MERIT RESEARCH JOURNALS
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Merit Research Journal of Agricultural Science and Soil Sciences (ISSN: 2350-2274) Vol. 2(5) pp. 064-069, May, 2014
Available online http://www.meritresearchjournals.org/asss/index.htm

Review

Antioxidants in dairy cattle health and disease

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Abstract

Oxygen is a vital substrate for aerobic energy generation in the biological systems of higher animals. However, minimal quantities of some toxic substances referred to as free radicals are generated during this biochemical process. These compounds have large capacity to oxidize biological structures, especially membranes rich in lipids and proteins, causing cellular necrosis. The recent growth in knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health. In fact, the discovery of the role of free radicals in chronic degenerative diseases is as important as the discovery of the role of microorganisms in infectious diseases.

Keywords: Antioxidants, Free radicals, Dairy cattle, Health.

INTRODUCTION

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals. Intracellular generation of ROS mainly comprises superoxide ($O2^{\cdot-}$) radicals and nitric oxide ($NO^{\cdot}$) radicals. Under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into $O2^{\cdot-}$ through mitochondrial respiration, phagocytosis, etc (Clarkson and Thompson, 2000). ROS percentage increases during infections, exercise, exposure to pollutants, UV light, ionizing radiation, etc. $NO^{\cdot}$ is an endothelial relaxing factor and neurotransmitter produced through nitric oxide synthase enzymes. ($NO^{\cdot}$) and ($O2^{\cdot-}$) radicals, are converted to powerful oxidizing radicals like hydroxyl radical ($OH^{\cdot}$), alkoxy radicals ($RO^{\cdot}$), peroxyl radicals ($ROO^{\cdot}$), singlet oxygen ($1O_2$) by complex transformation reactions. Some of the radical species are converted to molecular oxidants like hydrogen pe-roxide (H_2O_2), peroxynitrite ($ONOO^{\cdot}$) (Clarkson and Thompson, 2000; NRC-National Research Council, 2001; Wilde, 2006; Cetin et al., 2003).

Antioxidants are molecules that can easily and harmlessly give up an electron. Nature produces an array of antioxidants to prevent free radical formation or to limit their damaging effects in cells. These include enzymes to decompose peroxides, proteins to bind transition metals, and other compounds that scavenge free radicals. The most important biological antioxidants are vitamins A, C, E and selenium, a key component of glutathione peroxidase, Vitamin E is an important lipid soluble antioxidant that protects against free radical-initiated lipid peroxidation (NRC-National Research Council, 2001). (see table 1). Vitamin A and or other carotenoids are abundant in many animal feeds and inexpensive to supplement. Vitamin C is produced naturally in the tissues of farm animals and thus is not routinely supplemented (Clarkson and Thompson, 2000; Tesfamariam, 1994).

Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Pham-Huy et al., 2008).

Although cells are equipped with an impressive reservoir of antioxidant enzymes as well as small antioxidant molecules, these agents may not be sufficient enough to normalize the redox status during ox-idative stress (Willett and MacMahon, 1984). Under such

Table 1. Incidence of placental retention in dairy cows fed diets contain >.12 ppm of Se with or without 1000 IV of supplemental vitamin E during the last 40 d of gestation. (Modified from 54).

Year	Treatment control	Treatment Vitamin E
	(% of group)	
1988	26.7 6.9·	6.9*
1989	34.4 10.8··	10.8**
1990	52.9 22.0·	22.0*
1991	32.3 21.9	21.9*

*p< 0.0
**p <0.01

conditions supplementation with exogenous antioxidants is required to restore the redox homeostasis in cells. Recent epidemiological studies have shown an inverse correlation between the levels of established antioxidants (vitamin E and C) / phytonutrients present in tissue / blood samples and cardiovascular disease, cancer and with mortality due to these diseases (Willett and MacMahon, 1984; Sevanian and Ursini, 2000; Beckman and Ames, 1997). Since several plant products are rich in antioxidants and micronutrients, it is likely that dietary antioxidant supplementation protects against the oxidative stress mediated disease development. Therefore, to maintain optimal body function, antioxidant supple-mentation has become an increasingly popular practice. Researchers are now attempting to develop new antioxidants either of natural or synthetic origin (Aruoma et al., 2007; Popov and Lewin, 2000).

Oxidative stress and mastitis

Mastitis is one of the most costly diseases in the dairy industry (Cetin et al., 2003). Mastitis continues to be an economically vital disease all over the world. Due to its anatomic topography, the udder is exposed to environmental effects, leading to inflammatory and non-inflammatory diseases. The disease in small ruminants is very important because of its high mortality rate on acute and peracute forms even though it is seen relatively rarely (Jian-Mei and Ajay, 2004). The organism of a high-yielding dairy cow is often in the state of disturbed homeostasis caused principally by the generation and accumulation of free radicals, which are natural and inevitable final products of the intensive metabolism in cells, created in the mitochondrial chain of the transport of electrons or as a result of NADPH stimulation (Stocker and Keany, 2004).

The specificity of the oxidative stress and its relation to mastitis and other diseases is a complex question, to which no simple answers have been found as yet. One of the symptoms of mastitis is an increased NO level and a reduced content of ascorbic acid in the blood serum of animals (Kleczkowski et al., 2005; Wilde, 2006). This is in accordance with (Jóźwik et al., 2012a), who reported, compared to healthy animals, a significant reduction of the concentration of ascorbic acid in the blood serum and milk of cows with a subclinical form of mastitis caused by an infection with Staphylococcus aureus, Staphylococcus agalactiae or E. coli. As reported by (Jóźwik et al., 2004; Strzałkowska et al., 2009b) who indicated that oxidative stress can lead numerous diseases in cows, above all to the inflammation of the mammary gland (mastitis), especially in the perinatal period.

The mechanisms by which inflammation cause damage to mammary gland tissue during mastitis is still not fully understood. It is well known that inflammatory reactions, in which vascular permeability increases and leukocyte migration occurs involve free radicals, such as $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet -}$ (Jian-Mei and Ajay, 2004). Antioxidant supplementation could decrease the duration, incidence, and severity of clinical mastitis (Smith et al., 1984; Bowers, Provide year). Antioxidant nutrition is an important part of coliform mastitis prevention because of the critical role of these micronutrients in mammary resistance to this disease (Bowers, 1997; Malbe et al., 1995). Supplemental vitamin E and /or Se has been shown to reduce prevalence and severity of mastitis and reduce SCC (Wichtel et al., 1994; Bowers, 1997).

Administration (IM) of 0.1 mg Se/kg BW at 21 days before calving did not affect the incidence of clinical mastitis but reduced the duration of clinical symptoms in cows with clinical mastitis by 46%. Selenium administration in combination with oral supplementation of vitamin E (740 IU/day) was most effective, reducing incidence of mastitis and duration of clinical symptoms by 37% and 62%, respectively (Smith et al., 1984).

Copper very important in the antioxidant system through its involvement in the enzymes Cu–Zn superoxide dismutase (SOD) and ceruloplasmin. Copper-

Table 2. Summary of micronutrient effects on mammary gland immunity (Sordillo et al., 1997).

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

Zn SOD is responsible for dismutation of superoxide radicals to hydrogen peroxide in the cytosol (Torre et al., 1995). As seen in Table 2 different antioxidants have crucial role in animals health.

Oxidative stress milk yield and quality

Mastitis which can be a consequence of oxidative stress threatening the optimum milk production depending on the size of inflammation (Sawa et al., 2008; Strza£kowska et al., 2010). Can also lead to reduction in fat, casein proteins and calcium content with a simultaneous increase in the concentration of whey proteins, sodium and chlorine, also the activity of enzymes from the group of lipases, proteases, oxidases and the plasminogen increases in the milk can be reduced, negatively affecting its technological properties and eating quality of milk products (Horbañczuk and Krzy¯ewski, 2011; Miller et al., 1993).

In addition to that organoleptic parameters like taste might be affected and inactivation of many biologically active ingredients contained in it. As a rule, an increase in the number of somatic cells and bacteria in milk accompanies the inflammation of the mammary gland is noticed in cows suffering from mastitis (Horbañczuk and Krzy¯ewski, 2011; Lapointe and Bilodeau, 2003).

Oxidative stress and reproduction

About 9% of all calving in the U.S. resulted in retained fetal membranes (RFM), which has a cost of $100 to $280/case (Malbe et al., 1995). In many cases, RFM is an oxidative stress disease. The vitamin C concentration in maternal and fetal placental tissue is about 50% lower when cows have RFM than when they not have (Brezezinska-Slebodzinska et al., 1994). The water soluble antioxidative capacity which is a part of the total antioxidative capacity measured in the blood of cattle in different postpartum periods, reflects the internal physiological status of dairy cattle, it was noticed that the ACW values around parturition were lower than the values 17 weeks postpartum, the values starting to increase gradually after parturition (AmitKunwar and Priyadarsini, 2011). GSH levels and GSH-Px activity were lower in cows with retention of fetal membranes as opposed to without, with a tendency to increase towards delivery (Brezezinska-Slebodzinska et al., 1994; AmitKunwar and Priyadarsini, 2011), vitamin E deficiencies are frequently observed during the periparturient period. The periparturient period is (Smith et al., 1984) associated with increased incidence of mastitis in dairy herds. White muscle disease is a classic sign of a clinical deficiency of vitamin E. (Bowers, 1997). The deficiency of antioxidants results in a lower muscle tone and thus poorer contraction of the uterus, which inhibits the transport of semen to the oviduct and causes a retained placenta (Bendich, 1993). Successful fertilization and implantation rely on complex and progressive interactions between the maternal genital tract, gametes and fertilized oocytes. The oviducts function as a Sperm reservoir, a site of male gamete selection and a site of fertilization in cows and other species. Although reactive oxygen species (ROS: H_2O_2, $O_2^{\cdot -}$, $OH^{\cdot -}$, $NO^{\cdot}$) are known to play an important role in male fertility/infertility, in vitro oocyte maturation (IVM), in vitro fertilization (IVF) and in vitro embryo development, little is known about the control of ROS levels by antioxidants in the oviduct in vivo (Goff and Stabel, 1990). Supplementation of the diet of prepartum cows with vitamin E and selenium showed lower incidence of retained placenta than in unsupplemented animals (Bendich, 1993). The Se requirement of dairy cattle is approximately 0.3 mg/kg diet (NRC, 2001).

Oxidative stress and Immunity

The immune system is responsible for protection against infection by pathogens such as bacteria, viruses, and protozoan parasites. Pathogens, recognized as invaders of the body and as “non-self”, are then destroyed by immune cells and their secretions. Antioxidants can play a role in enhancing the functions of the immune system for example carotenoids can enhance immune functions independently of any provitamin A activity. The mechanisms of immuno enhancement may include the capacities of a number of carotenoids to quench free radicals and singlet oxygen (Hidiroglou et al., 1995). The reduced concentration of antioxidants on the other hand affects the immune system and phagocytic activity of cells and results in an increase in the incidence of mastitis and puerperal diseases in pregnancy, delivery, and post-partum periods (Hadden, 1987; Goff and Horst, 1997).

Since vitamin E acts as a tissue antioxidant and aids in quenching free-radicals produced in the body, any infection or other stress factors may exacerbate depletion of the limited vitamin E stores from various tissues. The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immunosuppressive. Vitamin E also most likely has an immune enhancing effect by virtue of altering arachidonic acid metabolism and subsequent synthesis of prostaglandin, for cows fed dietary treatments with low or intermediate thromboxanes and leukotrienes. Under stress conditions, increased levels of these compounds by endogenous synthesis or exogenous entry may adversely affect immune cell function (Goff et al., 2002; AmitKunwar and Priyadarsini, 2011).

Peripartum immunosuppression is multifactorial but is associated with endocrine changes and decreased intake of critical nutrients (Goff et al., 2002). Circulating concentrations of vitamins A and E decrease around calving (Hogan et al., 1992). Decreased phagocytosis and intracellular killing by neutrophils occur in parallel with decreased DMI, and decreased circulating vitamin E (α-tocopherol) concentration (Hogan et al., 1992). Vitamin E is a fat-soluble membrane antioxidant that enhances the functional efficiency of neutrophils by protecting them from oxidative damage following intracellular killing of ingested bacteria (Herdt and Stowe, 1991).

Another important fat soluble vitamin is vitamin A, which has been called the anti-infective vitamin for many decades. Overt vitamin A deficiency has been associated with increased risk of infections. Vitamin A is critical for the development and functioning of T and B lymphocytes. Thus, low vitamin A status understandably results in a reduction of cell-mediated immune responses and decreased specific antibody responses following immunization (Hidiroglou et al., 1995).

The role of dietary Cu on the immune system in Holstein heifers was evaluated by (Halliwell and Gutteridge, 1999), where Heifers were fed a control diet containing 6–7 mg Cu/kg diet or the control diet supplemented with 20 mg Cu/kg diet beginning at 84 days pre-partum and continuing into lactation. Neutrophils from heifers fed the low Cu diet exhibited reduced killing of S. aureus when blood samples were collected at approximately 35 days post-partum.

Oxidative stress and periparturient period

The transition period for dairy cows is characterized by increased risk of several metabolic and infectious diseases. One important causal factor is impaired immune function in peripartum cows (National Research Council, 2001), and cows vitamin A and vitamin E status are component factors in immune function (Jerry, 2008).

The transition period is particularly important for health and subsequent performance of dairy cows, which are exposed to drastic physiological changes and metabolic stress. Relationships between BCS and incidence of metabolic diseases have been exhaustively reported. It has been hypothesized that an involvement of oxidative stress during transition period is the etiology of some diseases and disorders in dairy cows (McDowell, 2002).

It was stated that vitamin A and E, and β-Carotene levels decreased in pregnant cows, reaching the minimum values at the birth period, and started to re-increase in the post-partum period. The decrease resulted from the utilization of the compounds for the colostrum and milk synthesis accordingly to the growing of the foetus. The reduced concentration of antioxidants affects the immune system and phagocytic activity of cells and results in an increase in the incidence of mastitis and puerperal diseases in pregnancy, delivery, and post-partum periods (Hogan et al., 1992). Cows fed supplemental β-Carotene plus vitamins A have decreased milk SCC during lactation (Morrissey et al., 1994). Optimal blood concentrations of antioxidants may be greater during periods of stress, such as parturition. Plasma vitamin E concentrations in dairy cows are normally lowest when neutrophils functions are depressed during the periparturient period. The decrease in plasma α-tocopherol during the periparturient period is related to changes in consumption of vitamin E and to decreased transport capacity for the vitamin in plasma (Floyd, 1990).

Supplementation of 3000 IU of vitamin E/day during the transition period prevented a decline in neutrophil superoxide anion production and interleukin 1 (IL-1) production after parturition compared with control cows not supplemented with vitamin E (Politis et al., 1995). Rapid recruitment of neutrophils is critical for maximizing host defence mechanisms. Vitamin E supplementation at 3000 IU/day prevented a decrease in chemotactic responsiveness of neutrophils beginning at 2 weeks prior to and continuing for 4 weeks after parturition (Politis et al., 1996).

Oxidative stress and meat quality

Milk quality is usually defined in terms of mastitis. Milk

Table 3**.** Effects of vitamin supplementation on immune responses. DTH = Delayed-type hypersensitivity; IL-2 = interleukin-2; NK = natural killer. (Bendich, 1993).

Oral supplement	Duration/Days	Immune effects
Vitamin E, 800 IU	30	Enhanced DTH, IL-2, and proliferation
β-Carotene, 30 mg	70	Prevented UV-induced depression in DTH in young adults
β-Carotene, 45 to 60 mg	60	Increased markers for helper T cells, NK cells, and IL-2 receptors
Vitamin A, 800 1U	28	Increased markers for helper T cells and total T cells; enhanced proliferation
Multivitamin mineral supplement	16	Enhanced DTH. Enhanced proliferation

with a low somatic cell count (SCC) and visibly normal appearance (no clots) is considered high quality. Most fluid milk is judged to have a good flavour up to 14 d of storage but off-flavuor (OF) of milk is still an important problem, In certain situations, OF can be detected in milk almost immediately following milking. Some antioxidants (for example, Cu) can increase susceptibility to oxidized flavour development, others reduce susceptibility. Milk with high concentrations of Cu is extremely susceptible to the development of OF, especially if the milk also is high in polyunsaturated fatty acids (Malbe et al., 1995).

Lipid oxidation is a major cause of deterioration in the quality of muscle foods. Oxidation leads to the production of off-flavours and odours, reduction of poly-unsaturated fatty acids, fat-soluble vitamins and pigments, lower consumer acceptability, and the production of compounds such as peroxides and aldehydes which may be toxic. Lipid oxidation is a free-radical-mediated process which occurs in raw muscle, and especially in cooked muscle. The process is believed to be initiated at the membrane level owing to the oxidation of the highly unsaturated membrane lipids (Floyd, 1990). Different antioxidants supplementation have different effects on the immune status of animals, see Table 3

Future work

Limited research with dietary Cu and immunity has been conducted in periparturient dairy cows. The correlation between antioxidative capacity and macro-elements must be taken into consideration in the future work. Also methods to enhance antioxidative capacity in dairy cattle using different herbal products are a promising field to be studied.

CONCLUSION

It is clear that the antioxidative capacity of animals in genaeral and especially dairy cattle screens the total animal health status, for this reason many researchers try to reduce health problems and increase the productivity of dairy cattle through manipulating (increasing) the antioxidative capacity, which reduces by the way the oxidative stress. More research is to be done to determine to which extent this manipulation is healthy.

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