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ROLE OF VITAMIN E AND ZINC IN CELLULAR ADAPTATION, OXIDATIVE STRESS AND METABOLIC STRESS IN DAIRY ANIMALS: *A REVIEW*

Pankaj Kumar Maurya*, O.K. Hooda[1], B.K. Chaudhari[1], A.K. Singh[1], A.K. Pandey[2] and R. Kushwaha[3]
[1]Dairy Cattle Physiology Division, [2]Animal Biochemistry Division, [3]Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal-132001 (Haryana) India

**Corresponding author:* dr.pankajmaurya3003@gmail.com

Periparturient cows undergo instance mammary growth, copious synthesis and secretion of carbohydrate, fat and proteins as well as marked accumulation of colostrum and milk. Since colostrum is rich in vitamins A and E, therefore, cows require increased supply of these vitamins prior to parturition. At parturition, due to increased colostrogenesis, there is diversion of zinc from plasma pool towards mammary gland. Decrease in serum zinc level at calving is also associated with an acute phase response due to inflammatory reaction in uterus. Stress at calving induces synthesis of zinc distribution protein metallothionein and zinc is redistributed from blood pool to other tissues such as liver. During periparturient period when there is significant decrease in vitamin E level and zinc, the cow's immunity status and neutrophil functions are depressed. Supplementation of vitamin E maintains proper antioxidant status of animals and improves the ability to resist infections. Zinc is an integral part of immune system. It has been indicated that zinc had an indispensable role in the development and maintenance of immunocompetence. Zinc is also known to be associated with superoxide dismutase (SOD) which is an important antioxidant enzyme involved in oxidative stress. Also role of vitamin E and zinc in energy metabolism like increase blood glucose level and decrease NEFA during early lactation when animals are under negative energy balance.

Keywords: Antioxidant enzymes, Oxidative stress, Metabolic stress, Vitamin E, Zinc

The transition phase from pregnancy to lactation is crucial for the profitability of the dairy cow (Grummer, 1995) and is characterized by a depleted antioxidant status. Endocrine and cellular adaptations during dry period and early lactation play an important role in animal productivity. Physiological changes during transition period associated with rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion are accompanied by a high-energy demand and an increased oxygen requirement (Gitto et al., 2002). This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Excessive production of free radicals and concomitant damage at cellular and tissue levels are controlled by cellular antioxidant defense systems. When ROS are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Trevisan et al., 2001). There are growing evidences that oxidative stress is a threat to transition period and an increase in its level may lead to calving-related complications in both man and animals (Castillo et al., 2005). Oxidative stress can contribute and/or lead to the onset of health disorders in cattle (Miller et al., 1993). Brezezinska et al. (1994) observed that during the transition period cows can experience oxidative stress which may contribute to periparturient disorders, and may be associated with metabolic diseases (Ronchi et al., 2000). The transition period is critical for the health of dairy cattle

(Drackley, 1999). Toyokuni (1999) reported that oxidative stress leads to peroxidative damage of lipids and other macromolecules with consequent alteration of cell membranes and other cellular components. Antioxidants can be broadly defined as any substance that delays, prevents, or removes oxidative damage to target molecules (Halliwell and Gutteridege, 2007). Vitamin E and some minerals like zinc and copper acts as antioxidant. There is a substantial decline in plasma vitamin A, beta carotene and α-tocopherol levels during periparturient period (Michiels et al., 1994; Weiss et al., 1997; Arechiga et al., 1998). α-Tocopherol functions as an antioxidant that terminates the chain of events of oxidative processes by donation of its phenolic hydrogen to chain propagating lipid peroxyl radicals, resulting in the enhanced formation of the less reactive α-tocopheroxyl radical (Zhang and Omaye, 2001). Zn is also known to be associated with enzymes involved in the phagocytic oxidative burst (Chandra and Au, 1980), in cellular maturation and functioning of B and T-lymphocytes. Several metalloenzymes such as superoxide dismutase (Cu, Zn, and Mn), catalase (Fe) and glutathione peroxidase (Se) are also critical in protecting the internal cellular constituents from oxidative damage. In fact, SOD is considered the first defense against pro-oxidants that convert the superoxide ($\bullet O^{-2}$) to hydrogen peroxide (H_2O_2), whereas glutathione peroxidase converts H_2O_2 into less dangerous reduced forms (Halliwell and Chirico, 1993). Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen.

Vitamin E status during dry period and early lactation

Goff and Stabel (1990) reported that the decrease in plasma α-tocopherol in cattle was from 1.8 μg/ml to 0.7 μg/ml during last two week of parturition to calving. Decrease in α-tocopherol from 3.5 to 1.8 μg/ml from dry period to calving in cows was also reported by Weiss et al. (1990). Plasma α-tocopherol typically decreases 7 to 10 day prior to calving and remains low for 2-3 weeks of lactation, even when the dietary vitamin E offered to cows is constant throughout this period (Weiss et al., 1990; Hogan et al., 1993). The α-tocopherol at parturition has been reported to decrease from 2.1 to 1.3 μg/ml and remained as such until 2-3 weeks post partum (Hogan et al., 1993). The decreased plasma α-tocopherol during periparturient period is related to changes in consumption of vitamin E and decreased transport capacity of vitamin E in plasma. During periparturient period when there is significant decrease in α-tocopherol, the cows' immunity status and neutrophil functions are depressed (Hogan et al., 1993), that's why 30 to 50% of clinical mastitis occurs during first month of lactation (Weiss et al., 1990).

Plasma vitamin E concentration in dairy cows is negatively correlated with the rate of intra mammary infection. Clinical mastitis did not occur when cows had more than 3 μg/ml α-tocopherol in plasma during calving (Weiss et al., 1997). Most of the fat soluble antioxidant vitamins such as retinol, α -tocopherol and β-carotene decrease at the time of parturition and are associated with severe health problems (Rajiv, 2001). Low plasma concentration of α-tocopherol at parturition has been documented as a significant risk factor for intra mammary infection (IMI) and mastitis during first week of lactation (Goff and Stabel, 1990; Kaur et al., 2002). A sharp decline in plasma α -tocopherol concentration to the extent of 57% was recorded in crossbred cows (Rajiv, 2001) as well from 30 days before parturition to date of calving (Chatterjee, 2002).

Chandra and Aggarwal (2010) reported in winter season a decrease of 47.22% in α -tocopherol concentration from 20 days before parturition to the day of calving in control cows where as significantly lower (20.19%) in treatment cows supplemented with 1000 IU α –tocopherol. Maurya (2011) reported that decrease in the plasma vitamin E concentration of control and treatment group cows was 36.30% and 25.25%, respectively 60 days before calving and on the day of calving. This decrease was more significant ($P<0.01$)

from 30 days before calving to the day of calving in control group than treatment group. The overall mean (±SEM) of plasma vitamin E concentration was found significantly (P<0.01) higher in vitamin E (1000 IU/day/cow) and zinc (60 ppm/day/cow) supplemented treatment cows as compared to control cows (2.60±0.05 vs. 2.38±0.06 µg/ml).

Zinc status during dry period and early lactation

In ruminants, normal plasma Zn level is 0.8 to 1.2 µg/ml. Concentration of Zn in plasma fluctuates with age, stress and infections. Zn content of colostrum and milk is 14 and 4 ppm, respectively (NRC, 2001). At parturition, due to increased colostrogenesis, there is diversion of Zn from plasma pool towards mammary gland. Drop in serum Zn level at calving is also associated with an acute phase response due to inflammatory reaction in uterus. Stress at calving induces synthesis of metallothionein, a protein associated with Zn distribution. As a consequence, Zn is redistributed from blood pool to other tissues such as liver (Meglia et al., 2001). During day 190 up to end of gestation, the foetus and uterus of cow retain about 12 mg Zn/day (NRC, 2001). Plasma Zn level of 0.90 µg/ml at 15 days prepartum decreased to 0.64 µg/ml on the day of parturition in dairy buffaloes, which increased to normal values at 15 days postpartum (Panda, 2003). Plasma Zn level of 1.51 µg/ml at 5 days prepartum decreased to 1.09 µg/ml on the day of parturition in cross bred cows (Chandra and Aggarwal, 2010). Maurya (2011) reported that decrease in the plasma Zn level of control and treatment (vitamin E @ 1000 IU/day/cow and zinc @ 60 ppm/day/cow) cows was 30.60% and 16.13% respectively from 60 days before calving to the day of calving. This decrease was more significant (P<0.01) from 7 days before calving to the day of calving in control group as compared to treatment group. After calving it's level remains low in early lactation.

Role of vitamin E and Zinc on oxidative stress and antioxidant enzymes

Oxidative stress

Vitamin E, as a powerful antioxidant, lowers oxidative stress and influences the health of dairy cows (Burton and Traber, 1990). Oxidative stress can contribute and/or lead to the onset of health disorders in cattle (Miller et al., 1993). Brezezinska et al. (1994) observed that during the transition period cows can experience oxidative stress which may contribute to periparturient disorders, and may be associated with metabolic diseases (Ronchi et al., 2000). The transition period is critical for the health of dairy cattle (Drackley, 1999). Toyokuni (1999) reported that oxidative stress leads to peroxidative damage of lipids and other macromolecules with consequent alteration of cell membranes and other cellular components. Oxidative stress resulting from increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defense, leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al., 2001).

Gitto et al. (2002) reported an imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to periparturient disorders in dairy cows. Williams et al. (2002) reported that oxidation is essential to nearly all cells in the body to provide energy for vital functions. Approximately 95 to 98% of the oxygen consumed is reduced to water during aerobic metabolism, but the remaining fraction may be converted to oxidative by-products-reactive oxygen species that may damage the DNA of genes and contribute to degenerative changes. In the transition period, blood concentrations of both vitamin E and certain oxidative stress products change. For example, the plasma

concentration of α-tocopherol decreases during the last month prepartum (LeBlanc et al., 2004) and oxidative stress increases around parturition (Castillo et al., 2005). Lohrke et al. (2005) reported that metabolic activity increases during the transition period, especially in the liver and mammary gland, the higher metabolic activity is accompanied by higher oxygen radical production which may cause greater concentrations of oxidative damage products if antioxidant status is inadequate.

Antioxidants Antioxidant is 'any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate'. This definition includes compounds of a non-enzymatic as well as an enzymatic nature (Halliwell and Gutteridge, 1989).

Antioxidants can be divided into 3 major groups.

1. Enzymatic antioxidants including superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) represents the main form of intracellular antioxidant defense.

In fact, SOD is considered the first defense against pro-oxidants that convert the superoxide ($^{\bullet}O^{-2}$) to hydrogen peroxide (H_2O_2), whereas erythrocyte glutathione peroxidase converts H_2O_2 into less dangerous reduced forms (Halliwell and Chirico, 1993). Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen.

2. Nonenzymatic protein antioxidants, is primarily found in plasma. Examples are albumin, L-cysteine, and homocysteine. Protein sulfhydryl groups are considered significant element of the extra-cellular antioxidant defense system against oxidative stress (Uleand et al., 1996; Frei et al., 1998).

3. The nonenzymatic low-molecular-weight antioxidants, is found in plasma and in other extracellular fluids, intracellular fluids, lipoproteins and membranes. The nonenzymatic low-molecular-weight antioxidants can be further subdivided into water-soluble and lipid-soluble antioxidants. Water-soluble antioxidants are ascorbic acid, glutathione, and uric acid. Lipid soluble antioxidants are α-tocopherol, β-carotene and retinol.

Tissue defense mechanisms against free-radical damage generally include vitamin C, vitamin E, and β-carotene as the major vitamin antioxidant sources. In addition, several metalloenzymes which include glutathione peroxidase (Se), catalase (Fe) and superoxide dismutase (Cu, Zn, and Mn) are also critical in protecting the internal cellular constituents from oxidative damage.

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) was first isolated by Mann and Keilis (1938) and thought to be a copper storage protein. Subsequently, the enzyme was identified by a number of names, erythrocuprein, indophenol oxidase, and tetrazolium oxidase until its catalytic function was discovered by McCord and Fridovitch (1969). SOD is now known to catalyse the dismutation of superoxide to hydrogen peroxide and oxygen.

$$^{\circ}O^{-}_{2} + {}^{\circ}O^{-2} + 2H^{+} \longrightarrow H_2O_2 + O_2$$

The major defense in detoxification of superoxide anion and hydrogen peroxide, are superoxide dismutase (SOD), catalase and glutathione peroxidase (McCord and Fridovich, 1969; Chance et al., 1979).

The role of intracellular SOD is to scavenge the superoxide ($\bullet O_2^-$) that is produced by a number of reaction mechanisms, including several enzyme systems, as a part of normal cellular functions (Fee et al., 1975). There are three distinct types of SOD classified on the basis of the metal cofactor: 1) Copper/zinc (Cu/Zn - SOD), 2) Manganese (Mn-SOD) and 3) Iron (Fe-SOD) isozymes (Bannister et al., 1987).

The oxidation or autooxidation of hemoglobin (Hb-Fe^{2+} into Hb-Fe^{3+}) into the erythrocytes results in the continuous formation of $\bullet O_2^-$ (Hebbel and Easton. 1989). SOD is a Cu/Zn-dependent enzyme and erythrocyte GPx is a Se-dependent enzyme (Sies, 1991). The higher erythrocyte SOD activity found in summer cows was probably a response to the higher $\bullet O_2^-$ generation. SOD catalyzes the dismutation of $\bullet O_2^-$ into oxygen and hydrogen peroxide (H_2O_2) and it is an important antioxidant defense mechanism in aerobic organisms (Halliwell and Chirico, 1993). The decomposition of H_2O_2 or its interaction with $\bullet O_2^-$ would generate hydroxyl radicals (OH•), these hydroxyl radicals can attack all biological molecules; including membrane lipids, and can result in initiation of lipid peroxidation (Halliwell and Chirico, 1993). In fact, the dismutation of $\bullet O_2^-$ results in a rise in H_2O_2. Since SOD activity increases H_2O_2 production, protection from reactive oxygen would only be conferred by a coordinated increase of catalase and GPx activities (Frei, 1994).

Reduction in zinc and copper availability in the early postpartum period (Muehlenbein et al., 2001) of dairy cows might explain the reduction of SOD activity (Michiels et al., 1994). Bernabucci et al. (2005) reported increased activity of SOD during the last 3 wk of pregnancy, and after calving, SOD activity rapidly declined. Kanna (2007) found that SOD activity was 4152.27 ± 71.19 and 4326.83 ± 81.85 (Units /g Hb/min) in medium BCS and high BCS cows, respectively. Chandra and Aggarwal (2009) reported that increase in the SOD activity of control and treatment group cows was 39.29% and 24.22%, respectively from 20 days before calving to the day of calving. Maurya (2011) reported that increase in the SOD activity of control and treatment (vitamin E @ 1000 IU/day/cow and zinc @ 60 ppm/day/cow) cows was 66.75% and 42.36%, respectively from 60 days before calving to the day of calving. This increase was more significant ($P<0.01$) from 15 days before calving to the day of calving in control group than treatment group (Sharma et al., 2011; Maurya, 2011).

Catalase (CAT)

Catalase is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen. The enzyme is found in all aerobic eukaryotes and is important in the removal of hydrogen peroxide generated in peroxisomes (microbodies) by oxidases involved in ß-oxidation of fatty acids and purine catabolism. Catalase was one of the first enzymes to be isolated in a highly purified state. In peroxisomes catalase takes care of the cytocylic and mitochondrial peroxides formed during urate oxidation (Oshino and Chance, 1977). Catalase located in cytosol and mitochondria of the cells. It is most efficient enzyme promoting the redox reaction (Chance et al., 1979). Catalase primarily found within peroxisomes of most cells, is an iron metalloenzyme which catalyses the conversion of hydrogen peroxide into water and oxygen (Chance et al., 1979). Mitochondrial SOD readily converts the bulk of mitochondrial superoxides ion to H_2O_2. Thus SOD and catalase protects the cell from the damage due to the secondary generation of highly reactive hydroxyl group from superoxide ion to H_2O_2 (Miyazaki et al., 1991).

Catalase is predominantly involved in removal of H_2O_2 in normal human erythrocytes (Mueller et al., 1997). H_2O_2 production was found to increase due to increased SOD activity during heat stress (Bernabucci et al., 2002) and this in turn results in a coordinated increase in catalase and glutathione peroxidase (Clemens and Waller, 1987; Frei, 1994; Kehrer and Smith, 1994) thus a positive and significant correlation exist between catalase activity and SOD activity. Mousa et al. (2002) observed a significant ($P<0.05$) increase in catalase activity of erythrocyte of goat which was orally fed 5.46 mg lead. Kumar (2005) observed a significant positive correlation of THI with the erythrocyte SOD and catalase activity

in Murrah buffalo and KF cattle. The highest increase was registered in KF followed by Murrah. Kanna (2007) reported catalase activity was significantly ($P<0.05$) higher in high BCS than medium BCS cows. Since SOD activity increases H_2O_2 production, protection from reactive oxygen would only be conferred by a coordinated increase of catalase and glutathione peroxidase activities (Frei, 1994; Kehrer and Smith, 1994; Sharma et al., 2011). In support of this conjecture, catalase activity was found to be increased in cows near parturition and also lower catalase activity in treatment (vitamin E @ 1000 IU/day/cow and zinc @ 60 ppm/day/cow) as compared to control group indicating less oxidative stress in treatment cows as compared to control cows (Maurya, 2011). Catalase activity is positively correlated with SOD and GPx activity where as negatively correlated with vitamin E (Maurya, 2011).

Glutathione Peroxidase (GPx)

Glutathione peroxidase is selenium dependent enzyme and it has also antioxidant property. It converts hydrogen peroxide to water. Glutathione peroxidase (GPx) catalyze the reduction of organic hydroperoxides, lipid peroxides, and hydrogen peroxide, using glutathione as the reducing agent, thereby also protecting cells from oxidative damage resulting from normal oxidative metabolism. There are four known GPx that contain selenocysteine at the active site. Glutathione peroxidase is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The protection offered to cellular membrane by Vitamin E may spare the requirement of GPx by oxidizing free radicals at the membrane, thereby preventing leakage of free radicals into cytosol and maintaining activity of cells at a high level thereby decreasing mastitis (Hogan et al., 1993). Brezezinska et al. (1994) observed that GPx tended to be higher in Vitamin E supplemented than Se offered or control animals (5.3, 5.0 and 5.1 U/ml). They suggested that when Se in the diet was adequate, its supplementation had no effect on GPx concentration. Plasma glutathione peroxidase is considered as an indicator of oxidative stress (Tüzün et al., 2002). Sordillo et al. (2007) reported that GPx activity increases in transition cows and could be used as an indicator of oxidative stress. Increase of plasma GPx activity might reflect an altered oxidative status in pre- and post calving periods (Bernabucci et al., 2005; Aitken et al., 2009). Sharma et al. (2011) had reported that activities of glutathione peroxidase were increased during early lactation. Maurya (2011) reported that increase in plasma glutathione peroxidase activity of control and treatment (vitamin E @ 1000 IU/day/cow and zinc @ 60 ppm/day/cow) cows towards parturition but there was no significant difference between the groups, however, a significant ($P<0.01$) difference between days was found. GPx-P activity was found to be increased in cows towards parturition indicating more oxidative stress at the time of parturition in both groups.

Thiobarbituric acid reactive substance (TBARS)

Lower antioxidant potential as a consequence of lactation stage can result from an excess accumulation of ROS, a depletion of antioxidant defences, or a combination of both. One way to determine if ROS-mediated damage is occurring within host tissues is to measure end products of free radical oxidative processes. For example, when ROS react with polyunsaturated fatty acids, lipid peroxidation occurs. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. The determination of lipid hydroperoxide levels in plasma would be an indication of early stages of this lipid peroxidation damage. Lipid peroxidation is commonly measured in terms of thiobarbituric acid reactive substance (TBARS). Erythrocytes being rich in polyunsaturated fatty acids (PUFA) and being exposed to high concentration of oxygen are highly susceptible to peroxidation damage (Clemens and Waller, 1987). Oxidative stress can lead to increase in TBARS (Halliwell and Chirico, 1993), TBARS can induce a reduction of membrane fluidity and increase

erythrocyte membrane fragility (Chen and Yu, 1994). The increase of TBARS immediately before and after calving confirms that cows during the transition period are under oxidative stress conditions. TBARS and plasma GPx are the better measures of oxidative status in transition dairy cows (Bernabucci et al., 2005). TBARS activity increased after calving and there was no significant difference in overall average values of TBARS in high BCS and medium BCS cows (Bernbucci et al., 2005; Kanna, 2007). Lipid peroxidation is one of important consequences of oxidative stress (Kumaraguruparan et al., 2002). The determination of lipid peroxidation products allows for the estimation of the intensity of this process; moreover, it can be used for the evaluation of oxidative stress severity (Halliwell and Whiteman, 2004). Lipids are the most susceptible for peroxidative damage due to low energy necessary for the initiation of the process as well as the presence of unsaturated bonds (Balasinska, 2004).

Lipid peroxidation was significantly ($p<0.001$) higher in early lactating cows than advanced pregnant cows (Saleh et al., 2007; Sharma et al., 2011). Oxidative stress in cows is a contributory factor to increase disease susceptibility since metabolic demands associated with late pregnancy, parturition and initiation of lactation would be expected to increase the production of reactive oxygen species (ROS), resulting oxidative stress. A relationship between oxidative stress (lipid peroxidation) and antioxidant status (catalase) was found significantly positive in advanced pregnant cows, while non-significant negative correlation was found in early lactating cows (Saleh et al., 2007; Sharma et al., 2011). Lipid hydroperoxides increased significantly from calving through the first 3 weeks of lactation when compared to the pre-partum measurements (Sordillo et al., 2007), these findings are consistent with other reports in periparturient animals where lipid hydroperoxides and biomarkers of lipid peroxidation, such as thiobarbituric acid-reactive substances (TBARS), were found to increase from calving through early lactation (Bernabucci et al., 2002; Castillo et al., 2005). Maurya (2011) reported that TBARS level was observed lower in vitamin E and zinc treated group than non treated group cows.

Role of vitamin E and Zinc on energy metabolites

During early lactation, energy used for *de novo* fatty acid synthesis and esterification to triglycerides is reduced in adipose tissue while the lipolysis increases considerably. The consequence is a mobilisation of fat deposited during gestation, which results in a rise in free fatty acids (NEFA) and glycerol in plasma. In adipose tissue as well as muscular tissue, the glucose intake is reduced, and instead the use of fatty acids and ketone bodies is increased. Despite the reduced use of glucose in these tissues and a quite considerable increase in the gluconeogenesis in liver tissue and kidney tissue, the glucose concentration normally drops postpartum in multiparous cows. The increased ketogenesis in hepatic tissue generally increases the level of ketone bodies, especially in second lactation and older cows resulting fatty liver and ketosis (Ingvartsen and Andersen, 2000).

NEFA

As a consequence of the extensive mobilization of adipose tissue in early lactation there is a manifold rise in plasma concentration of NEFA (Pullen et al., 1989). NEFA is one of the most sensitive metabolites to environmental stress. The increased NEFA concentration during early lactation in cows suggests mobilization of free fatty acids (NEFA) from adipose tissue due to negative energy balance to meet energy requirements (Pullen et al., 1989). Bahga and Gangwar (1992) reported that season of calving also influence blood levels of free fatty acids. NEFA levels were significantly higher in animals parturated in summer compared to those parturated in winter during 6 to 57 days of lactation. Highest values were obtained on day 8 postpartum in both summer and winter seasons (55.38 and 33.81 mg/100ml) which declined consistently with number of days in both

the seasons. The gradual increase in plasma NEFA concentrations from week-3 to week-1 has been suggested as a feed intake effect, while the rapid increase in the immediate precalving period may be hormonally regulated (Grummer, 1993). The liver plays an important role in fat metabolism, removing NEFA from the blood. In early lactating cows, about 50% of NEFA are oxidised to ketone bodies or reesterified to triglycerides in the liver (Bell, 1995). Bell (1995) estimated that in the immediate postpartum period, approximately 50% of circulating NEFA are either oxidized or incorporated into milk fat. Pal (1996) reported plasma levels of NEFA to be around 534 to 299 µ mol/l up to day 19 of lactation and declined gradually till day 54 of lactation in buffaloes to 256 µmol/l.

Dairy cows undergo tremendous changes during the transition from late gestation to early lactation. Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones. For example, growth hormone (GH) is increased around parturition and in early lactation (Grum et al., 1996), which increases responsiveness of adipose tissue to lipolytic signals such as nor-epinephrine. The resulting increase of NEFA from adipose tissue is used as alternate fuels for much of the rest of the body. Doepel et al. (2002) reported that cow with the lowest intake at calving (2.9 kg) had the highest NEFA concentration (2172 µmol/L). High BCS and greater decline of BCS are related to plasma NEFA concentration (Rukkwamsuk et al., 1998) and possibly, to incidence of metabolic disorders (Cameron et al., 1998). Plasma NEFA concentrations were in the range of 100 to 2000 µeq/litre in cows and were low in low producing cows. Kokkonen et al. (2005) reported increased level of NEFA in lactating cows as compared to non-lactating cows.

Bernabucci et al. (2005) observed that after calving, cows that had high BCS at calving and high lipid mobilization have a more pronounced alteration of oxidative status. These conditions can make cows more sensitive to oxidative stress. Kanna (2007) reported that after calving, HBCS cows had more lipid mobilization as indicated by higher NEFA levels and more pronounced alteration of oxidative status indicative of higher oxidative stress in HBCS cows. Valde et al. (2007) found that cows in a fatter condition at calving lost more BW and body condition over a longer period of time than cows in a thinner condition at calving. Chandra and Aggarwal (2010) and Singh (2010) found that cows supplemented vitamin E @1000 IU/day have lower NEFA level in comparison to non supplemented cows. Maurya (2011) also reported a significant ($P<0.01$) increase in the NEFA level of control and treatment (vitamin E @ 1000 IU/day/cow and zinc @ 60 ppm/day/cow) cows towards parturition but there found a significantly ($P<0.01$) lower NEFA level in treatment group than control group.

Glucose

Glucose concentration found maximum at calving, the peak at calving may be related to the release of glucocorticoids immediately before calving that stimulate glycogenolysis and gluconeogenesis (Vazquez-Anon et al., 1994). The decreased glucose concentrations postpartum are probably related to low DMI, and the concomitant reduction in propionate absorption, along with an increased glucose requirement for milk synthesis. Itoh et al. (1997) found an increase in plasma glucose concentration in cold exposed (0^0C) cows. Plasma glucose concentrations were different between the hot (79.4 mg/dl) and cold (90.5 mg/dl) environments. Glucose concentrations that peaked at calving were lower postpartum than prepartum (Dann et al., 2005). Kanna (2007) reported glucose levels were significantly ($P<0.1$) higher in high BCS than medium BCS cows (54.03 ± 3.02 vs 43.88 ± 2.33 mg/dl). Chandra and Aggarwal (2010) and Singh (2010) found that cows supplemented vitamin E @1000 IU/day have higher glucose level in comparison to non supplemented cows during the transition period. Maurya (2011) also reported higher glucose level in treatment (vitamin E @ 1000

IU/day/cow and zinc @ 60 ppm/day/cow) group as compare to control group.

CONCLUSION

Oxidative damage can lead to cell dysfunction and cell death which result higher maintenance costs for the animal to repair those tissue, decreasing productivity and increasing susceptibility to infection. More oxidative stress occurs during transition period in dairy animals. At that time high producing animals undergo negative energy balance and sometimes animal undergo metabolic stress. Antioxidant enzymes like SOD, catalase and glutathione peroxidase are true indicator of oxidative stress. Activity of these antioxidant enzymes increases near parturition and peaked up on the day of parturition. Vitamin E, as the primary Iipid-soluble antioxidant is important for the body's defence against oxidative stress. The activity of SOD, catalase and glutathione peroxidase was significantly decreased by supplementation of vitamin E and zinc indicating improvement in the antioxidant activity and decrease oxidative stress to animals. Supplementation of vitamin E and zinc has also role in improved energy metabolism. Supplementation of vitamin E and zinc during transition period decreases NEFA level and increases plasma glucose level indicating improvement in the metabolic status of the animal and decreases chances of metabolic diseases.

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