

## Role of antioxidant vitamins and trace elements in mastitis in dairy COWS

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### ABSTRACT

Mastitis is associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk. Antioxidant vitamins and minerals protect the body from free radicals either by directly scavenging free radicals or by inhibiting the activity of oxidizing enzymes. The supplementation of mastitic dairy cows with antioxidant vitamins as vitamin A (VA) and  $\beta$ -carotene (BC), vitamin C (VC), vitamin E (VE), and antioxidant minerals as selenium (Se), Zinc (Zn) and copper (Cu) is very important to help the animal recover early. The aim of this review was to discuss the oxidative stress in dairy cows' mastitis, and the roles of VA and BC, VC, VE, Se, Zn, and Cu in mastitis of dairy cows. Before deciding to supplement dairy cow rations with the levels of vitamins and minerals, dairy farmers should have their animal feeds tested and their rations evaluated by a competent dairy cow nutritionist and a trustworthy laboratory to be sure what levels of supplementation may be warranted. While inadequate intake and absorption of certain nutrients may result in a weakened immune system and perhaps more mastitis during the lactation period, unjustified supplementation can be expensive and lead to other animal health problems.

### Keywords

Antioxidant, Cow, Mastitis, Trace-element, Vitamin

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### INTRODUCTION

Inflammatory reaction of mammary gland (i.e., mastitis) usually caused by microorganism, is considered as the most costly disease for dairy cattle. Both clinical mastitis (CM) and subclinical mastitis (SCM) produce great economic losses. CM has elevated body temperature, inappetence, a red, swollen and/or painful udder and/or abnormal milk. However, SCM has no apparent clinical signs but accompanied with elevation of somatic cells count (SCC) in milk (Huijps et al., 2008). The SCC in milk is considered as a well-known indicator that reflects milk quality and health status of mammary gland. Excessive amount of neutrophils, various epithelial cells, macrophages, lymphocytes, eosinophils of mammary tissue in milk is considered as indicative of response of mammary tissue to microorganisms (Atakisi et al., 2010).

Vitamins and minerals have long been recognized as antioxidants in the animal health and production. However, they also have specific roles in Mastitis of Dairy cows, such as vitamin A (VA) and  $\beta$ -carotene (BC), vitamin C (VC), vitamin E (VE), selenium (Se), Zinc (Zn) and copper (Cu). As described in Table 1, different antioxidants have crucial role in animals' health. Previous studies revealed that increase in lipid peroxidation in mastitis may cause decrease in levels of some antioxidant molecules leading to an increase in oxidative stress (Weiss et al., 2004). Oxidative stress is generally described as an imbalance between oxidant and antioxidant levels (Lykkesfeldt and Svendsen, 2007). When the production of oxidants exceeds the capacity of antioxidant defense, a condition of oxidative stress is produced resulting in oxidative

**Table 1. Summary of micronutrient effects on mammary gland immunity (Hayajneh, 2014).**

Micronutrient	Observation
Vitamin A	Decreased SCC. Moderated glucocorticoid levels
Beta Carotene	Increased bactericidal function of phagocytes. Increased mitogen-induced proliferation of lymphocytes
Vitamin E	Increased neutrophil bactericidal activity Decreased incidence of clinical mastitis
Se	Decreased efficiency in neutrophils function. Improved bactericidal capabilities of neutrophils. Decreased severity and duration of mastitis
Cu	Deficiency decreased neutrophil killing capability. Deficiency increased susceptibility to bactericidal infection
Zn	Deficiency decreased leukocyte function. Deficiency increased susceptibility to bacterial infection

damage to macromolecules such as lipids, DNA and proteins (Sordillo et al., 2009).

Milk with higher SCC is positively associated with malondialdehyde (MDA) level in milk (Suriyasathaporn et al., 2006), and the mean level of MDA is significantly higher in SCM milk than in normal milk (Yang et al., 2011b), consequently more free radicals being released and a state of oxidative stress arise (Su et al., 2002). During the past decade, significant advances have been made in understanding the roles of antioxidants in mastitis. The purpose of this paper was to review the current knowledge of the roles of vitamins and minerals in mastitis of dairy cows.

## OXIDATIVE STRESS IN MASTITIS OF DAIRY COWS

Mastitis could induce the increase of free radicals formation in milk and leading to oxidative stress (Gu et al., 2009), especially during the early lactation period of dairy cows (Sordillo et al., 2007). Both CM and SCM are associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk (Atakisi et al., 2010).

During lactation, mammary epithelial cells exhibit a high metabolic rate and thus produce large amounts of reactive oxygen species (ROS) and lipid peroxides in vivo (Jin et al., 2014). Jhambh et al. (2013) reported that significant ( $p < 0.05$ ) decrease in blood superoxide dismutase (SOD) and catalase activities, reduced glutathione (GSH) concentration and an increase in erythrocytic lipid peroxides was observed in cows with clinical mastitis.

Nitric oxide (NO) is one of the most important reactive nitrogen intermediates, which operates in a variety of tissues to regulate a diverse range of physiological

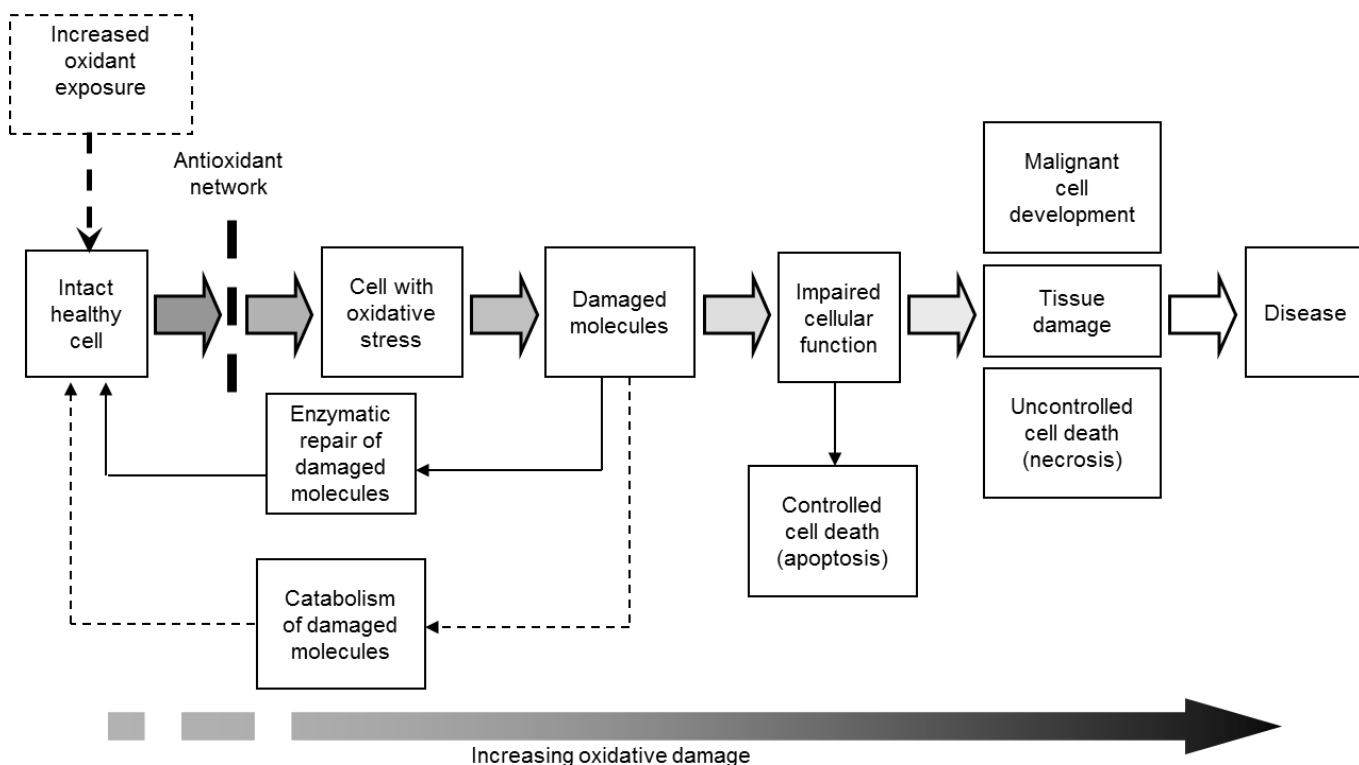
processes such as inflammatory response (Dawson and Dawson, 1995). During inflammation, epithelial cells and macrophage of mammary gland produce a significant amount of NO; this inducible NO mediates inflammation during mastitis (Bouchard et al., 1999). Another source for NO is the mammary epithelial cells and/or mononuclear phagocytes, which contribute to NO production upon stimulation with lipopolysaccharide and cytokines (Boulanger et al., 2001).

NO was reported to increase in CM milk (Atakisi et al., 2010) and in milk and plasma after intramammary infusion of *Escherichia coli* or endotoxin produced by *E. coli* (Komine et al., 2004). Milk SCC is considered as a well-known indicator that reflects mammary health and milk quality; thus, a positive correlation between SCC and NO concentration had been reported (Atakisi et al., 2010).

NO has an important role in mediating microbistatic and/or microbicidal activity, as the activated macrophages synthesize NO (Jungi, 2000), which is considered as a primary defence system that eliminate intracellular pathogens (O'Flaherty et al., 2003). The antimicrobial property of NO is attributed to peroxynitrite, a reactive nitrogen metabolite, derived from oxidation of NO (Beckman et al., 1990). In severe mastitis, peroxynitrite is produced in excess, which may result in alterations in the antioxidant balance (Chaiyotwittayakun et al., 2002). This means that excessive release of NO results in oxidative damage to mammary gland secretions (Komine et al., 2004; Atakisi et al., 2010).

The cells contain a variety of antioxidants that play an important role in the protection against excessive release of reactive oxygen species in blood and tissues,

including the udder tissue and milk in mastitis (Scaletti et al., 2003; Abd Ellah, 2013). Antioxidants protect the



**Figure 1.** Schematic outline of cellular defenses against oxidative stress-mediated cellular damage. Increased oxidative stress is initially counteracted by the antioxidant network. Damaged molecules are either repaired or categorized. Controlled cell suicide can be initiated if further oxidative damage leads to impaired cellular function. When these signaling cascades are damaged or the oxidative damage exceeds the capacity of the defense mechanisms, uncontrolled cell death, tissue damage and malignant cell development can progress into disease (Lykkesfeldt and Svendsen, 2007).

body from free radicals either by directly scavenging free radicals or by inhibiting the activity of oxidizing enzymes (Abd Ellah et al., 2009).

## MECHANISMS OF DEFENSE AGAINST OXIDATIVE STRESS

Due to the substantial background exposure to oxidants resulting from a life depending on molecular oxygen, aerobic organisms have adapted to constantly fighting a battle against oxidative stress. Advanced cellular defense strategies have evolved and gradually expanded the possible lifespan for the individual species. The cellular defense mechanisms can be divided into at least three levels according to their function of quenching oxidants, repairing/removing oxidative damage or encapsulating non-repairable damage (Figure 1).

As a first level of defense against oxidants, the cell is equipped with a so-called antioxidant network.

Antioxidants are capable of donating electrons to oxidants, thus quenching their reactivity under controlled conditions and making them harmless to cellular macromolecules. A second and highly important level of defense is the ability to detect and repair or remove oxidized and damaged molecules. Finally, if the extent of the oxidative damage exceeds the capacity of repair and removal, the organism is equipped with one final weapon, controlled cell suicide or apoptosis. The ability to induce programmed cell death is of major importance in a variety of bodily functions, including control of tissue growth, and is apparently under control by several signaling pathways. However, one of these appears to be that apoptosis is induced by increased oxidative stress and thus constitutes a final resort to encapsulate and isolate the damaged cells (Lykkesfeldt and Svendsen, 2007).

## THE ROLES OF VITAMIN A AND B-CAROTENE IN MASTITIS OF DAIRY COWS

VA and its precursor-BC are important in maintaining epithelial tissue health and play a vital role in mucosal surface integrity and stability (Sordillo et al., 1997). These functions may affect cow resistance to pathogen entry into the mammary gland as well as resistance post-entry. VA is an important factor in improving immune function and attenuating oxidative stress (Jin et al., 2014). In addition, BC appears to function as an antioxidant, reducing superoxide formation within the phagocyte, and it play an important role in protecting udder tissue and milk from the harmful effect of free radicals. This is potentially important due to phagocytosis and internal killing of bacteria once engulfed are primary mechanisms used by phagocytic cells to eliminate bacteria. Both VA and BC also appear to have stimulatory effects on immune cells (Heinrichs et al., 2009). Cows with higher California Mastitis Test (CMT) scores had significantly lower plasma VA and BC concentrations than cows with CMT scores indicating no mastitis (Yang et al., 2011a).

BC functions independently of VA in mastitis and reproduction. It was reported that, low concentrations of plasma VA (<0.8µg/mL) and BC (<2µg/mL) were linked with severity of mastitis (Jukola et al., 1996). Jukola et al. (1996) reported that VA and BC supplementation have an effect on udder health only when the plasma level of VA is lower than 0.4 mg/L and BC is lower than 3.0 mg/L. LeBlanc et al. (2004) showed a 60% relative reduction in the risk of clinical mastitis for every 100 ng/mL increase in serum retinol. Data showing the association of VA and BC with mastitis have been variable at best, and supplementation is likely not needed in excess of normal recommendations. However, several studies have shown no impact of supplementing BC on intramammary infection level (Heinrichs et al., 2009).

### THE ROLES OF VITAMIN C IN MASTITIS OF DAIRY COWS

Ascorbic acid is the most abundant and important water-soluble antioxidant for mammals (Sauberlich, 1994). Even though it can be synthesized in the body of most mammals except primates and guinea pigs which have a dietary vitamin C requirement. Thus, ascorbic acid is not a required nutrient for dairy cows; some data are accumulating that suggest vitamin C is related to mastitis. The cows suffering from mastitis have lower concentrations of vitamin C in their milk and plasma (Weiss et al., 2004; Kleczkowski et al., 2005). The severity of clinical signs is related with magnitude of the decrease in concentrations (Weiss et al., 2004).

Ascorbic acid scavenges aqueous reactive oxygen species by rapid electron transfer, thus inhibiting lipid peroxidation, and represents one of the important antioxidant defences against oxidative damage. In bovine mastitis, it has been identified as oxidative stress biomarkers. The number of the leukocytes per milliliter blood was correlated positively with VC content of plasma. Decreased concentration of ascorbic acid has been recorded from mastitis milk of cows (Naresh et al., 2002). Many studies had been reported that its milk concentration significantly decreased in acute mastitis and SCM especially when the condition is accompanied by an increase in the levels of lipid hydroperoxide in erythrocytes (Weiss et al., 2004; Kleczkowski et al., 2005; Ranjan et al., 2005).

Ascorbic acid along with cupric ions was found successful to prevent and treat the mastitis of dairy cows as teat dip or intramammary infusion (Naresh et al., 2002). Vitamin C administered to cows by subcutaneous injection may have therapeutic value in mastitis (Ranjan et al., 2005). However, its therapeutic effect decreased in the presence of lipid peroxidation with moderate improvement in clinical signs of mastitis (Ranjan et al., 2005).

### THE ROLES OF VITAMIN E IN MASTITIS OF DAIRY COWS

The VE is the most important lipid soluble membrane antioxidant, and the biologically active form is known as  $\alpha$ -tocopherol. The VE is an integral component of lipid membrane, and has an important role in protecting lipid membranes from attack of reactive oxygen (Rice and Kennedy, 1988). VE enhances the functional efficiency of neutrophils by protecting them from oxidative damage following intracellular killing of ingested bacteria (Herdt and Stowe, 1991). Fresh green forage is an excellent source of VE; however, concentrates and stored forages (hays, haylages, and silages) are generally low in VE (NRC, 2001). The best understood the role of VE on mastitis is that it acts as a lipid soluble cellular antioxidant, free radical scavenger, and protects against lipid peroxidation (Yang et al., 2011a).

Many experiments have shown that plasma concentrations of  $\alpha$ -tocopherol in dairy cows are low at parturition. Cows are immunosuppressed during the time when plasma concentrations of VE are low. Supplementation of antioxidant vitamins may reduce inflammatory response and oxidative stress during mastitis. Smith et al. (1984) were the first to report that

supplemental VE and Se reduced CM. That study was conducted in Ohio where the soil concentration of Se is very low, and used dry cows fed hay-based diets (should be very low in VE). Cows were either injected with a placebo or 50 mg of Se at 21 days before calving and were fed either 0 or 1000 IU/day of supplemental VE (dl- $\alpha$ -tocopheryl acetate). Se without supplemental VE reduced the incidence and duration of CM, but the largest response was caused by VE with or without Se. However, Chandra et al. (2013) indicated that supplementation of vitamin E and Zn in food of Sahiwal cows during peripartum period enhanced milk production by reducing negative energy balance.

The VE deficiencies are frequently observed in peripartum dairy cows. Most cases of CM occur during the first month of lactation and originate in the dry period (Green et al., 2002), and coincide with the lowest VE blood concentration (Goff and Stabel, 1990). It was suggested that maintaining an optimal VE level, together with low levels of oxidative stress is an important factor in dry cow management and improvement of udder health.

## THE ROLES OF SELENIUM IN MASTITIS OF DAIRY COWS

Se is recognized as an essential trace element for domestic animals, the majority of Se in body tissues and fluids is present as either selenocysteine, which functions as an active center for selenoproteins, or selenomethionine, which is incorporated into general proteins and acts as a biological pool for Se (Juniper, 2006). It functions in the antioxidant system as an essential component of the glutathione peroxidase (GSH-Px), which is responsible for reduction of H<sub>2</sub>O<sub>2</sub> and free O<sub>2</sub> to H<sub>2</sub>O (NRC, 2001). It also plays a vital role in protecting both the intra- and extra-cellular lipid membranes against oxidative damage. The activity of GSH-Px in milk varies with the species and diet (Fox and Kelly, 2006). GSH-Px catalyzes the reduction of various peroxides, protecting the cell against oxidative damage (Abd Ellah et al., 2007) and protects milk lipids from oxidation (Bhattacharya et al., 1988). Vitamin E and selenium supplements in diets appear to have a preventive effects against acute infections in which high polymorphonuclear response occur in the infected gland (Ata and Zaki, 2014).

Dairy cattle have several known endogenous antioxidant defense mechanisms that can counteract the harmful effects of ROS accumulation. The Se-dependent selenoproteins had been studied extensively

with respect to mammary gland health (Miller et al., 1993). Se supplementation to periparturient cows reduces the incidence and severity of mastitis (Smith et al., 1984). The effects of feeding organic Se in the form of selenized yeast and sodium selenite were compared in a feeding experiment on 100 dairy cows. Feed was supplemented at a rate of 0.2 ppm for 8 weeks. The results showed that the yeast Se yielded the greater blood level of Se. The GSH-Px level went from 0.22 to 3.0 and to 2.3 microKat/g of hemoglobin from Se yeast and selenite, respectively. Blood GSH-Px continued to increase up to 10 weeks after the supplementation had stopped. The bioavailability of yeast Se was found to be superior to selenite. The percentage of quarters harboring mastitis dropped along with milk SCC. It is important to note that the test cows began the trial with a significantly low blood Se status (Malbe et al. in 1995). Moeini et al. (2009) reported that milk SCC of heifers at 8 weeks lactation was significantly decreased (193,000/mL vs. 179,000/mL,  $p < 0.05$ ) by 20mg Se and 2000 IU dl- $\alpha$ -tocopheryl acetate supplemental at 4 and 2 weeks before expected calving. The beneficial effects of Se supplementation are thought to be due to the actions of certain antioxidant Se-dependent enzymes (Papp et al., 2007). NRC (2001) recommended the level of Se in dairy diets is 0.3 mg/kg dry matter (DM) and should be closely monitored to ensure that over supplementation does not occur.

## THE ROLES OF ZINC IN MASTITIS OF DAIRY COWS

Zn is an essential trace mineral found to be an integral component of over 300 enzymes in metabolism (Yang et al., 2011a), the functions of Zn include tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity, and generalized host defense (Gropper et al., 2005). The mammary gland is an organ that is derived from the skin, thus making Zn necessary to maintain the integrity of the keratin that lines the streak canal. Zn has a significant effect on gene expression and cellular growth.

Cell mediated immunity has also been found to be altered by Zn deficiency. Zn deficiency has been associated with reduced formation of both T and B lymphocytes and phagocytes (Sherman, 1992). T and B cells are the major cellular components of the adaptive immune response. Once they have recognized an invader, the cells generate specific responses that work to eliminate pathogens or pathogen infected cells. As previously discussed, Zn is also involved in the removal of free radicals by superoxide dismutase

(SOD). Extracellular and cytosolic SOD requires both Zn and Cu. Another important factor to note in regard to the cow's immune response is that immunoactive substances such as VA have been found to react with Zn in several ways. Zn is necessary for the hepatic synthesis of retinol-binding protein, which transports VA in the blood (Gropper et al., 2005).

Zn is required for the formation of Mn-Zn SOD, deficiency of Zn affect the activity of SOD in blood and tissues, which results in increased superoxide radicals. It is known that mastitis associated with increased SCC counts in milk, which act as a source for free radicals and hence oxidative stress. Low Zn status leads to low quality milk with high SCC and increased incidence of mastitis (Gaafar et al., 2010). An experiment was done that included 12 lactation trials addressing Zn supplementation. The result indicates that SCC was reduced from 294 000/mL to 196 000/mL (as a 33.3% reduction,  $p < 0.01$ ) in cows receiving Zn methionine complex (Kellogg et al., 2004). In an experiment, Popovic (2004) replaced 33% of the supplemental inorganic Zn-sulphate with organic Zn for 45 days of pre-calving until 100 days of post-calving. The cows receiving organic Zn had significantly ( $p < 0.05$ ) lower SCC at day-10 of lactation (158,840/mL vs. 193,530/mL), and at the end of the trial (62,670/mL vs. 116,440/mL). In addition, Kinal et al. (2005) reported that replacing 30% of the inorganic Cu, manganese (Mn), and Zn for 6 weeks pre-calving until 305 days of lactation in dairy cows could result 34% reduction in SCC (270,000/mL vs. 409,000/mL,  $p < 0.01$ ). NRC (2001) recommended the level of Zn supplementation for lactating dairy cows at 40-60 mg/kg DM.

## THE ROLES OF COPPER IN MASTITIS OF DAIRY COWS

Cu is a component of ceruloplasmin, which facilitates iron absorption and transport. In addition, Cu is considered as an important part of SOD, an enzyme that protects cells from the toxic effects of oxygen metabolites produced during phagocytosis (Yang et al., 2011a). Therefore, lactating cows were recommended for Cu supplementation at 11 mg/kg DM (NRC, 2001). Cu deficiency in cattle is generally due to the presence of dietary antagonists, such as sulfur, molybdenum and iron that reduce Cu bioavailability (Spears, 2003).

As a modulator of the inflammatory process, ceruloplasmin serves as an acute-phase protein. Acute phase proteins rise in the blood with infection and other inflammatory events (Gropper et al., 2005). The

enzyme SOD, which is found both in the cytosol of cells and extracellularly, is Cu and Zn dependent. Without the presence of SOD, superoxide radicals can form more destructive hydroxyl radicals that damage both unsaturated double bonds in cell membranes, fatty acids, and other molecules in cells. Therefore, SOD assumes a very important protective function (Gropper et al., 2005). A study was conducted on first lactation Holstein heifers to assess potential role of dietary Cu enhancing resistance to *E. coli* mastitis, and conclusions were made that Cu supplementation reduced the severity of clinical signs during experimental *E. coli* mastitis but the duration of mastitis was unaffected (O'Rourke, 2009). Experimental studies approved that Cu supplementation reduced the severity of clinical signs of *E. coli* mastitis (Scaletti et al., 2003).

## CONCLUSION

Mastitis is associated with release of free radicals, increased total oxidant capacity and decreased total antioxidants capacity in milk. Antioxidant vitamins and minerals protect the body from free radicals either by directly scavenging free radicals or by inhibiting the activity of oxidizing enzymes. The supplementation of mastitic dairy cows with antioxidant vitamins as vitamin A, C, E and  $\beta$ -carotene, and antioxidant minerals as selenium, Zinc and copper is very important to help the animal recover early. Before deciding to supplement dairy cow rations with the levels of vitamins and minerals indicated above, dairy farmers should have their animal feeds tested and their rations evaluated by a competent dairy cow nutritionist and a trustworthy laboratory to be sure what levels of supplementation may be warranted. While inadequate intake and absorption of certain nutrients may result in a weakened immune system and perhaps more mastitis during the lactation period, unjustified supplementation can be expensive and lead to other animal health problems.

## REFERENCES

- Abd Ellah MR (2013). Role of Free Radicals and Antioxidants in Mastitis. Journal of Advanced Veterinary Research, 3: 1-7.
- Abd Ellah MR, Okada K, Goryo M, Oishi A, Yasuda J (2009). Superoxide dismutase activity as a measure of hepatic oxidative stress in cattle following ethionine administration. The Veterinary Journal, 182: 336-341.
- Abd Ellah MR, Rushdi M, Keiji O, Jun Y (2007). Oxidative stress and bovine liver diseases: Role of

- glutathione peroxidase and glucose6-phosphate dehydrogenase. *Japanese Journal of Veterinary Research*, 54: 163-173.
- Ata N, Zaki MS (2014). New Approaches in Control of Mastitis in Dairy Animals. *Life Science Journal*, 11: 275-277.
- Atakisi O, Oral H, Atakisi E, Merhan O, Pancarci SM, Ozcana A, Marasli S, Polat B, Colak A, Kaya S (2010). Subclinical mastitis causes alterations in nitric oxide, total oxidant and antioxidant capacity in cow milk. *Research in Veterinary Science*, 89: 10-13.
- Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proceedings of the National Academy of Sciences of the United States of America*, 87: 1620-1624.
- Bhattacharya ID, Picciano MF, Milner JA (1988). Characteristics of human milk glutathione peroxidase. *Biological Trace Element Research*, 18: 59-70.
- Bouchard L, Blais S, Desrosiers C, Zhao X, Lacasse P (1999). Nitric oxide production during endotoxin-induced mastitis in the cow. *Journal of Dairy Science*, 82: 2574-2581.
- Boulanger V, Bouchard L, Zhao X, Lacasse P (2001). Induction of nitric oxide production by bovine mammary epithelial cells and blood leukocytes. *Journal of Dairy Science*, 84: 1430-1437.
- Chaiyotwittayakun A, Erskine RJ, Bartlett PC, Herdt TH, Sears PM, Harmon RJ (2002). The effect of ascorbic acid and L-histidine therapy on acute mammary inflammation in dairy cattle. *Journal of Dairy Science*, 85: 60-67.
- Chandra G, Aggarwal A, Singh AK, Kumar M, Upadhyay RC (2013). Effect of Vitamin E and Zinc Supplementation on Energy Metabolites, Lipid Peroxidation, and Milk Production in Peripartum Sahiwal Cows. *Asian-Australasian Journal of Animal Sciences*, 26: 1569-1576.
- Dawson TM, Dawson VL (1995). Nitric Oxide: Actions and Pathological Roles. *Neuroscientist*, 1: 7-18.
- Fox PF, Kelly AL (2006). Indigenous enzymes in milk: Overview and historical aspects-Part 2. *International Dairy Journal*, 16: 517-532.
- Gaafar HMA, Basiuoni MI, Ali MFE, Shitta AA, Shamas ASE (2010). Effect of zinc methionine supplementation on somatic cell count in milk and mastitis in Friesian cows. *Archiva Zootechnica*, 13: 36-46.
- Goff JP, Stabel JR (1990). Decreased plasma retinol,  $\alpha$ -tocopherol, and zinc concentration during the periparturient period: effect of milk fever. *Journal of Dairy Science*, 73: 3195-3199.
- Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ (2002). Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *Journal of Dairy Science*, 85: 2589-2599.
- Gropper SS, Smith J, Groff J (2005). *Advanced Nutrition and Human Metabolism: Copper transport and uptake*. 4th ed. Wadsworth. Belmont, CA; pp 449-451.
- Gu BB, Zhu YM, Zhu W, Miao JF, Deng YE, Zou SX (2009). Retinoid protects rats against neutrophil-induced oxidative stress in acute experimental mastitis. *International Immunopharmacology*, 9: 223-229.
- Hayajneh FMF (2014). Antioxidants in dairy cattle health and disease. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine*, 71: 104-109.
- Heinrichs AJ, Costello SS, Jones CM (2009). Control of heifer mastitis by nutrition. *Veterinary Microbiology*, 134: 172-176.
- Herdt TH, Stowe HD (1991). Fat-soluble vitamin nutrition for dairy cattle. *Veterinary Clinics of North America: Food Animal Practice*, 7: 391-415.
- Huijps K, Lam TJGM, Hogeveen H (2008). Costs of mastitis: facts and perception. *Journal of Dairy Research*, 75: 113-120.
- Jhambh R, Dimri U, Gupta V K, Rathore R (2013). Blood antioxidant profile and lipid peroxides in dairy cows with clinical mastitis. *Veterinary World*, 6: 271-273.
- Jin L, Yan SM, Shi BL, Bao HY, Gong J, Guo XY, Li JL (2014). Effects of vitamin A on the milk performance, antioxidant functions and immune functions of dairy cows. *Animal Feed Science and Technology*, 192: 15-23.
- Jukola E, Hakkarainen J, Saloniemi H, Sankari S (1996). Blood Selenium, Vitamin E, Vitamin A, and  $\beta$ -Carotene Concentrations and Udder Health, Fertility Treatments, and Fertility. *Journal of Dairy Science*, 79: 838-845.
- Jungi TW (2000). Research from the Division of Immunology: production of nitric oxide (NO) by macrophages in ruminants. *Schweizer Archiv Ffur Tierheilkunde*, 142: 215-217.
- Juniper DT, Phipps RH, Jones AK, Bertin G (2006). Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine, and feces. *Journal of Dairy Science*, 89: 3544-3551.

- Kellogg DW, Tomlinson DJ, Socha MT, Johnson AB (2004). Review: effects of zinc methionine complex on milk production and somatic cell count of dairy cows: twelve-trial summary. *The Professional Animal Scientist*, 20: 295-301.
- Kinal S, Korniewicz A, Jamroz D, Zieminski R, Slupczynska M (2005). Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. *Journal of Food, Agriculture and Environment*, 3: 168-172.
- Kleczkowski M, Kluciński W, Shaktur A, Sikora J (2005). Concentration of ascorbic acid in the blood of cows with subclinical mastitis. *Polish Journal of Veterinary Sciences*, 8: 121-125.
- Komine K, Kuroishi T, Komine Y, Watanabe K, Kobayashi J, Yamaguchi T, Kamata S, Kumagai K (2004). Induction of nitric oxide production mediated by tumor necrosis factor alpha on staphylococcal enterotoxin C-stimulated bovine mammary gland cells. *Clinical and Diagnostic Laboratory Immunology*, 11: 203-210.
- LeBlanc SJ, Herdt TH, Seymour WM, Duffield TF, Leslie KE (2004). Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *Journal of Dairy Science*, 87: 609-619.
- Lykkefeldt J, Svendsen O (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals. *The Veterinary Journal*, 173: 502-511.
- Malbe M, Klaassen M, Fang W, Myllys V, Vikerpuur M, Nyholm K, Sankari S, Suoranta K, Sandholm M (1995). Comparisons of Selenite and Selenium Yeast Feed Supplements on Se-incorporation, Mastitis and Leucocyte Function in Se-deficient Dairy Cows. *Journal of Veterinary Medicine Series A*, 42: 111-121.
- Miller JK, Brzezinska-Slebodzinska E, Madsen FC (1993). Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science*, 76: 2812-2823.
- Moeini MM, Karami H, Mikaeili E (2009). Effect of selenium and vitamin E supplementation during the late pregnancy on reproductive indices and milk production in heifers. *Animal Reproduction Science*, 114: 109-114.
- Naresh R, Dwivedi SK, Swarup D, Patra RC (2002). Evaluation of ascorbic acid treatment in clinical and subclinical mastitis of Indian dairy cows. *Asian-Australasian Journal of Animal Sciences*, 15: 905-911.
- NRC (2001). Subcommittee on Dairy Cattle Nutrition, Committee on Animal Nutrition, National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- O'Flaherty C, Beorlegui N, Beconi MT (2003). Participation of superoxide anion in the capacitation of cryopreserved bovine sperm. *International Journal of Andrology*, 26: 109-114.
- O'Rourke D (2009). Nutrition and udder health in dairy cows: a review. *Irish Veterinary Journal*, 62:S15.
- Papp LV, Lu J, Holmgren A, Khanna KK (2007). From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxidants Redox Signaling*, 9: 775-806.
- Popovic Z (2004). Performance and udder health status of dairy cows influenced by organically bound zinc and chromium. Ph.D. Thesis, University of Belgrade.
- Ranjan R, Swarup D, Naresh R, Patra RC (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. *Veterinary Research Communications*, 29: 27-34.
- Rice DA, Kennedy S (1988). Assessment of vitamin E, selenium and polyunsaturated fatty acid interactions in the aetiology of disease in the bovine. *Proceedings of The Nutrition Society*, 47: 177-184.
- Sauberlich, H. E. 1994. Pharmacology of vitamin C. *Annu. Revista De Nutricao-Brazilian Journal of Nutrition*, 14:371-391.
- Scaletti RW, Trammell DS, Smith BA, Harmon RJ (2003). Role of Dietary Copper in Enhancing Resistance to *Escherichia coli* Mastitis. *Journal of Dairy Science*, 86:1240-1249.
- Sherman AR (1992). Zinc, copper, and iron nutrition and immunity. *Journal of Nutrition*, 122: 604s-609s.
- Smith KL, Harrison JH, Hancock DD, Todhunter DA, Conrad HR (1984). Effect of Vitamin E and Selenium Supplementation on Incidence of Clinical Mastitis and Duration of Clinical Symptoms. *Journal of Dairy Science*, 67:1293-1300.
- Sordillo LM, O'Boyle N, Gandy JC, Corl CM, Hamilton E (2007). Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. *Journal of Dairy Science*, 90: 1186-1192.
- Sordillo LM, Shafer-Weaver K, DeRosa D (1997). Immunobiology of the mammary gland. *Journal of Dairy Science*, 80: 1851-1865.
- Spears JW (2003). Trace mineral bioavailability in ruminants. *Journal of Nutrition*, 133: 1506S-1509S.
- Su WJ, Chang CJ, Peh HC, Lee SL, Huang MC, Zhao X (2002). Apoptosis and oxidative stress of infiltrated neutrophils obtained from mammary glands of



- goats during various stages of lactation. American Veterinary Medical Association, 63: 241-246.
- Suriyasathaporn W, Vinitketkumnuen U, Chewonarin T, Boonyayatra S, Kreausukon K, Schukken YH (2006). Higher somatic cell counts resulted in higher malondialdehyde concentrations in raw cows' milk. International Dairy Journal, 16: 1088-1091.
- Weiss WP, Hogan JS, Smith KL (2004). Changes in vitamin C concentrations in plasma and milk from dairy cows after an intramammary infusion of Escherichia coli. Journal of Dairy Science, 87: 32-37.
- Yang FL, Li XS, He BX (2011a). Effects of vitamins and trace-elements supplementation on milk production in dairy cows: A review. African Journal of Biotechnology, 10: 2574-2578.
- Yang FL, Li XS, He BX, Yang XL, Li GH, Liu P, Huang QH, Pan XM, Li J (2011b). Malondialdehyde level and some enzymatic activities in subclinical mastitis milk. African Journal of Biotechnology, 10: 5534-5538.

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