.

Baldi *et al.* (2000) also reported that significantly lower somatic cell count (log 10) in cows fed 2,000 IU day^{-1} supplemental vitamin E vs 1,000 IU day^{-1} 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^{-1}. 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^{-1} during the prepartum period can be beneficial to dairy cows in terms of udder health and milk quality.

Sharma *et al.* (2004) also reported that antibiotic (Cefotaxime at the rate of 5 mg/kg/b. wt. for 4 days I/m) in vitamin E (at the rate of 500 IU/animal/day) and selenium (at the rate of 6 mg/animal/day during early lactation) supplemented cows is more effective in the control of sub clinical mastitis as compared to unsupplemented animals.

Sharma and Maiti (2005) dietary supplemented vitamin E (at the rate of 500 IU/animal/day) and selenium (at the rate of 6 mg/animal/day) in sub clinical mastitic cows during early lactation caused 40% reduction in the infection rate. Both somatic cell count and total bacterial count were gradually reduced in supplemented groups. Supplementation of vitamin E (at the rate of 1000 IU/animal/day) and selenium (at the rate of 3 mg/animal/day) during dry period caused 50 and 71.42% reduction in incidence of sub clinical mastitis after calving on animal and quarter wise basis, respectively.

STATUS OF VITAMIN E AND SELENIUM LEVEL IN BLOOD OF MASTITIC COWS

Vitamin E status has a positive relationship with udder health in dairy cows (Smith, 2000 ; Weiss, 1998). Plasma concentrations of α-tocopherol are significantly lower during the prepartum period than during lactation and gestation. The concentration of α-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 α-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 L^{-1}. 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 L^{-1} were 9.4 times more likely to have clinical mastitis than cows with concentrations greater than 3 mg L^{-1} (Weiss *et al.*, 1997). Concentration of selenium in whole blood or plasma is a reliable indicator of selenium status. Adequate level of Se is >0.075 and >0.20 μg mL^{-1} in plasma and whole blood of dairy cows respectively while deficient level is <0.05 μg mL^{-1} in plasma. Deficient means the animal is at risk for deficiency syndrome and adequate means there is little possibility of benefit from additional supplementation. 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^{-1} b. wt. S/C.

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