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Effect of vitamin E, Zn and Cu supplementation on Immune status, reproductive health and production in dairy animals: A review

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Abstract
Vitamin E and trace minerals are essential for immune status, reproductive health, and productivity in dairy animals. Vitamin E is an essential, fat-soluble antioxidant that is necessary for maintaining immunity and reproductive health. Zinc and Copper essential trace minerals. In order to maintain and develop immunocompetence, zinc is essential, Copper is also importantto maintain immunocompetence. Copper is a co-factor in hundreds of enzymatic activities as lysyl oxidase, cytochrome c oxidase that have a role in collagen synthesis, erythropoiesis, energy production, collagen synthesis, hormone production, and oxidative damage protection. Zinc and Copper are parts of cytosolic superoxide dismutase, an enzyme that shields cells from reactive oxygen species (ROS) damage. Along with vitamin E, zinc and selenium, copper is important for maintaining a healthy udder. Vitamin E and Zinc are also known to be associated with milk production and composition.A substantial drop in vitamin E levels occurs during the periparturient phase, which affects the neutrophil functions and immunity of the cow. Micronutrients and vitamin E supplements are considered to be essential to maximizing animal production and health. To produce a significant amount of colostrum and milk, periparturient cows show great mammary development and profuse synthesis and release of carbohydrates, fats, and proteins. Cows require a higher supply of vitamins A and E before calving because colostrum is loaded with these nutrients. During early lactation period cows require the highest Cu and Zn supplementation. Due to an increase in colostrogenesis, zinc is diverted from the plasma pool and directed toward the mammary gland after parturition. Acute phase response due to a drop in serum Zn concentration at the time of parturition results in uterine inflammation. When sufficient Cu is supplied, this is especially significant for phagocytic cells and could be the main mechanism of action for decreased infectious diseases.

Keywords: Immunity, reproductive disorders, milk yield, vitamin e, zinc and copper

Introduction
Periparturient period is a very crucial phase for the dairy animals and is distinguished by a weakened immune system and low antioxidant levels. Animal production is significantly influenced by endocrine and cellular changes made during the dry season and the early stages of lactation. Elevated energy demand and use of oxygen are linked to physiological changes during the transition period associated with fast secretory parenchyma differentiation, intense development of mammary glands, and profuse milk production (Gitto *et al*., 2002) [21]. Brezezinska *et al*. (1994) [6] found that oxidative stress can occur in cows during the transition phase, which could potentially cause periparturient diseases. For the health status of cows, the period between 3 weeks before and 3 weeks after calving is crucial (Drackley, 1999) [16]. Toyokuni (1999) [76] observed that peroxidative degradation of lipids along with other macromolecules caused by oxidative stress results in changes in membrane and other component of the cell. A substance that delays, prevent, or eliminate oxidative damage of the target molecule is considered an antioxidant. Antioxidant includes vitamin E and trace elements like copper and zinc. Vitamin E and selenium plays the role of antioxidants and help to scavenge ROS (Reactive Oxygen Species) in the body. In dairy cattle, vitamin E and selenium both have properties of antioxidant and immune responses in reproduction

(Xiao *et al.,* 2021) [84]. The periparturient phase shows a significant decrease in plasma concentrations of vitamin A, α-tocopherol, and beta-carotene (Weiss *et al*., 1997; Arechiga *et al*., 1998) [80, 3]. Particularly in the transition period of cows, α-tocopherol prevents the formation of reactive oxygen species, which shields cells from oxidative damage(Xiao *et al.,* 2021) [84]. Zinc is an essential trace element that is necessary for the development and function of the immune system (Wessels *et al.,* 2021) [82]. The phagocytic oxidative burst enzymes are also associated with zinc (Chandra and Au, 1980) [12], in cellular development and activity of T and B lymphocyte. A number of metalloenzymes, including glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Zn, Cu & Mn), are also essential in preventing oxidative damage to the internal components of cells. Trace minerals such as Zn, Mn, Cu and Co have various roles in protein synthesis, vitamin metabolism, connective tissue formation and immune responses (Miller *et al*., 1988) [46]. For dairy cattle to be fertile and retain the integrity of their claws, copper is important (Miller *et al*., 1988; NRC, 2001) [46, 51]. Reduced neutrophil count, impaired neutrophil function, decreased macrophage antibacterial activity, reduced proliferation splenocyte, impaired B cell antibody formation, and impaired activity of helper T cells and cytotoxic T lymphocytes the ways that a copper deficiency affects the development and functioning of the immune system (Cheng *et al*., 2022) [14].

Effect of vitamin and micronutrients during different physiological stages

Vitamin E status during peripartum period

Goff and Stabel (1990) [22] found that during the very last two weeks of gestation to calving, in cattle, the level of α-tocopherol in plasma dropped from 1.8 µg per millilitre to 0.7 µg per millilitre. Even when the dietary amount of vitamin E is fed to cows constantly, the plasma α-tocopherol usually drops 7 to 10 days before calving and stays lower for 2 to 3 weeks after calving (Hogan *et al*., 1993) [28]. It has been observed that the level of α-tocopherol drops from 2.1 - 1.3 • g/ml at parturition and remains low for two to three weeks after parturition (Hogan *et al*., 1993) [28]. Reduced plasma levels of α-tocopherol in the periparturient period are correlated with variations in vitamin E intake and a reduction in the vitamin E transport ability in plasma. During the periparturient period, the immunity status and functions of neutrophils are decreased due to a significant drop in α-tocopherol (Hogan *et al*., 1993) [28], For this reason, the lactation period's first month has a higher incidence of clinical mastitis. There is a negative correlation between the intramammary infection rate and vitamin E levels in plasma in dairy cattle. When cows had plasma levels of α-tocopherol above 3 g/ml during parturition, clinical mastitis was not observed in them (Weiss *et al*., 1997) [80].

Low levels of α-tocopherol in the plasma during parturition have been identified as a major risk factor for mastitis and intramammary infections (IMI) in the first 7 days of lactation (Goff and Stabel, 1990; Kaur *et al.,* 2002) [22, 33]. A significant decrease in the level of plasma α-tocopherol was found in the very last 30 days of the gestation period to the date of parturition (Chatterjee, 2002) [13]. Control cattle had a 47.22% fall in α-tocopherol level from 20 days before calving to the date of calving, whereas treatment cattle supplemented by 1000 IU of α-tocopherol in the winters observed a 20.19% decrease (Chandra and Aggarwal, 2010) [11]. Maurya (2011) [44] found that 60 days before parturition and on the date of parturition, the drop in vitamin E level in the plasma of the cattle in the treatment group was 25.25% and in the control groups was 36.30%.When comparing vitamin E (1000 IU/d/cattle) and Zn (60 ppm/d/cattle) supplemented treatment cattle to control cattle (2.60±0.05 vs. 2.38±0.06 µg/ml), the overall mean (±SEM) of vitamin E level in plasma was found to be considerably ($p<0.01$) higher.

Zinc status during peripartum period

Normal zinc levels in plasma in ruminants range from 0.8 µg/ml to 1.2 µg/ml. Zinc levels vary in plasma with stress, age, and diseases. Milk and colostrum contain 4 and 14 parts per million of zinc, respectively (NRC, 2001) [51]. Zinc is diverted from the plasma pool and directed into the mammary gland during parturition as a result of enhanced colostrogenesis. A decrease in zinc concentration in the plasma during parturition is related to an acute response due to inflammation in the uterus. A protein linked to the distribution of zinc, metallothionein, is synthesized in response to stress during calving. Zn is thus transferred from the blood pool to other tissues, primarily the liver (Meglia*et al.,* 2001) [45]. About 12 mg of zinc per day are retained by the cow's uterus and foetus during the very last 190 days of gestation. (NRC, 2001) [51]. Dairy buffaloes had a plasma zinc level of 0.90 µg/ml at 15 days before parturition, which dropped to 0.64 µg/ml at the time of parturition and then returned to normal levels 15 days after parturition (Panda, 2003) [53]. In cross-bred cows, zinc level of the plasma 1.51 µg/ml at five days before parturition decreased to 1.09 µg/ml on that day of calving (Chandra and Aggarwal, 2010) [11]. Maurya (2011) [44] found Zn level dropped by 30.60% and 16.13%, in control and treatment group (Zn @ 60 ppm/d) respectively, between 60 days before parturition and the day of parturition. In comparison to the treatment group, this drop was more pronounced ($p<0.01$) in the control group from seven days before parturition to the day of parturition. This level stays low in the early stages of lactation after calving.

Copper status during peripartum period

Hepatic Cu concentrations of the dairy herd were found much lower in dry cattle than in cattle in late lactation (Waterman *et al.,* 1991) [79]. Copper status of cows under actual feeding conditions can undergo a drastic change throughout the dry period (Waterman *et al.,* 1991) [79]. Sathler *et al.* (2017) [65] recommended that in cattle, daily dietary Cu intake vary from 8 - 20 mg per kg of dry matter and depend on different factors. Brown *et al.* (1990) [8] reported that in primiparous Holstein cows without Cu supplementation, liver Cu content was lower 30 days prepartum compared to 30 and 90 days postpartum. Kromm and Cardoso (2020) [37] reported that cattle have less copper tolerance than previously thought; copper already accumulates in bovine liver at the recommended dietary level, as indicated by recent research. At 190 days of gestation, the conceptus of Holstein cows (average BW = 715 kg) contained about 20 mg Cu (House and Bell, 1993) [29]. From 190 days of pregnancy to calving, the conceptus accumulated 1.6 mg per day or 2.3 µg Cu per kg of maternal Body weight/day (House and Bell, 1993) [29]. For an average lactating Holstein cattle (35 kg milk production, 650 kg Body weight, 150 days gestation), the total daily requirement for absorbed Copper is 11.0 mg/day, up from 11.4 mg in the seventh revised edition (NRC, 2001) [51]. The daily requirement for a 700-kg nonlactating cattle at 260 days of pregnancy are 11.7 mg/d (about 20 mg dietary Copper per kg assuming a daily intake of 13.5 kg), compared to 7 mg per day calculated using the seventh revised edition (NRC 2001) [51]. Cattle require the highest Cu and Zn (mg/kg DM) during early lactation (NRC, 2001) [51].

Importance of vitamin E, Zinc and Cu in immunity

During calving, dairy cattle are prone to infectious diseases such as mastitis than at other times. Usually, immunity decreases during peripartum period in dairy cattle. In cattle, vitamin E enhances the killing potential of the circulating neutrophils and improves the activity of macrophages (Xiao *et al.*, 2021) [84]. It has been observed that vitamin E increases the ability of bovine blood neutrophils to destroy *Staphylococcus aureus* and *E. coli* intracellularly (Hogan *et al.*, 1990) [26]. Supplementing with vitamin E boosted the production of IgM and interleukin by bovine peripheral mononuclear cells *in vitro* (Judith *et al.*, 1992) [32]. Because prostaglandins reduce a cell's sensitivity to mitogen activation and subsequent cellular differentiation, they limit the activity of macrophages and lymphocytes. This indicates that prostaglandins could influence immune function by suppressing the cytokine synthesis involved in immune response control while enhancing differentiation and proliferation of cells. Peripheral blood lymphocytes and macrophages of bovine mammary gland function are increased by vitamin E (Ndiweni and Finch, 1995) [48]. Vitamin E (3000 IU/cow/day) oral supplementation prevented the inhibition of macrophage and blood neutrophil activity in the early postpartum period. Vitamin E is necessary for humoral immunity as well as cellular immunity. This might be induced by its impact on the stability of cell membranes and its regulatory function in the production of different inflammatory mediators (Smith *et al.*, 1997) [73]. According to Kuhn and Sordillo (2021) [38], in bovine, 10 μM α-tocopherol significantly prevents the loss of the mammary endothelial cell barrier's integrity, that is induced by pro-oxidants. Oxidant challenge-induced damage and loss of function in bovine mammary endothelial cells are successfully prevented by the antioxidant activities of α-tocopherol. Vitamin E may have a protective impact on animal health throughout the prepartum and postpartum period by reducing the immunosuppressive glucocorticoids. Important host immunological responses such as lymphocyte proliferation, antibody formation, and cytokine generation are known to be suppressed in dairy cattle during the post-partum period (Sordillo, 2005) [75]. Recent studies have concentrated on the use of nutrition to boost cows' immune systems against infections. When provided in adequate amounts, minerals like zinc, copper, and selenium improve cows' immune systems by making them more resistant to diseases and reducing their infection severity. Zinc is an essential component of the immune system. Research indicates that zinc plays an essential part in the maintenance and development of immunocompetence. Zinc is involved in almost every element of immunity. Zinc has also been associated to the phagocytic oxidative burst-related enzymes (Chandra and Au, 1980) [12], in the development and functioning of T lymphocyte and B lymphocyte. Zinc impacts the immune system in a number of ways, including lymphoid cell apoptosis, mitosis, and gene expression. The major zinc-dependent enzyme is DNA polymerase, which is important in DNA replication (Shankar and Prasad, 1998) [68]. Prasad (1998) [59] found that zinc contributes to immune maintenance in a variety of ways. The enzyme deoxy-thimidinekinase, which is involved in DNA synthesis, requires zinc as a necessary component. Impaired cell cycle and DNA synthesis are signs of zinc deficiency. Zinc is part of thymulin, a hormone that depends on the thymus, and Zn is essential for the expression of its biological activity. Since T-cells have thymulin receptors on their surface, zinc plays a role in maintaining cell-mediated immunity. A zinc deficiency is related to a decrease in the number of blood lymphocytes and a reduction in macrophage phagocytosis and killing (Fraker and King, 1999) [18] and caused the thymus and spleen atrophy. In the animals that are deficient in zinc, T-lymphocyte response to mitogen is suppressed. Broadway *et al.* (2021) [7] reported that monocyte counts were affected by zinc supplementation in mixed way with a propensity to decrease in quantity. Mucosal surfaces are lined by epithelial barriers, preventing invasion by pathogens, particularly in the digestive tract. Supplementing with zinc oxide nanoparticles (0.8 micrograms/ml) improvedthe viability of intestinal cells of bovine (Li *et al.*, 2023) [39]. Zinc supplementation in livestock either has no effect on the overall diversity of the gut microbiome or decreases it a little (Sanjuan *et al.*, 2022) [64]. Reduced overall microbiota activity may benefit the host by limiting the colonization and proliferation of pathogenic bacteria (like coliforms). Zn supplementation tended to alter the antioxidant capacity or concentration of oxidative stress markers, but had no effect on the levels of immunoglobulins (Oconitrillo*et al.*, 2024) [52]. Smerchek *et al.* (2023) [72] found that Zinc supplementation of 1,000 mg per day had no effect on the expression of the activation surface markers (CD44, CD25, CD16, CD2, and CD45RO) in natural killer (NK) cells in steers that are weaned. When CD8+ NK cells were evaluated using the same surface markers, CD16 expression increased. This indicated that supplementation with zinc can alter the phenotypic activation markers.

Non-specific cellular immune effectors including macrophage, monocyte and neutrophil play significant role in phagocytosis and killing of microbial pathogen. Peripheral blood granulocyte collected from sheep given a Cu deficient diet exhibited equal phagocytic ability but were less effective at killing the consumed *Candida albicans* than Cu appropriate controls (Jones and Suttle, 1981) [30]. In Cu-rich calves, neutrophil killing capacity rises by 100 percent but stays low in Cu-deficient animals (Jones and Suttle, 1981) [30]. Mice that were infected with *Pasteurella hemolytica* showed reduced red blood cell and super oxide dismutase (SOD) activities due to a copper deficiency (Jones and Suttle, 1983) [31].

Copper, along with vitamin E and A, as well as zinc and selenium, is important for maintaining a healthy udder. Scaletti *et al*. (2003) [67] discovered that administering copper reduced the mastitis severity produced by *E. coli*, but had no effect on the duration of disease. Furthermore, an adequate copper content in the diet of animals is required to optimise the functioning of complete immune system, as Cu lowers the danger for oxidative stress and metabolic stress in dairy cattle (Cortinhas *et al.,* 2010) [15]. Kromm and Cardoso (2020) [37] reported that cattle have less copper tolerance than previously thought; copper already accumulates in bovine liver at the recommended dietary level, as indicated by recent research.

Lymphocyte proliferation

The immediate pre & post-partum periods saw less mitogen induced multiplication in the peripheral lymphocytes taken from sows that fed a diet low in vitamin E (Waryastuti *et al.,* 1993) [78]. Pollock *et al.* (1994) [58] observed, 15 calves that were 7 months old were split up into 4 groups. First group calves were fed a basal feed with low levels of vitamin E and selenium (4.9 nM/g β-tocopherol acetate and 0.44 nM/g Selenium). The group 2nd, 3rd and 4th were fed a basal diet enriched with0.46μm per gramα-tocopherol acetate, 2.53nM per gram Selenium (as sodium selenite), and Selenium (2.53 nM per gram) + α-tocopherol acetate (0.46 μM per gram), respectively. The proliferation of lymphocytes was observed during weeks 33rd, 35th, and 38th of treatment. At every step of the trial, the calves fed a diet enriched with vitamin E had a greater response to mitogen, regardless of selenium. Only Se supplemented diet had no impact on the proliferation of lymphocytes. Lymphocyte proliferation was shown to be considerably lower in mastitic cows compared to normal cows, and it was positively related to plasma Vit E level (Sato *et al.,* 1995) [66]. Male rats with a deficit in Cu had less pan-T cells and CD4+ cells in their spleen (Bala *et al.,* 1991) [4]. However, the extent to which Cu deficiency inhibited MNCs sensitivity to T-cell mitogens far outweighed the reduction in the relative number of mature T cells, implying one or more abnormalities in Cu deficient cell function. Because expression of IL-2 receptor, transferrin receptor, and class II MHC molecule increased similarly, in rats with copper deficiency and control rat's concanavalin-A stimulated splenic MNCs, it showed up that trace-metal deficiency had no effect on early T-cell activation events.

Total immunoglobulin

Maurya *et al.* (2013) [43] reported that the control cow's total immunoglobulin level in plasma was 45.09±1.27 mg/ml 60 days before parturition, and it dropped to 27.24±0.57 mg/ml on the day of parturition while 60 days before parturition, the total immunoglobulin level in plasma in the group supplemented with Zn and vitamin E was 46.91±1.02 mg/ml, and on the day of parturition, it dropped to 31.96±0.46 mg/ml. In the analysis, the drop in total immunoglobulin level in plasma before parturition to the day of parturition in the control group was 39.59%, and in the treatment group it was 31.87%. When comparing the treatment group to the control group (37.66±0.90 vs. 34.65±1.14 mg/ml)., the overall mean ± SEM of total immunoglobulin level in plasma was found to be considerably (p<0.01) higher. The total immunoglobulin concentration in plasma began to rise after 7 days of calving and continued to do so for 60 days of lactation. The immunoglobulin level changes during transition period due to the change in concentration of immunoglobins in the mammary glands. Chandra and Aggarwal (2009) [10] reported, 20 days before parturition to the day of parturition in the summer, there was a 25.61% and 14.53% drop in the total immunoglobulin levels in plasma in the control group and treatment group cattle, respectively. Supplementing with vitamin E boosted the production of interleukin and Ig M by bovine peripheral mononuclear cells *in vitro* (Judith *et al.,* 1992) [32]. Roshanzamir *et al.* (2020) [62] reported that, compared to cows that were not supplemented, cows that were supplemented with organic Copper, Manganese, and Zinc immediately after calving had elevated level of immunoglobulin A & M. Plasma IgA concentrations increased after Zinc supplementation (32 mg/kg dry matter) in the form of nano-particles (Pandey *et al.,* 2023) [54]. The optimal immunoglobulin production perhaps sustained only by Zinc methionine supplementation at 76 mg/kg of dry matter (Cytotoxic T lymphocyte) (Oconitrillo *et al.,* 2024) [52]. Copper's redox characteristics make it a catalytic cofactor for a variety of enzymes, permitting protein interaction via complexation with lower molecular substances and histidine, cysteine, and methionine (Robinson and Winge, 2010) [61].

Importance of vitamin E, Zinc and copper in reproduction

The periparturient period is particularly crucial for dairy cows' health and performance. Dairy cow's reproductive health has improved when vitamin E and selenium supplements were given before parturition (Erskine *et al.,* 1997) [17]. Supplementing selenium during the transition period and milking period enhances the reproductive performance, it may improve the first-service conception rate (Ullah *et al.,* 2020) [77]. Selenium supplementation after parturition stimulates the ovulatory follicles, the growth of the uterus, and stabilizes hormonal balance, so days in the open period reduce (Ahn *et al.,* 2021) [2]. According to Lizarraga *et al.* (2019) [41], bovine oocytes that developed in the presence of selenium showed a decrease in apoptotic cells and an increase in embryo hatching rates compared to non-supplemented bovine oocytes. Molecules like vitamin E and β-carotene are examples of antioxidant systems; they function as membrane antioxidants to protect phospholipids from oxidative damage & lipid peroxidation. An increase in free radical production could deplete antioxidant defence systems and disrupt cellular functions (Arechiga *et al.,* 1998) [3]. Due to the sensitivity of spermatozoa, pre-implanting embryos, and ovarian steroidogenic tissue to free radical damage, the production of free radicals may be a contributing factor to infertility. Arechiga *et al.* (1998) [3] reported that A single intra-muscular shot of 680 IU of vitamin E given to lactating cattle 30 days after calving significantly decreased the number of services required for each conception as well as the time between calving and conception. It was also observed that the pregnancy rate in cows had improved at the second service. The total antioxidant capacity of cows supplemented with an organic methionine-chelated source of copper, manganese, and zinc increased immediately postpartum than in cows supplemented with inorganic or glycine-chelated or not supplemented with copper, manganese, and zinc (Roshanzamir *et al.,*2020) [62]. Zinc is an important component for the production of LH which is required for normal growth of ovaries. Campbell *et al*. (1999) [9] reported that, supplemented crossbred cattle with 359 mg Zinc as zinc methionine, 125 mg Copper Lys, and 26 mg Cobalt glucoheptonate. It was found that the cattle supplemented with complex trace minerals had fewer days to very first oestrus, first luteal activity, and first CL than the control group. Panda (2003) [53] found that supplementing 1500 IU of vitamin E per day to buffaloes from 60 days before to 30 days after parturition, significantly lowered the number of days needed for postpartum oestrus to occur and the number of days open. Occurrence of reproductive complications like retained placenta and metritis were also not observed in supplemented buffalo. Zinc plays an important role in reproductive health, being required for oocyte maturation, fertilization, and the development of the embryo and placenta in female (Garner *et al.,* 2021) [20]. Phiri *et al.* (2007) [56] observed throughout a two-year trial, giving 400 mg of Zn supplements to cross-bred dairy cattle improved their reproductive efficiency by shortening the time from parturition to first oestrous, parturition to conception, and calving-interval days. Wooldridge *et al.* (2019) [83] reported that inner cell mass & total cell numbers increased with supplementation of Zn during the first eight days after fertilization that is an indicator of better embryo quality. Griffiths *et al*. (2007) [23] supplementing cattle from 35 days before to 230 days after calving with 360 mg zinc, 200 mg manganese, 125 mg copper, and 12 mg cobalt from complex sources and observed a betterment in the conception rate in comparison to the control group. In the same way, there were a reduced number of non-pregnant cattle, and the number of cows in the treatment group that did not conceive in 48 days of mating was also reduced. Maurya *et al.* (2013) [43] reported that supplementation of vitamin E and zinc reduces metritis, retained placenta and pyometra cases in Karan Fries cows.

Copper supplementation affects the reproductive performance of cattle. Deficits in zinc, copper, and iodine have been associated to cow's sensitivity to disease and reproductive issues (Balamurugan *et al*., 2017) [5]. Galbat (2020) [19] reported that hypocupremic cows had a mixed mineral deficiency cobalt, selenium and iodine, all animals with mineral deficiencies suffered unresponsive ovaries, silent heat, and postpartum reproductive difficulties. Nazari *et al.* (2019) [47] reported that normal luteal activity in dairy cattle showed a higher concentration of Cu than cows with anovulation, delayed ovulation, shortened luteal phase, and a long luteal phase. Additionally, it was reported that, in compression to cow that was not pregnant, pregnant cattle had higher circulatory concentrations of copper. The average Zn and Cu contents in the fluid of cystic and normal follicles in cattle and heifers were 3 mg/litre Zn and 2.1 rag/litre Cu, according to Petkov *et al*. (1969) [55]. There were no differences in follicular fluid content of each element between cows and heifers and there were no significant associations between liver and follicular fluid Cu and Zn. Zinc and copper concentrations in anestrus cow follicular cyst fluid drop as fluid volume increases, demonstrating that content per follicle remains constant and implying that both of these nutrients are intracellular in the follicle. The uterine fluid of cattle with cystic endometrial hyperplasia contains 13 mg/litre Zn and 12 mg/litre Cu. There was an inverse relation between the Zn and Cu concentrations and volume of fluid (Petkov *et al.,* 1969) [55]. Copper deficiency might affect parturition. Regular breeders had much greater levels of copper and zinc in their sera than repeat breeders (Manickam *et al.,* 1977) [42]. Increased dietary iron promotes copper deficiency, which impairs reproduction, whereas high selenium levels can cause abortions and stillbirths due to toxicity (Abramowicz *et al.,* 2019) [1].

Importance of vitamin E, Zn and Cu in milk production

Rajala *et al*. (1999) [60] observed that mastitis has a persistent impact on milk production. Despite curing their mastitis, the cows did not produce as much milk as they had before for the remaining lactation period. Sinek *et al.* (2000) [69] observed an increase in milk production of 13.8% in animals supplemented with vitamin E at a dose of 9000 IU/kg in the concentrate mixture. It was believed that the shielding of mammary tissues from free radicals was the cause of the increase in milk production. Kaur *et al.* (2001) [34] reported a 20% rise in milk yield in cows supplemented with 1000 IU per cow per day during the dry period. It was proposed that the decreased cases of clinical and subclinical mastitis in supplemented cattle were the cause of the rise in milk production. Chatterjee (2002) [13] suggested that in order to lower the risk of mastitis during parturition, vitamin E supplemented at a dose of 1000 IU per day should begin at least thirty days before to parturition, hence a rise in milk production. Singh *et al.* (2020) [70] reported that α-Tocopherol (1 g /cow/day) supplementation for 30 days before and up to 60 days after parturition remarkably raised the milk yield and decreased the cases of mastitis in Jersey cattle. Oconitrillo *et al.* (2024) [52] reported that in the first half of the experiment, increasing the dietary zinc methionine supplementation 76.0 to 97.0 ± 2.5 mg per kg of dry matter over 70 days in cattle resulted in lower milk production, but during the second half of the experiment, this was associated with raised milk production. Salama *et al*. (2003) [63] reported that the milk produced by dairy goats supplemented with Zn-met was marginally (2.5%) higher. The supplementary group's milk fat level was lower than the control group. The dilution effect was most likely the reason of this tendency, as supplemented goats produced marginally greater quantity of milk than control goats. According to dietary treatment, a differential rise in somatic cell count was caused by hand milking. In the Zn-Met-supplemented group, SCC was lower than in control goats. This indicated that dairy goats supplemented with zinc methionine have less damage to their udders during hand milking. Zn supplementation reduces somatic cell count in dairy goats and also udder infection. Kellogg *et al.* (2003) [35] observed that The SCC of milk decreased by 12.1% when complexed trace mineral supplements (360 mg Zn Met, 200 mg Mn Met, 125 mg Cu Lys, and 25 mg Co glucoheptonate) were given to periparturient cattle from 21 days before to 200 days after calving. When cattle were supplemented with 1000 IU per day of α-tocopherol acetate, their milk output elevated by 17.14% as compared to a control group (Chandra and Aggarwal, 2010) [11]. Over the duration of 60 days of lactation, the group supplemented with zinc and vitamin E had a 20.90% elevation in milk production compared to the control group (Maurya, 2011) [44]. Copper sulphate supplementation in dairy cattle has been found to lower the incidence and severity of IMI in response to experimentally produced mastitis using endotoxin (Harmon *et al.,* 1994) [24] or live *Escherichia coli* (Scaletti *et al.,* 2003) [67] in primiparous Holstein heifers.

Importance of vitamin E and Zinc in milk composition

Colostrum contains a significant amount of vitamin E. The calf has to consume colostrum to get vitamin E after calving since α-tocopherol does not pass the placenta in significant quantities. Approximately 6–7 times more α-tocopherol is present in colostrum compared to milk (Hidiroglou, 1989) [25]. The supplementation of 0.2 ppm Se and 70 IU vitamin E per kg DM to the diet of dry cows resulted in a 40% rise in α-tocopherol content in colostrum i.e. from 5.3 - 7.5 µg per g of milk (Weiss *et al.*, 1990b) [81]. Njeru *et al*. (1994) [50] found that linear rise secretion of vitamin E in the colostrum of cattle given different amounts of vitamin E supplements. Nicola *et al*. (1996) [49] observed that before 4-5 weeks of calving, cows were fed 25 IU dl-α-tocopheryl acetate per 100 kg BW and 5 mg of selenium as sodium selenite, which resulted in a 22 percent rise in colostrum yield from 7.2 to 8.8 Litre per day. Similarly, Weiss *et al*. (1997) [80] supplemented 66 Jersey & Holstein cattle at 100 IU/day, 1000 IU/day, and 4,000 IU/day throughout the dry period and observed that in the respective groups, the α-tocopherol level in colostrum was 5.62µg/ml, 9.98 µg/ml, and 10.49 µg/ml of milk and 103.1µg/g, 136.9µg/g, and 222.7 µg/g of fat; however, there was no rise in colostrum yield throughout this trial. During lactation, cows with elevated leptin levels during pregnancy had greater percentages of fat in their milk. In cows with greater leptin levels during pregnancy, the first few months of lactation were associated with greater percentages of lactose in the milk, and higher leptin levels all the time were associated with higher percentages of protein in the milk. No notable variations were seen for the yields of milk constituent (lactose, fat and protein in kg), most likely due to the fact that the percentage of these constituents are often reduced at larger milk yields (Liefers *et al.*, 2003) [40]. Other than a drop in milk fat percentage and an increase in total milk fat (kg) in the group supplemented with Zn and vitamin E, there was no discernible change in the composition of the milk (Maurya, 2011) [44].

Somatic Cell Count

Essential micronutrients such as vitamin E, selenium, zinc, and copper have been demonstrated to improve udder health and reduce SCC in milk (Smith *et al.*, 1997) [73]. Oconitrillo *et al.* (2024) [52] reported that, in response to Zn supplementation, the WBC counts stayed unchanged, but the somatic cell counts in milk improved. For older cattle, the milk production dropped by 2.04 kg per day for every unit rise in $\log_{10}$ SCC. The percentages of milk yield lost were 8%, 15%, and 27% for SCC of 1 x 106/ml, 2 x 106/ml, and 4 x 106/ml of milk (Jones and Suttle, 1981) [30]. In 66% of the cases, SSC in heifers supplemented by 1000 mg of vitamin E daily throughout the dry period decreased to less than 2 lakhs (Smith *et al.*, 1985) [74]. Smith *et al*. (1997) [73] reported that SCC up to 2 lakhs may not be a sign of inflammation, for healthy quarters SCC should be less than 100,000 cells per ml of milk. SCC more than 2 lakhs indicates sub-clinical mastitis in that quarter and is suggestive of inflammation, infection, and decreased manufacturing properties. Hogan *et al*. (1994) [27] reported a significant decrease in SCC in the mastitic cows given 3000 IU of vitamin E with antibiotics; however, no change in the rates of bacteriological cure was seen. Animal vitamin E levels in plasma and sub-clinical mastitis have been found to significantly correlate negatively (Ndiweni and Finch, 1995) [48]. Politis *et al*. (1995) [57] reported that milk SCC was significantly reduced by supplementation of vitamin E at a rate of 3000 mg per cow per day from 4 weeks before to 8 weeks after parturition. SSC and the occurrence of clinical mastitis can be decreased by vitamin E and selenium dietary supplementation in high levels than what is necessary for adequate nourishment. Koeldeweij *et al.* (1999) [36] reported that for cows in their first lactation, each cow's milk loss was 1.29 kg per day for every unit rise in $\log_{10}$ SCC.

International recognition exists for somatic cell count as a metric for evaluating the health of the udder and the quality of the milk. Leucocytes from blood and udder epithelial cells that have shed due to natural wear and tear are components of SCC. About 20% of leucocytes and 80% of epithelial cells are found in normal milk, whereas over 90% of all cells are leucocytes in milk from the unhealthy quarter or mastitic udder. A rise in SCC indicates inflammation in the udder and lowers milk quality. Milk that has a SCC which may not always related to the clinical signs of mastitis carries a price penalty. Although milk pricing in India is not currently dependent on the SCC, this is expected to change in the near future as a result of globalization and the entry of international corporations into the dairy industry. Therefore, producing milk of a high enough quality to meet international standards is one of the challenges of the new millennium. In the buffalo milk average SCC value is 0.97 to 1.15 x 105 cells per ml, and there is no significant difference of parity and lactation stage (Singh *et al.*, 1994) [71]. It is conceivable to interpret the SCC as a physiologically normal secretion of the cow udder up to 100,000 cells per ml. SCC increase exceeding 100,000/ml is expected to result in a decrease in milk processing qualities, also large loss in production of milk. At 15 days of lactation, there was a substantial drop in SCC in the milk in cattle supplemented by 1000 IU vitamin E per day throughout the dry period (Kaur *et al.*, 2002) [33]. Maurya (2011) [44] observed that the SCC of milk in the vitamin E and Zn-supplemented group was considerably lower than the control group.

Conclusion

The transition period is critical for health and subsequent performance due to the depleted immune status during this period in dairy animals and high-producing animals at that time may suffer from various metabolic and reproductive disorders. Studies shows that supplementation of vitamin E, zinc and copper during transition period improves energy status of the animal and increases milk production significantly without any significant change in milk composition. Supplementation of Zn and vitamin E increases total plasma immunoglobulin, which indicates an improvement in the animal's immune status. Vitamin E, zinc and copper reduces chances of reproductive disorders like mastitis, retained placenta, abortion and pyometra and are essential for reproductive health and production.

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