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Invited Review Article

Role of micronutrients in managing bovine udder health in changing climate scenario of Indian subtropical regions

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Abstract

The impairment of mammary defense during summer and hot-humid season resulted oxidative stress to impair mammary gland defense by reducing activity of polymorphonuclear neutrophils (PMNs) and lymphocytes together with decreased level of certain cytokines. Supplementation of micronutrients such as vitamin E, Vitamin A/beta-carotene, selenium, copper and zinc have a beneficial effect to reduce the occurrence or severity of mastitis by improving udder immunity and antioxidant status. But, the bioavailability and efficacy of these compounds depend upon the source, dose and time of supplementation. Most of the studies recommended supplementing the micronutrients during peripartum and dry periods. A combination of vitamins and minerals is recommended than supplementing individual compounds. Traditional feeding practices and lack of awareness among Indian dairy farmers are the major constraints to achieve optimum responses of nutritional supplementation to promote udder health. Therefore, an appropriate nutritional strategy needs to be implemented together with other hygienic practices to reduce mastitis under changing climatic conditions of the Indian sub-tropical region.

Key words: Climate change, Mastitis, Micronutrient, Udder health

Highlights

- Poor immunity of mammary gland during stressful conditions resulted mastitis.
- Dietary supplementation of micronutrients during peri-partum and dry period are reported to improve udder health.
- Vitamin E, selenium, copper, chromium, zinc and vitamin A provide antioxidant and cellular defense of mammary gland.
- Nutritional management together with other hygienic practices need to be followed to control mastitis in India.

INTRODUCTION

The meteorological variables of India have changed a lot and are projected to change faster in the coming days (till the end of this century) (Krishnan *et al*., 2020). In the dairy sector, this climate change and geographical variability are a great matter of concern that may influence the prevalence of mastitis-causing pathogens (Guzmán-Luna *et al*., 2022) as environmental factors play a major role in the incidence of mastitis (Sato *et al*., 2008; Wani *et al*., 2022) along with the various predisposing factors like; housing (Østeras and Lund, 1988), nutrition (Barkema *et al*., 1999), milking procedures (Schukken *et al*., 1990) and procedure of drying the cows (Berry and Hillerton, 2002). The first report on mastitis in India was published in 1962 by Dhanda and Sethi (1962). Since then, the annual losses due to mastitis had a whopping from Rs. 52.9 crores to Rs. 7165.5 crores in 2009 (Bansal and Gupta, 2009). The environmental impact of mastitis occurrence in

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India has been documented by Jingar *et al.* (2014). Extreme climatic conditions may cause inflammatory sequelae in mammary alveolar cells due to high oxidative stress and make the udder more vulnerable to mastitis-causing pathogens (Bae *et al.*, 2017). The economic losses under mastitis include decreased production, poor milk quality, culling of animals, labour charges and therapeutic expenses (Hogeveen *et al.*, 2011). Asian countries possess the highest number of thermo-tolerant cattle, and a steady increase in total milk production was reported compared to other regions despite of adverse effect of climate change globally (Wankar *et al.*, 2021). But a study in India, the annual loss in milk production due to heat stress in sub-tropical North Indian Plains was reported to be equivalent to INR 11.93 to INR 12.44 billion (Choudhary and Sirohi, 2022). Mastitis also has public health significance as the antibiotics used to treat mastitis can lead to antibiotic resistance (White and Mcdermott, 2001) which is a serious global health concern. The use of antimicrobial therapy for bovine mastitis leads to emergence of antibiotic resistance pathogens (Hogan, 2017). Nutritional interventions to reduce mastitis by improving udder health have been investigated in several countries (Machado *et al.*, 2013; Ganda *et al.*, 2016). Traditional feeding methods used in India and a lack of farmer's understanding led to nutritional imbalance, jeopardizing the ultimate goal of obtaining a farm with sound health. Here, in this review, we have tried to summarize the studies on the nutritional interventions to improve udder health in Indian perspective, which could be a good option for the prevention of mastitis under changing climatic scenarios.

Impairment of mammary gland immunity during environmental stress and incidence of mastitis

The mammary gland is equipped with anatomical, cellular and humoral defense factors that facilitate pathogen recognition, leukocyte migration and phagocytic killing of intramammary pathogens and termination of inflammation (Mukherjee and Das, 2019). Neutrophils are the first line of defense against intramammary pathogens (Riollet *et al.*, 2002) that enter into the mammary gland from peripheral circulation after crossing the physical barrier (Mohapatra *et al.*, 2012) reflected in increased somatic cell count (SCC) of milk. Higher somatic cell counts of milk were reported during summer and hot-humid season (Khate and Yadav, 2010; Mukherjee *et al.*, 2013) of the Indian tropical climate with an increased incidence of clinical mastitis during the hot-humid season. The incidence of clinical mastitis was reported to be associated with critical thresholds of temperature humidity index (THI) (Bertocchi *et al.*, 2014; Zeinhom *et al.*, 2016). Impaired mammary defense during summer and hot-humid season may be due to reduced activity of polymorphonuclear neutrophils (PMNs) (Mukherjee *et al.*, 2015; Lecchi *et al.*, 2016) and lymphocytes (Mukherjee *et al.*, 2015) that are not potential enough to fight with pathogens in mammary tissue and make the animal susceptible to intra-mammary infection (IMI) (Rakib *et al.*, 2020). The reduced expression of interleukin-10 was reported in cows challenged with intra-mammary infusion of *S. uberis* under heat stress to suppress immunity (Thompson *et al.*, 2014). Heat stress during the dry period results mammary gland involution by apoptosis and autophagy and decreases the amount of mammary epithelial cells to reduce milk yield (Pragna *et al.*, 2017). Higher prolactin and decreased estrogen also affect the mammary gland involution during the dry period (Tao *et al.*, 2017). During summer, *Trueperella pyogenes* causes summer mastitis, and the infection is associated with a fly named *Hydrotaea irritans* (Rzewuska *et al.*, 2019). Heat stress is associated with the activation of the HPA (Hypothalamic-Pituitary-Adrenal) axis which increases the secretion of glucocorticoids. Glucocorticoids reduce the expression of cell adhesion molecules required for leukocyte migration (Roth *et al.*, 1982) along with the inhibition of cytokines secretion (interleukins and tumor necrosis factors) to reduce the activity of immune effector cells of the mammary gland such as neutrophils, macrophages and epithelial cells (Bagath *et al*., 2019). A negative correlation between plasma cortisol level and *in vitro* activity of milk leukocytes has been reported in crossbred cattle (Mukherjee *et al*., 2015). The oxidative damage of mammary epithelial cells during environmental stress induces cellular inflammation and makes the animals more prone to intra-mammary infections (Bae *et al*., 2017; Habeeb, 2018). In a recent study, Guo *et al*. (2021) reported decreased protein synthesis by mammary epithelial cells during heat stress induced oxidative damage in mammary gland. The nutrition supplementation to improve udder health should be targeted either to improve the mammary cellular and antioxidant defense system.

Micronutrient and udder health

Vitamins and micro minerals were reported to have immunomodulatory roles to augment general as well as udder immunity in cows (Paterson and MacPherson, 1990; O'Rourke, 2009). The micro-minerals such as copper, zinc, and selenium along with vitamin E and vitamin A were reported to influence udder immunity in cows (O'Rourke, 2009). Though the plasma micronutrient concentrations were reported to be unaltered (Pradhan *et al*., 2015), still pre-partum periods supplementation had significant positive impact on the post-partum udder health in Indian crossbred cows (Pradhan *et al*., 2017). *In vitro* studies have demonstrated that supplementation of Cu and Zn significantly improved the phagocytic activity of neutrophil and lymphocyte proliferation response in cows approaching parturition (De *et al*., 2015).

Copper: Copper (Cu) is an integral component of the antioxidant enzyme superoxide dismutase that protects the cells from reactive oxygen produced due to respiratory burst activity during phagocytosis (Yang *et al*., 2011). Supplementation of copper (6.5 ppm) from 60 days pre-partum was reported to decrease milk bacterial counts and clinical severity of *E. coli* mastitis in Holstein cows (Scaletti *et al*., 2003). The efficacy of copper supplementation depends upon the copper source. Copper proteinate (10 ppm) exerts higher response to reduce milk bacterial counts than copper sulphate (10 ppm) and copper in the basal diet (6.5 ppm) (Scaletti *et al*., 2002). Supplementation of copper together with vitamin E and zinc had a more pronounced effect on general immunity and udder health compared to copper alone in Sahiwal cows (Mutoni *et al*., 2012; Dang *et al*., 2013). Supplementation of copper (10 mg/kg dry matter) along with selenium (0.3-0.4 ppm in total ration), manganese (13 mg/kg dry matter) and zinc (43 mg/kg dry matter) from 45 days pre-partum to 45 days post-partum were reported to decrease milk somatic cell counts along with improvement of lymphocyte proliferation response and phagocytic activity of milk neutrophils in crossbred cows (Pradhan *et al*., 2017). In an *in vitro* study, De *et al*. (2015) demonstrated that the addition of 2.360 μM Cu in culture potentiated the phagocytic activity of neutrophils and *in vitro* lymphocyte proliferation response in crossbred cows supplemented with copper (20 ppm) around parturition suggesting additional supplementation of copper over recommended level to augment immunity in cows. Copper was reported to improve humoral and cell mediated immunity in heat stressed dairy cattle (Marins *et al*., 2021). Injectable copper supplementation (Warken *et al*., 2018) was reported to reduce somatic cell counts in milk by stimulating the immune system and antioxidant responses of dairy cows.

Zinc: The beneficial effects of zinc in the mammary gland are many folds. It helps to establish keratin in the teat canal and promotes anatomical defense against pathogens (Spain *et al*., 2005), regulates the integrity of mammary epithelial cells (Weng *et al*., 2018) and toughens the epithelial barrier to infection (Keshri *et al.*, 2019). Zinc helps to protect cells from oxidative damage as a component of the dismutase enzyme (SOD) along with the improvement of cell-mediated immunity and generalized host defense (Yang and Li, 2015; Libera *et al.*, 2021). Dietary zinc supplementation in the form of zinc methionine reduced milk somatic cell counts (Kellogg *et al.*, 2004). However, Spain *et al.* (1993) and Popovic (2004) reported that organic zinc was more beneficial over the inorganic form of zinc source. Several *in vitro* and *in vivo* studies have demonstrated the potential role of zinc alone or in combination with other nutrients to augment udder health in indigenous and crossbred cows (Mutoni *et al.*, 2012; Dang *et al.*, 2013; De *et al.*, 2015; Pradhan *et al.*, 2017). The studies of Mutoni *et al.* (2012) and Pradhan *et al.* (2017) demonstrated peri-partum zinc supplemented with copper, cobolt and vitamin E had more pronounced effects on the udder health in indigenous and crossbred. Supplementation of zinc was reported to improve heat stress-induced impairment of the integrity of the mammary epithelium by stimulating claudin 1 and zonula occludens 1 and 2 (Weng *et al.*, 2018). Supplementation of zinc significantly improved hormonal and lipid peroxidation status in heat stressed crossbred cows during peri-parturient period (Patel *et al.*, 2017). In related study, Sheikh *et al.* (2017) suggested that inorganic zinc supplementation reduced heat shock protein response and augments immunity in periparturient dairy cows habituating tropics.

Vitamin E and selenium: Vitamin E is one of the first lines of defense in body tissues and a chain-breaking antioxidant that prevents lipid peroxidation to protect cell membranes at the initial stage of free radical attack (Morissey, 1994). Selenium is an integral component of the antioxidant enzyme glutathione peroxidase that prevents lipid peroxidation (Surai and Fisinin, 2016). Supplementation of vitamin E and selenium results rapid influx of neutrophils into milk for the killing of intra-mammary pathogens by peroxidation of AA to its unstable metabolites that facilitate PMN function and inflammatory response after pathogen invasion in the tissues including the mammary gland (Craven and Williams, 1985; Smith *et al.*, 1997). Vitamin E deficiency was associated with the incidence of mastitis (Politis *et al.*, 2012). The duration of clinical mastitis was reduced in response to pre-partum supplementation of vitamin E (320 IU/day) and selenium (0.1 mg Se/kg body weight) (Smith *et al.*, 1984). In a related study, Smith *et al.* (1985) reported that supplementation of selenium (0.3 ppm) and vitamin E (1,000 IU/d total intake) from 60 days pre-partum resulted in reduced udder infection and lower milk somatic cells. The concentration of selenium in blood was negatively correlated with somatic cell counts (Weiss *et al.*, 1990) and selenium dependent glutathione peroxidase (GSH-Px) activity (Erskine *et al.*, 1987). Organic selenium conjugated with yeast was reported to have a better response than sodium selenite (Grasso *et al.*, 1990). In a meta-analysis, Moyo *et al.* (2005) reported that vitamin E supplementation resulted in 14% less intra-mammary infection risk and 0.70 times less milk somatic cell counts which consequently reduced the prevalence of clinical mastitis by 30%. Supplementation of vitamin E ((1000IU) and zinc (60ppm) from 60 days prepartum to 90 days postpartum, reduced somatic cell counts and incidence of subclinical mastitis in indigenous Sahiwal cows (Chandra *et al.*, 2015). Supplementation of selenium and vitamins E decreased incidence of bovine mastitis during the periparturient period by improving the antioxidant status (Khan *et al.*, 2022). The selenium inhibits activation of TLR2, NF-κB and MAPK signaling pathways in *Staphylococcus aureus*-induced inflammation in bovine mammary epithelial cells (Wang *et al.*, 2018).

Vitamin A and β-carotene: The association between the status of vitamin A and β-carotene and the occurrence of clinical mastitis has been documented in several studies (Chew *et al.*, 1982 and 1985; Dahlquist *et al.*, 1985), and it was concluded that lower vitamin A and β-carotene were associated with the incidence of clinical mastitis. Vitamin A promotes proliferation of mammary epithelial cells in the mammary epithelial (Cabezuelo *et al.*, 2020). Vitamin A and β-carotene can be supplemented during the dry period or lactation. Supplementation of vitamin A and β-carotene from 3 weeks before calving to 10 weeks of lactation decreased milk somatic cells (Chew and Johnston, 1985). In a similar study, Dahlquist and Chew (1985) supplemented vitamin A (53,000 IU/day along with β-carotene (300 mg /day) three weeks before drying-off and throughout the dry period decreased incidence of clinical mastitis. In contrary, Oldham *et al.* (1991) and Prom *et al.* (2022) reported that vitamin A and β-carotene didn't influence udder health in cows. Higher dose of vitamin A (220 IU/kg BW) resulted significant reduction in the somatic cell count of the milk (Jin *et al.*, 2014).

Chromium: Chromium (Cr) has been reported to influence the metabolism of glucose (Stahlhut *et al.*, 2006), and increase milk production (McNamara and Valdez, 2005) and immunity in cows, particularly under stress conditions (Spears, 2000). Chromium regulates dairy cow metabolism and improves lactation performance by enhancing antioxidant activity (Wu *et al.*, 2021). Chromium supplementation can be very effective during early lactation when the cows are under severe metabolic stress and have poor glucose reserves (Garcia *et al.*, 2017). The organic form of chromium such as chromium-yeast and chromium-propionate has a better response in terms of milk yield in dairy cows (Spears *et al.*, 2012). Supplementation of the chelated chromium in the form of chromium methionine significantly improved total antioxidant enzyme activity such as plasma glutathione peroxidase and superoxide dismutase in a linear manner. But, on contrary, Zhang *et al.* (2014) reported that Cr supplementation didn't improve the antioxidant capacity of mid-lactating cows during heat stress.

Conclusion

Extreme climatic conditions impose cellular inflammation in mammary alveolar cells due to high oxidative stress and make the udder more vulnerable to mastitis-causing pathogens. Supplementation of vitamin E, vitamin A/beta-carotene, selenium, copper and zinc have a beneficial effect to reduce the occurrence or severity of mastitis by improving udder immunity and antioxidant status. The efficacy of micronutrient supplementation depends upon the form, dose and period of supplementation. Most of the studies depicted that supplementation of micronutrient during heat stress improves general and udder innate immunity by modulating cytokines and other signaling molecules. Further, antioxidants like selenium and vitamin E improve antioxidant status and protect mammary epithelial cells from oxidative injury. As per the recommendation of the National Academy of Agricultural Sciences that "nutrition plays an important role in the control of mastitis" a holistic approach to mastitis control should be implemented by incorporating nutritional management by supplementing micronutrients as per the recommended together with other hygienic practices during the stressful climatic conditions for sustainable milk production.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: AM, KD, JM: Conceived the idea and share their work related to the topic; AM: Drafted the manuscript; KD, JM: Corrected the manuscript.

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