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Review Article

Essentiality of antioxidant vitamins for ruminants in relation to stress and reproduction

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Summary

The naturally occurring antioxidants: vitamin E, β-carotene and vitamin C are known to ameliorate the oxidative stress generated during various physiological and pathological conditions. The oxidative stress may occur at different sites, different times, and by different mechanisms in the animal system. Lower levels of the anti-oxidant vitamins are associated with poor fertility and production levels in ruminants. These antioxidants need to be replenished/regenerated or supplemented at appropriate timings in order to optimize the ruminant health and productive/reproductive performance.

Key words: Antioxidants, β-carotene, Vitamin C, Vitamin E, Ruminants

Introduction

The terms oxidative stress, oxidative damage, free radical and antioxidant have become an integrated part of the variety of scientific discussions in issues related to chemistry, biology and research in biosciences. Free radical reactions are the integral part of normal metabolism. Oxidative stress is experienced by the living organisms from both exogenous and endogenous sources in terrestrial as well as marine environments (Cross *et al*., 1998; Lesser, 2006). Production of oxidants together with the ability of organisms to respond to oxidative stress is intricately connected to ageing and life span (Finkel and Holbrook, 2000). The description, terminology, definitions, basic chemical characteristics of the species involved in oxidative stress phenomena and the defense mechanisms of the organisms against these reactive metabolites have been discussed in detail (Kohen and Nyska, 2002). The dietary and tissue balance of antioxidant nutrients is important in protecting tissues against free radical damage. The antioxidant function could, at least in part, enhance immunity by maintaining the structural and functional integrity of the important immune cells. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to diseases, thereby leading to increased animal morbidity and mortality (McDowell, 2002). In the last few years, the detection of free radical damage and the protection against it has become very important in the studies related to ruminant production/reproduction as the level of lipid peroxidation and antioxidant status give complementary information about the metabolic status of the animal rather than metabolic parameters alone (Castillo *et al*., 2003). The requirement/involvement of antioxidants is evident from the results of studies during different stages in the life of the animal such as protective effect of retinol on oocytes during heat stress (Lawrence *et al*., 2004), the regulation of cellular metabolic pathways of bovine embryo by oxygen (Harvey, 2007) and inadequate provision of

vitamin E to new born calves in colostrum of cows not supplemented with vitamin E during the dry period (Quigley III and Drewry, 1998).

Fetal and neonatal stage

Oxidative stress is involved in the etiology of defective embryo development. Embryo metabolism generates reactive oxygen species *via* several enzymatic mechanisms, which may alter most of cellular molecules and induce developmental block and retardation (Guérin *et al*., 2001). Reactive oxygen species may be produced at early developmental stages, necessitating anti-oxidative defense mechanisms. The pre-implantation embryo is sensitive to the environment in which it is developing *in vivo* in response to maternal diet. This sensitivity may lead to alteration in the fetal and neonatal growth (Fleming *et al*., 2004). Under normal conditions, the developing embryo is capable of coping with oxidative stress but this may fail under various pathological conditions, leading to embryonic damage (Zaken *et al*., 2000). Antioxidants can obviate these effects through modification of gene expression, transcription factor signaling and cell cycle alterations (Dennery, 2007). Retinoids influence follicular development, oocyte maturation and early embryonic development and have a beneficial antioxidant effect during embryo culture (Livingston *et al*., 2004).

Glucose-6-phosphate dehydrogenase is a developmentally critical cytoprotective enzyme for both endogenous and xenobiotic-initiated embryopathic oxidative stress and DNA damage. Glucose-6-phosphate dehydrogenase deficiency, and hence a disturbance in redox homeostasis may have a broader biological relevance as important determinant of infertility, in utero and post natal death, and teratogenesis along with altered susceptibility to viral infection and increased susceptibility to degenerative diseases (Nicol *et al*., 2000; Ho *et al*., 2007). During early embryo development, glutathione plays a role as a key cofactor free radical scavenger in the antioxidant actions of other cellular protectants, for example helps maintaining cellular ascorbate levels (Henshel, 2004).

Maternal concentrations of vitamin E during early and late pregnancy are positively associated with fetal growth (Scholl *et al*., 2006). Vitamin E is necessary to protect the newborn against oxidative stress whereas vitamin A is required for growth and development; and both vitamins are essential for immune system development. Adding vitamin E to diets at pharmacological level seems beneficial for decreasing bovine respiratory disease morbidity in feedlot cattle (Duff and Galyean, 2007). The antioxidant vitamins A and E, and the micronutrients zinc, iron and copper have the greatest impact on pregnancy outcome as both copper and iron may elicit changes in prenatal development through the generation of free radicals whereas zinc deficiency can alter retinol metabolism or stimulate the up-regulation of antioxidant enzymes. Micronutrient imbalance can affect pregnancy outcome through alterations in maternal and conceptus metabolism, as a consequence of their essential role in enzymes and transcription factors and through their involvement in signal transduction pathways that regulate development. Micronutrient-induced disturbances in the balance between the generation of free oxygen radicals and the production of antioxidants that scavenge free radicals may provide an additional mechanistic explanation. Greater understanding of the role of key micronutrients and the impact of antioxidant status at key stages of development will provide new opportunities to refine nutritional guidelines to enhance pregnancy outcome (Ashworth and Antipatis, 2001).

Metabolic disorders associated with negative energy balance may impair fertility, immune status and antioxidant status. Oxidative stress continues to be a problem in transition cows. During lactation onset, mammary gland epithelial cells are exposed to significant levels of free radicals because of higher oxygen demand for milk synthesis and secretion. The antioxidant system is an integrated system and deficiencies of one component can affect the antioxidant efficiency of others. Vitamin E, β-carotene and selenium are effective dietary antioxidants in ruminants. Innovative approaches are needed to enhance the

antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands (Baldi *et al*., 2008; Sordillo and Aitken, 2009).

Reproductive function

Reproductive problems in cattle are of significant economic concern in dairy farming. Free radicals and reactive oxygen species play a number of significant and diverse roles in reproductive biology (Riley and Behrman, 1991) and have been implicated in fetal dysmorphogenesis, abortions and intrauterine growth restriction (Agarwal *et al*., 2006). In mammals, radical oxygen species (ROS) are essential factors of cell division, differentiation and growth, notably during prenatal stage. Vitamins and trace minerals have dual effects: (a) control of metabolic pathways or gene expression and (b) ROS trapping activity. Their deficiency may induce high rates of ROS production resulting in different disorders of gestation (Aurousseau *et al*., 2006). Deficiency may also be due to different kinds of stress (McDowell *et al*., 2007). Vitamin E-selenium injections given before mating significantly increased the incidence of oestrus response, fecundity rates and lamb body weight at 60 days of age in ewes (Koyuncu and Yerlikaya, 2007). Supplementation with vitamin E and selenium may be beneficial in reducing oxidative stress in dystocia-affected buffaloes in the immediate post-partum period (Sathya *et al*., 2007). It can be hypothesized that antioxidants and oxidative stress may influence the timing and maintenance of a viable pregnancy as threshold levels of female oxidative stress exist, depending on anatomic location and stage of preconception (Ruder *et al*., 2008).

In bovine corpus luteum, collagen is produced as a component of tissue growth, supported by the concurrent accumulation of its biosynthetic cofactor ascorbic acid (Luck and Zhao, 1993). The ovary has long been recognized as a site of ascorbic acid accumulation, which is probably due to the high rates of tissue remodeling and collagen synthesis that is required for follicular growth, for repair of the ovulated follicle, for corpus luteum development and steroidogenesis (Luck *et al*., 1995). There is feed dependent accumulation of β-carotene in bovine luteal tissue (Arikan and Rodway, 2001). The β-carotene is spread over the entire volume of luteal cells with higher levels occurring at distinct sites, including the surface (Arikan *et al*., 2002). In cattle, β-carotene levels in the plasma, corpus luteum and follicular fluid were influenced by the stage of the estrous cycle or the pregnancy and were related to the bovine luteal function without depending on vitamin A (Haliloglu *et al*., 2002). Beta-carotene supplementation positively affects ovarian activity in goats (Arellano-Rodriguez *et al*., 2007). Both β-carotene and α-tocopherol accumulate in the corpus luteum secondary to the uptake of lipoprotein-bound cholesterol during steroid synthesis. Lowest retinol levels were observed at highest metabolic activity of the corpus luteum indicating a possible consumption of retinol during steroidogenesis. Hence the supplementation should be sufficient enough to support an accumulation of β-carotene in the corpus luteum as a local precursor of retinol (Schweigert, 2003).

Retinoids have been shown to enhance developmental competence of the oocyte in cattle, sheep and pigs. Retinol has teratogenic effect on the intrafollicular oocyte in the cow (Hidalgo *et al*., 2005). Vitamin A injection before mating altered development of oocytes and embryos by decreasing the percentage at the germinal vesicle stage and increasing the percentage at advanced stage (Whaley *et al*., 2000). Aging of the unfertilized oocyte inevitably occurs following ovulation, limiting its fertilizable life span. Reactive oxygen species may be produced in the oocyte micro-environment in response to infections, certain medications, radiation and pollutants. Compromise in the oocyte's cellular mechanisms could be due to the deficiency of vitamin E, vitamin C, uric acid, glutathione, taurine, albumin or a group of enzymes that help to scavenge the oxygen radicals throughout the female reproductive tract (Goud *et al*., 2008). In cattle, β-carotene from forages and retinol ester from formula feed, is metabolized and transported to the oocytes and cumulus-granulosa cells in ovarian follicles through binding to various interacting molecules. The β-

carotene may enhance oocyte cytoplasmic maturation by its antioxidant property and retinoic acid may promote the same *via* its modulatory effect on the gene expression of gonadotrophin receptors in cumulus-granulosa cells (Rodgers *et al*., 1995; Ikeda *et al*., 2005).

The cellular origin of oxygen radicals in corpora lutea from resident and infiltrated leukocytes may lead to the risk of ovarian pathology which may be exacerbated under condition of reduced antioxidant status (Behrman *et al*., 2001). Reactive oxygen species may damage luteal cell membrane and may also affect progesterone production by interrupting trans-mitochondrial cholesterol transport, impairing LH receptors or cytochrome P450 enzymes activities (Kato *et al*., 1997). Role of oxidative stress in etiologies of female infertility has been reviewed in detail (Agarwal *et al*., 2005). The corpus luteum of the mammalian ovary is a unique endocrine organ; hormones regulate not only its function but also its existence. In luteal cells, $PGF_{2\alpha}$ mobilizes intracellular calcium, generates reactive oxygen species, depletes ascorbic acid levels, inhibits steroidogensis and ultimately induces cell death (Pepperell *et al*., 2003). $PGF_{2\alpha}$-induced and naturally occurring functional luteal regression is associated with accumulation of reactive oxygen species and/or decrease in protective enzymes, antioxidant vitamins, and radical scavengers (Stocco *et al*., 2007). The correlation of the antioxidant capacity with progesterone levels indicates that antioxidative mechanisms are activated to cope with sreoidogenesis dependent oxyradical formation in the bovine corpus luteum (Rapaport *et al*., 1998). Steady state level of vitamin E is inversely related to the steroidogenic activity of the corpus luteum and level of HDL-receptors does not change after functional regression. This is consistent with the hypothesis that a diminished consumption (by oxidative radicals, most likely generated during steroidogensis) rather than increased uptake is the reason for the increase in vitamin E levels that follows functional regression (Aten *et al*., 1994).

The corpus luteum of early pregnancy may be rescued from luteolysis through increasing activities of key antioxidant enzymes. Levels of antioxidant enzymes in the corpus luteum throughout pregnancy may be linked to reactive oxygen species generated continuously in the steroidogenically active luteal cells, and may be involved in the maintenance of luteal steroidgenic activity and cellular integrity (Al-Gubory *et al*., 2004). Al-Gubory *et al*. (2008) have provided the first firm evidence for the role of ovarian steroid hormones, estrogen and progesterone, in the regulation of the activities of key antioxidant enzymes in the endometrium of mammalian females, which has become central dogma.

Reactive oxygen species and superoxide dismutase work as local regulators of the corpus luteum function (Sugino, 2005, 2006; Sander *et al*., 2008). Antioxidant defenses in the female reproductive tract may have some regulatory role in fertility (Jean-François and Marc-André, 2001). Activities of antioxidant enzymes superoxide dismutase, nitric oxide synthase, glutathione reductase and glutathione-S-transferase in sheep corpus luteum are subjected to major changes during the estrous cycle (Al-Gubory *et al*., 2005). Expression of copper-zinc superoxide dismutase in bovine oviduct is influenced by the hormonal milieu and variations among oviduct sections in protein expression profile of superoxide dismutase suggested its important role in preserving and capacitating sperm (Roy *et al*., 2008). Placental luteotropins increased superoxide dismutase expression in the corpus luteum and stimulated progesterone suggesting that superoxide dismutase is involved in the maintenance of corpus luteum function by placental luteotropins (Takiguchi *et al*., 2000). The mRNA expression and distribution of enzyme activities e.g. glutathione peroxidase, superoxide dismutase and catalase in the bovine oviduct during the estrous cycle indicated their physiological role in the events leading to successful fertilization and implantation *in vivo* (Jérôme and Jean-François, 2003). Prepartum supplementation with vitamin E and selenium has been shown to reduce the incidence of retained fetal membranes, metritis and cystic ovaries. Vitamin E deficiency is directly associated with embryonic loss in cows, and through its role in immune system may also affect the rate of

uterine involution after calving (Noakes *et al*., 2001). Prepartum injections of vitamin E-selenium cause transient increase in the post-partum levels of vitamin E and β-carotene in buffaloes (Nayyer *et al*., 2006).

Stress

Free radical oxidation is activated in animals under various types of stresses and lipid peroxidation products accumulate in various organs (Yarovan, 2008). Free radicals generated in steroidogenic cells and mononuclear phagocytes in the corpus luteum may influence progesterone synthesis and could cause reproductive problems. Anoestrous and repeat breeder buffaloes have been reported to have lower plasma concentration of vitamin E and higher levels of lipid peroxidation and activities of superoxide dismutase and glucose-6-phosphate dehydrogenase. The supplementation of vitamin E-selenium to repeat breeder and anoestrous buffaloes relieved the oxidative stress as shown by the reduced levels of lipid peroxidation, activities of superoxide dismutase and glucose-6-phospahate dehydrogenase along with increased vitamin E and β-carotene and improved blood biochemical composition in vitamin E-Se supplemented animals (Nayyar *et al*., 2002, 2003a, b; Anita *et al*., 2003, 2004). The stress factors induce an inhibition of progesterone synthesis in repeat breeder cows (Rizzo *et al*., 2007). Short term supplementation of β-carotene to goats may result in enhanced ovarian function and progesterone synthesis (Arellano-Rodriguez *et al*., 2008).

Seasonal periods of heat stress reduce milk yields and fertility in lactating dairy cows. Heat stress during follicular recruitment suppresses subsequent growth to ovulation, accompanied by decreased LH receptor level and estradiol synthesis in the follicles (Ozawa *et al*., 2005; Roth, 2008). Heat stress induces systemic changes in redox status in the maternal body, and the resultant increase in oxidative stress in the oviduct is possibly involved in the heat stress induced early embryonic death (Matsuzuka *et al*., 2005). Seasonal rather than nutritional factors have a more pronounced effect on oxidative stress markers (Di Trana *et al*., 2006). Reduced glutathione is decreased in heat-stressed cattle (Lakritz *et al*., 2002). Retinol and other oxidants may protect oocytes from some of the deleterious effects of heat stress (Lawrence *et al*., 2004). Although cattle can synthesize vitamin C in the liver, a large reduction in plasma vitamin C concentration was reported in heat stressed cows (Padilla *et al*., 2006). The supplementation of ascorbic acid has been shown to enhance the plasma ascorbic acid concentration and immune response of the cattle subjected to environmental stressors (Padilla *et al*., 2007). However, moderate heat stress does not modify cell-mediated immunity, the protective value of colostrum and passive immunization of the offspring as observed in a study done by Lacetera *et al*. (2004). During the peripartum period, high-yielding dairy cows experience metabolic and oxidative stress which alters their homeostasis, and exposes the cows to illness (Colitti and Stefanon, 2006). The feeding of vitamins E and/or C in calves is associated with alterations in the concentrations of other antioxidants (Cusack *et al*., 2005). The important infectious diseases e.g. mastitis, pneumonia etc. in farm animals are thought to be associated with the oxidative stress which can be theoretically prevented with the antioxidants (Ellah *et al*., 2007; Lykkesfeldt and Svendsen, 2007), yet the use of antioxidants therapy remains controversial.

Conclusion

Since ascorbate, tocopherol and β-carotene supplements are inexpensive, it is essential that while focusing on the nutritional components for improving production/reproduction, the antioxidant status of the ruminants must be maintained at the proper times (during growth, pregnancy, deficiency or under stress), in addition to energy, protein and mineral balance to optimize the ruminant production/reproduction. Hence the evaluation of the oxidative stress and antioxidant status i.e. lipid peroxidation level and total antioxidant capacity (enzymatic and non-enzymatic) is necessary in addition to the metabolic profile while conducting physiological, biochemical and

nutritional studies in ruminants.

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