Oxidative stress and health disorders in periparturient dairy cows

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INTRODUCTION

Dairy cattle are susceptible to increased incidence and severity of several infectious and metabolic disease during the periparturient period (Kelton et al., 1998). Health disorders occurring during this time may greatly impact the productive efficiency of dairy cattle in the ensuing lactation. A major contributing factor to increased health disorders is thought to be oxidative stress that contributes to dysfunctional immune and inflammatory responses. Oxidative stress occurs when cellular macromolecules (including lipids, proteins, and DNA) are damaged as a consequence of an imbalance between oxidants and antioxidants (Valko et al., 2007). Oxidants consist of reactive oxygen metabolites that are capable of oxidizing macromolecular substrates (Villamena, 2013). Antioxidant defenses scavenge pre-formed reactive oxygen metabolites or delay the formation of oxidants (Valko et al., 2007). At physiological concentrations, oxidants are essential for intracellular signaling and other beneficial cellular processes. When oxidants form at concentrations that overwhelm antioxidant defenses, however, oxidative stress can occur, resulting in damage to immune cell populations needed to protect against infectious pathogens. Indeed, the progressive development of oxidative stress in transition dairy cattle is thought to be a significant underlying factor leading to dysfunctional inflammatory responses associated with several economically important diseases such as mastitis and metritis. Understanding more about the underlying causes of oxidative stress during the periparturient period may facilitate the design of nutritional regimes that will reduce the severity and duration of disease as a function of dysfunctional inflammatory responses.

WHAT IS OXIDATIVE STRESS?

Oxidative stress is generally defined as excessive oxidant challenge that causes damage to cellular macromolecules including nucleic acids, proteins, and lipids (Sies et al., 2017).

Oxidative damage to these cellular constituents is now recognized as a significant pathophysiological event leading to many diseases processes in both human and veterinary medicine. For example, potent pro-oxidants can damage to essential all components of the DNA molecule and lead to gene mutations and abnormal protein synthesis (Valko et al., 2006, Halliwell and Gutteridege, 2007). Indeed, oxidant-induced mutations of nucleic acids is a major underlying cause of many different forms of cancer in humans. Other targets of oxidative damage include the amino acid side chains of proteins. Cysteine and methionine residues of proteins are particularly susceptible to oxidation and can lead to the reversible formation of mixed disulfides between a variety of thiol groups (Eaton, 2006). Oxidative modification of protein thiol groups can regulate the function of proteins and influence multiple metabolic, enzymatic, and receptor-mediated processes of cells. Lipids, and polyunsaturated fatty acids in particular, are most susceptible to the impact of excessive pro-oxidant challenge due to the multiple double bonds structure. Oxidative attack of membrane phospholipids causes a lipid peroxidation chain reaction. Since lipid peroxidation is a self-propagating event, the initial oxidation of only a few lipid molecules can eventually result in significant tissue damage. Peroxidation of lipids within cellular membranes can lead to changes in fluidity and cause damage to intracellular organelles. As such, the destruction of membrane lipids and the end-production of lipid peroxidation chain reactions have been implicated in immune dysfunction that leads to several health disorders of dairy cattle including mastitis and metritis (Sordillo and Raphael, 2013).

Although excessive accumulation of pro-oxidants can cause significant damage to cells and tissues, it is important to distinguish between the concepts of oxidative stress and normal redox biology. Redox reactions are characterized as the transfer of electrons where one chemical species is undergoing oxidation (loss of electrons) while the other is being reduced (gain of electrons). Indeed, redox reactions play important roles in many aspects of biology and medicine. The expression of moderate amounts of pro-oxidants, for example, are critical for many normal physiological responses including those regulated by redox sensitive signaling pathways such as NFkB, NrF2, and the MAPKs. The ability to maintain moderate amounts of pro-oxidants is now recognized as an essential way for cells to respond to the microenvironment and regulate essential

physiological functions such as the immune system (Halliwell and Gutteridge, 2007, Sordillo and Aitken, 2009).

WHAT ARE PRO-OXIDANTS?

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that contribute to this pro-oxidant challenge. In general, the term ROS is used as a collective term to include a wide variety of radicals and non-radical molecules including superoxide, hydrogen peroxide, hydrogen radical, singlet oxygen, peroxyl radical, alkoxyl radical, lipid hydroperoxides, peroxynitrite, hypochlorous acid and many others (Li et al., 2016). Formation of ROS occurs as byproducts of normal cellular metabolism of oxygen. A major source of ROS are the free radicals formed as a normal end product of cellular metabolism arising from either the mitochondrial electron transport chain or from stimulation of NADPH (Valko et al., 2007). The majority of ROS found in most healthy tissues likely results from increased cellular metabolism and energy generation by the mitochondria. The generation of ATP in the mitochondria through the Krebs' cycle and the electron transport chain generates O_2^- and H_2O_2 as byproducts will increase during times of enhanced metabolism. Approximately 1-3 % of electrons during the mitochondrial oxidative phosphorylation reactions are transferred are to oxygen to form superoxide (O_2^-) (Holmstrom and Finkel, 2014). Reactive metabolites also increase during regulated inflammatory processes to levels necessary for effective innate and adaptive immune functions (Sordillo and Aitken, 2009). For example, the NADPH oxidase system found in phagocytic immune cells generates significant amounts of ROS during the respiratory burst to kill microbial pathogens (Babior, 1999). There are various cellular enzymes that also can contribute to the overall ROS pool during inflammation. Some of these include xanthine oxidoreductase, nitric oxide synthase, cytochrome P450 monooxygenase (CYP450), lipoxygenase (LOX), and cyclooxygenase (COX)(Sordillo and Aitken, 2009, Mavangira and Sordillo, 2018). For example, membrane phospholipids can undergo enzymatic oxygenation through the COX, LOX, or CYP450 pathways resulting in not only a highly reactive lipid hydroperoxide, but also superoxide anion as a byproduct of the reaction (Raphael and Sordillo, 2013). The production of some ROS from these various sources is essential for the regulation of normal cellular processes including those that control immune and inflammatory responses (Sordillo, 2018).

WHAT ARE ANTIOXIDANTS?

The production of ROS during oxygen metabolism has necessitated the development of antioxidant defenses that can effectively trap reactive intermediates before causing oxidation to macromolecules or to reduce biomolecules that already have been oxidized. As such, antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecules (Halliwell and Gutteridge, 2007). Antioxidant defenses are diverse, can be either synthesized *in vivo* or derived from the diet, and are localized transiently throughout tissues and different cell types. The various antioxidant defense mechanisms also can be classified on the basis of several criteria, such as on their solubility in lipids and water or on their chemical and physical characteristics (i.e., enzymatic or nonenzymatic) (Sordillo and Aitken, 2009). Among the most efficient antioxidants are those enzymes that can directly catalyze the reduction of ROS. For example, the dismutation of superoxide to H_2O_2 and $^3O_2$ or H_2O_2 to H_2O and $^3O_2$ are catalyzed by superoxide dismutase and catalase, respectively. The selenium-dependent antioxidant enzymes, however, are the most widely studied systems with respect to dairy cattle health and well-being. Many of the beneficial health effects of Se are mediated by antioxidant selenoenzymes, such as glutathione peroxidase and thioredoxin reductase, which have selenocysteine residues incorporated into their active sites (Sordillo, 2013). The non-enzymatic antioxidants are represented by tocopherols, ascorbic acid, carotenoids, lipoic acid, and GSH to name just a few (Papas, 1999, Halliwell, 2007). Vitamin E, and □-tocopherol specifically, is a predominant antioxidant found in biological membranes. The tocopherols are able to disrupt radical chain reactions that lead to auto-oxidation of adjacent membrane-associated fatty acids. For example, vitamin E can act as a scavenger of both lipid radicals and lipid peroxy radical by donating a hydrogen ion with the formation of a tocopheroxyl radical. The tocopheroxyl radical is then regenerated back to its reduced form by vitamin C. Ascorbic acid (vitamin C) is a water-soluble antioxidant that plays a key role in maintaining the redox state of cells. In addition to recycling vitamin E, ascorbic acid can reduce several other oxidized biomolecules and act as a direct scavenger of free radicals. Thus, ascorbic acid plays a key role in maintaining the redox state of cells in addition to functioning as a free radical scavenger for other oxidized biomolecules (Sordillo and Aitken, 2009). The vitamin A precursor, □-carotene, is another important free radical scavenger. The carotenoids are

especially effective at quenching singlet oxygen and can prevent the subsequent formation of secondary ROS. Lipoic acid is a component of the pyruvate dehydrogenase complex and has a central role in energy metabolism. However, lipoic acid also can function as a metal chelator and ROS scavenger. The reduced form of lipoic acid, dihydrolipoic acid, can further prevent ROS accumulation by recycling vitamins C and E (Sordillo, 2016).

Although micronutrients are essential components of the antioxidant defense network, it is significant to note that plasma concentrations of vitamins and mineral tend decrease in dairy cows around the time of parturition. The decrease in available serum-derived micronutrients is likely a combination of reduced dietary intakes as well as increased rates of utilization associated with metabolic stress in transition cows (Sordillo, 2016). Concentrations of vitamins and minerals that should be supplemented to dairy cattle diets should consider not only what is adequate to maximize production efficiency, but also what is required by the immune system to prevent oxidative stress and optimize immune cell functions.

OXIDATIVE STRESS IN TRANSITION COWS

Several studies have documented important changes in the antioxidant potential and prooxidant status in the transition dairy cattle (Bernabucci et al., 2002, Castillo et al., 2005b, Sordillo et al., 2007). Antioxidants can be found as water-soluble or lipid-soluble molecules that are localized transiently throughout tissues and various cell types (Drackley, 1999). Given the multiplicity of antioxidant pathways, their centrality in the prevention of oxidative stress, and the influences of diet on overall antioxidant capacity, it is important to be able to quantitatively measure the total antioxidant capacity or antioxidant power within biological specimens. Impairment of blood and milk leukocyte function has long been linked with increased susceptibility to mastitis around the time of calving when oxidative stress is increased. However, remarkably few studies have examined in any detail the redox status of important immune cell populations during this time. Results from our laboratory indicate that the antioxidant potential of isolated peripheral blood mononuclear cells (PBMC) remained relatively constant from 3 wk prior to calving and through calving, but dropped significantly (P <0.05) by 21 days in milk

(Figure 1). These finding are consistent with reports in both humans and dairy cows that showed a relationship between the physiological changes during the periparturient period with a loss in overall antioxidant potential in several different tissue compartments (Gitto et al., 2002, Bernabucci et al., 2005, Castillo et al., 2005a).

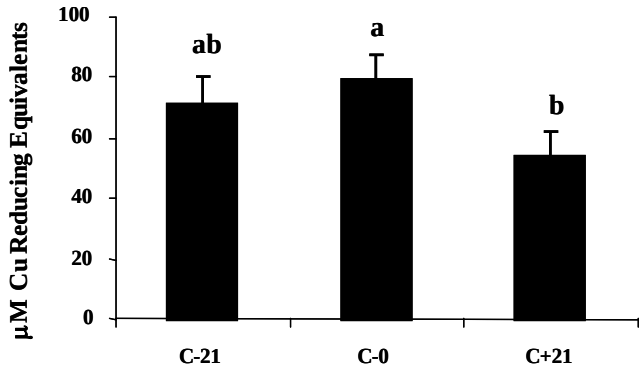

Figure 1. An assay used to determine total antioxidant potential of PBMC obtained from transition cows was based upon the reduction of Cu^{++} to Cu^{+} by the combined action of all antioxidants present in the sample. Changes in total antioxidant potential of white blood cells obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means ± SE. [a,b]Bars with different superscripts differ (P< 0.05) (Sordillo et al., 2007).

Lower antioxidant potential as a consequence of lactation stage can result from an excess accumulation of ROS, a depletion of antioxidant defenses, 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. We showed that measurement of lipid hydroperoxides increased significant (P < 0.05) from calving through the first 3 wk of lactation when compared to the pre-partum measurements (Figure 2). 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 and through 25 DIM (Bernabucci et al., 2005, Castillo et al., 2005b).

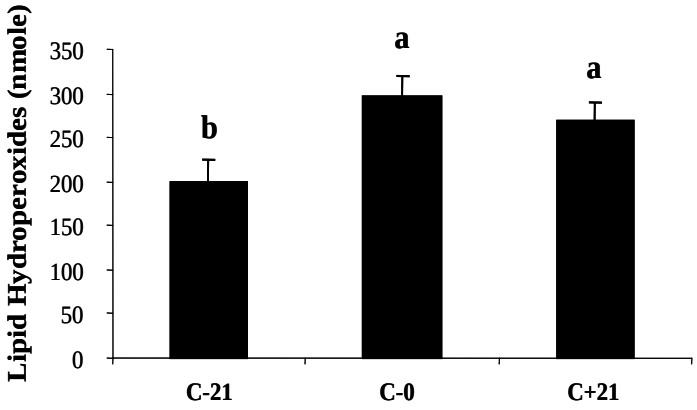

Figure 2. Changes in lipid hydroperoxide levels in plasma samples obtained approximately 21 d prior to calving (C-21), at calving (C-0), and 21 d after calving (C+21). Data are expressed as least square means ± SE. [a,b]Bars with different superscripts differ (P< 0.05) (Sordillo et al., 2007).

ASSESSING OXIDATIVE STRESS

A major obstacle in mitigating the detrimental impact of oxidative stress on transition cow health is the lack of an accurate way to assess the amount of oxidative injury that is associated with subsequent disease outcomes. Although all cows will experience some degree of oxidative stress around the time of calving, only those cows with severe oxidative injury will succumb to subsequent health disorders (Sordillo and Aitken, 2009). To date, however, there is no standardized way of measuring oxidative stress in cows or assessing critical thresholds of oxidative injury that will lead to health disorders. Primary targets of free radical-induced oxidative damage are cellular lipids that are converted to lipid peroxides. Isoprostanes are a family of prostaglandin-like compounds formed through the free radical-induced peroxidation of arachidonic acid in cellular membranes (Mavangira et al., 2016, Sordillo, 2018). In human medicine, measurement of plasma 15-F_{2t}-isoprostane has proven to be ideally suited for assessing lipid peroxidation and concentrations increased proportionate to oxidative injury and disease incidence (Milne et al., 2015). Indeed, chromatography-based measurement of plasma 15-F_{2t}-isoprostane is considered the gold standard method of assessing oxidative stress in humans (Klawitter et al., 2011). Our group was the first to use chromatography-based analytics (liquid chromatography tandem mass spectrophotometry; LC/MS/MS) to measure 15-F_{2t}-isoprostane concentrations in bovine plasma during the transition period and during mastitis (Mavangira et al., 2016, Kuhn et al., 2018). Unfortunately, threshold concentrations of plasma 15-F_{2t}-isoprostane that could be used as a biomarker for subsequent disease susceptibility in transition dairy cows are currently unknown. The

availability of tools to detect health risk early enough in the transition period will allow time to intervene effectively with antioxidant micronutrients before diseases occur.

CONCLUSION

Oxidation and the production of free radicals are an integral part of aerobic metabolism. Considerable evidence supports the contention, however, that oxidative stress during the periparturient and early lactation period may contribute to a number of health disorders in dairy cattle. Dairy cattle management practices and emphasis on genetic selection to maximize milk production has increased the metabolic stresses associated with parturition and the onset of copious milk synthesis and secretion. The antioxidant requirements of cows will likely increase as production demands continue to escalate within the dairy industry. The performance of high producing dairy cattle can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transitions cows. Innovative approached are needed to enhance the antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands. A better understanding of how antioxidants may preventative immune dysfunction and prevent oxidative damage to host tissues may lead to more effective strategies to control health disorders in the transition dairy cow. However, strategies to mitigate the detrimental impact of oxidative stress on dairy cattle health are limited by available methodologies for accurately evaluating oxidative damage.

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