Association between acute phase response, oxidative status and mastitis in cows

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Introduction

Inflammation of the mammary gland is a widespread disease in dairy cattle and can be considered one of the greatest health issues in dairy cows (Peeler et al., 2002). It is a physiological, protective response and a reaction to any kind of injury or mammary tissue destruction. Inflammation of the mammary gland caused by various microorganisms, including bacterial pathogens, is characterized by pathophysiological changes in body, udder and milk (Cvetnić et al., 2016). Clinical and subclinical forms can be distinguished (Wu et al., 2005; Đuričić et al., 2014), with an estimate that the ratio between clinical and subclinical forms is 15 to 40 (Cook et al., 2002).

The initial response of the body to foreign agents (e.g. bacteria) is usually localised at the site of infection and involves neutrophils, macrophages, and other immune cells. This response includes the release of small proteins, known as cytokines, from macrophages and other neutrophils, which act as intercellular messengers to regulate cellular response (Wesson et al., 2000). Some researchers have found that the degree of mammary gland tissue damage can be evaluated by the systemic release of inflammatory mediators (Perl et al., 2003). The synthesis and release of plasma acute phase proteins (APP), [1-proteinase inhibitor (1-PI), ceruloplasmin (Cp), complement components, and serum amyloid-A (SAA)], are regulated by inflammatory mediators (Raynes, 1994). Interleukin-6 (IL-6) is a pro-inflammatory cytokine that appears to be essential for initiating the systemic inflammatory response (Riollet et al., 2000), and mediating APP production (Hayes, 1994).

Antioxidative status helps to protect mammary gland cells against free radical damage and is a key factor for normal milk production (Turk et al., 2011). The transition metal ions play an important role in the antioxidative and prooxidative activity accompanying an inflammatory response (Kleczkowski et al., 2002). Nutritional factors are believed to play an important role in increased sensitivity to cellular oxidative damage in cows (Kleczkowski et al., 2003a). For example, lowering the daily dietary supply of zinc to one-third to one-tenth

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of the values required to fully meet the demands of cattle, results in a 20% decrease in the activity of superoxide dismutase (Underwood and Suttle, 1999). An increased concentration of zinc or molybdenum in fodder leads to a limitation of copper absorption, upon which SOD activity depends (Stehbens, 2003). On the other hand, copper possesses dual properties. In its ionized form, the element as a transitory metal is a strong prooxidant. However, when in the composition of superoxide dismutase, it plays an antioxidant role. Both copper and zinc can influence and modulate inflammation processes, indicating the multidirectional influences of transition metal ions (Kleczkowski et al., 2002). This can result in providing a stimulating factor or inhibitory antioxidant activity in liquids or tissues, and can have a modulating influence on inflammation states (Chi Chen et al., 2001).

An increased concentration of free radicals can stimulate the release of copper reserves in the liver, which are indispensable for enzymatic activities and the copper-dependent anti-oxidative mechanisms (Puig and Thiele, 2002). However, inadequate Cu levels may influence the magnitude of tissue injury that occurs during inflammation (Scaletti et al., 2001). Many other mechanisms that are yet unknown should not be excluded. Recent investigations have demonstrated a novel property in copper homeostasis for XIAP (X-linked inhibitor of apoptosis protein) acting through the ubiquitous copper regulating factor (MURP1). It has been observed that MURP1 plays a role in the regulation of copper homeostasis in mammals. The mechanism of MURP1, involving binding to XIAP, is observable by caspases (Bratton et al., 2002). Copper is important to the enzymatic activity of many housekeeping genes, including Cu and Zn superoxide dismutases. Copper also plays a role in pathophysiology in oxidative stress damage, by its ability to induce free radical formation (Burnstein et al., 2004).

Acute phase response

Acute phase response is the inflammatory response of the host occurring shortly after any tissue damage. Acute phase proteins occur in cattle and are known to increase in concentration following infection, inflammation, and trauma. Monitoring these proteins is an effective and valuable tool in the diagnosis of disease in cattle. It was recently discovered that acute phase proteins are present in milk from cows with inflammation of the mammary gland and the mechanism for this pathophysiological process and its potential usefulness in diagnosis are currently the subject of study. The proteinases that play a role in inflammatory tissue injury include the gelatinases, collagenases stromelysins, and metalloproteinases (Eckersall et al., 2001; Perkins et al., 2002).

The clinical form of mastitis may be preceded by the acute phase response (APR). The initiation of APR occurs at the site of injury. APR occurs immediately and helps to maintain physiological homeostasis after injury. APR is a non-specific reaction; the cause of injury can be metabolic (oxidative), toxic, immunological, or traumatic. The role of APR is to prevent further injury to an organ, to isolate local injury, to remove harmful molecules and debris and to destroy microorganisms. Additionally, it helps to activate the repair processes necessary for returning the organ to its normal function. The inflammatory response occurs in the vascularized connective tissue including circulating cells, blood vessels, cellular, and extracellular constituents. The inflammatory process can be characterized by the exudation of fluids and proteins and by the migration of leukocytes, especially

neutrophils. APR is not dependant on the origin of inflammation; it is a physiological state that occurs in the initial stages of any inflammatory process. In animals suffering from an acute form of mastitis, the diagnosis and the choice of treatment depends on observation of both local and systemic clinical signs, which are in fact consequences of APR. It is therefore important for veterinarians to know and understand the nature of this phenomenon, in order to correctly interpret the results of the physical examination. APR is responsible for changes in the concentration of a wide range of serum or plasma proteins, including antioxidative indices, which are associated with the host response. These changes result from alterations in protein synthesis in the liver. Malnutrition can attenuate APR. However, feed restriction does not influence serum TNF-α concentrations. Therefore, we can consider that cattle diseases, including inflammation of the mammary gland, may result from decreased bioavailability of Cu and Zn.

The acute phase proteins are produced by the liver and other tissues in response to inflammation or a toxic challenge. During the acute phase, proteins can release inflammatory mediators such as cytokines (interleukin, tumour necrosis factor), lipid mediators, vasoactive amines (histamine, serotonin), uterocalin, haptoglobin, serum amyloid A, reactive oxygen species, proteases, nitric oxide and products of the complement and coagulation cascades (Ohtsuka et al., 2001; Gračner et al., 2006). Cytokines, the pro-inflammatory mediators, are multipotent polypeptides produced by various cell types. The following are examples of cytokines: interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor-a (TNFa), and interferon-γ (IFN-γ) (Byrum et al., 1999). In the local reaction area, cytokines are able to activate stromal cells, such as endothelial cells and fibroblasts, to start secondary release. Two transcription factors, activator protein-1 (AP-1) and necrosis factor $_{k}$B (NF-$_{k}$B), can both be regulated by intracellular reactive oxygen intermediates (ROI). Metallothioneins (MT) and superoxide dismutase (SOD) induced by tumour necrosis factor (TNF) and interleukin 6 (IL-6) exert antioxidative activity during APR. This process protects the tissues of the mammary gland from oxidative injury. Haptoglobin and serum amyloid A are the most sensitive acute phase proteins. Increased levels of these proteins are found in serum and milk of cows during acute clinical mastitis and subclinical mastitis (Grönlund et al., 2003; Turk et al., 2012). They may be associated with different cytokine profiles, which in turn may be dependent on the duration and type of inflammatory stimuli in the mammary gland. Finally, *in vitro* studies indicate that bacterial factors cause apoptosis in the bovine mammary gland cell line and can activate pro-apoptotic factors-bax and interleukin-1ß (Kerr and Wellnitz, 2003).

Inflammatory mediators are the cause of both local and systemic inflammatory reactions. The local reactions comprise an increase in capillary permeability, and passage or infiltration of leucocytes to the site of inflammation (Kluciński et al., 1997). Recent studies have demonstrated an interaction between leukocytes and specific endothelial cell adhesion molecules. These molecules, which include many plasma proteins such as transport proteins, proteinase inhibitors, and other binding proteins, are allowed to migrate by circulation to sites of tissue injury due to increased capillary permeability. Adhesion of leukocytes to the endothelium helps to regulate the migration of leukocytes to the site of inflammation. The adhesion surface receptors of capillary endothelial cells and leukocytes express a response to inflammatory mediators. Macrophages

and neutrophilic granulocytes have an important function in eliminating foreign antigens. This function depends on lysosomal hydrolases, phagocytosis and oxygen radicals.

Reactive oxygen molecules can react with inflammatory mediators (cytokines). The hydroxyl radical is often responsible for oxidative modifications of proteins. On the other hand, cytokines can exert antioxidative reactions during acute phase inflammation of the mammary gland. Peroxynitrite ($ONOO^-$) has recently been implicated as a major cytotoxic agent, and is converted from nitric oxide (NO), two oxygen radicals, and the superoxide anion (O_2^-) (Freeman, 2002). Antibiotics also have the ability to influence inflammatory mediators, which in turn can modify their activity.

Prooxidant status

Oxidation provides energy for vital functions to almost all cells in the body. Nearly 95–98% of oxygen is reduced during aerobic metabolism, whereas the rest may be converted to oxidative by-products, reactive oxygen species (ROS), which can damage DNA and contribute to degenerative changes, including aging and inflammation (Harrison, 2002).

Some studies have defined prooxidants or free radicals as compounds that can destroy tissue, which are similar to unstable solar systems, as they lack a planet in their outer orbit. Free radicals are molecules with one or more unpaired electrons that are formed during normal metabolic cell function. However, they are also produced by the body in response to inflammatory reactions, infectious conditions, environmental pollutants, sunlight, and radiation exposure, high ingestion of fats and even overly aggressive exercise or prolonged physical activity. Free radicals may be also produced during some pathological processes. The hydroxyl radical is one of the most reactive among these compounds. It is formed via a mechanism called the Fenton reaction, which involves the catalysing breakdown of hydrogen peroxide by transition metals (Kleczkowski et al., 2002). The activation of white cells is a common source of free radical production, whereas one of the most common mechanisms in the production of free radicals during disease is the generation of the superoxide anion by white blood cells (McCord, 1994). Leukotrienes and prostaglandins are important mediators in inflammation; they are synthesised by cyclooxygenase and lipoxygenase enzymes, which also produce free radicals. This is part of the synthetic process, and in this way, these radicals can inactivate enzymes. Over 100 diseases have been associated with reactive oxygen intermediates (free radicals), including inflammation of the mammary gland, bovine respiratory tract diseases, and bovine retained placenta (Kankofer, 2002).

The attack of lipid molecules by free radicals leads to lipid peroxidation, which is dangerous for lipid membranes (Kankofer, 2001a; Kleczkowski et al., 2002; Folnožić et al., 2015). They also affect protein synthesis and the inflammatory response. Reactive oxygen species (ROS) cause peroxidative damage to proteins. Protein peroxidation leads to modification of amino acid residues and to the loss of biological activity. When proteins are under attack, free radicals can modify their physico-chemical properties (Kankofer, 2001b). Uterocalin might decrease oxidative and carcinogenic activity in the mammary gland (Nilsen-Hamilton et al., 2003). Attack of free radicals on nuclear DNA occurs during normal cell metabolism. Increased rates of DNA damage result from either high prooxidant status or ineffective repair (Bashir et al., 1993). Damage to DNA can lead to serious alterations, resulting in mutations. On the other hand, gene expression may be al-

tered by the activation or inhibition of transcription factors. Vitamins A, E, C, D, and β-carotene in excessive doses can be prooxidative and kill healthy cells. Vitamin A deficient cattle have depressed activity of natural killer cells, decreased antibody production, decreased responsiveness of lymphocytes to mitogenic stimulation, and increased susceptibility to infection (Rajaraman et al., 1998).

It is widely known that the presence of transition metal ions can catalyse the peroxidation of organic biomolecules and production of reactive oxygen species or reactive nitrogen species. Selenium is highly prooxidative at elevated concentrations. However, increased occurrence of mastitis and chronic renal failure is associated with low selenium levels. This action may be observed in an increase of somatic cell counts in milk. Some of this activity may also be associated with high doses of vitamin E (Smith et al., 1985). Some studies have shown that high iron stores impose a risk of many diseases, among them inflammation and heart disease (McCord, 1994). A deficiency of iron appears primarily to affect antibody formation associated with B-cell lymphocytes, although studies with T cells have shown a slight reduction in rosette-forming cells (Kuvibidila and Sarpong 1990). Low dietary intake of copper may produce prooxidant effects (Strain, 1994). Some studies have found a relationship between low copper levels and the development of mastitis associated with factors such as ceruloplasmin. They also found that the antioxidant function of copper likely has greater practical significance than its prooxidant activity (Johnson and Fischer, 1994).

Antioxidant status

In response to prooxidative processes both in constitutional liquids and in cells, the antioxidant mechanisms begin to fulfil their protective function against the unfavourable results of oxidative stress. Antioxidants function in several ways: they may reduce the energy of free radicals, stop free radicals from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals (Turk et al., 2011). We can evaluate the antioxidative activity in cow's blood using direct and indirect antioxidant metabolic indices. The most important parameters are: glutathione peroxidase activity, superoxide dismutase activity, total antioxidant status, and concentrations of ascorbic acid, ceruloplasmin, albumin, copper and molybdenum as well as zinc indices. Deficiency in any of these components can cause a reduction in the antioxidant status, which is important in controlling several diseases, e.g. retained placenta and inflammation. Antioxidative activity in cow's blood during inflammation of the mammary gland has been recently studied by proteomic analysis (Turk et al., 2012). It would be advantageous to determine the risk of occurrence of oxidative stress and mastitis and the efficacy of treatment of dairy cows (Kankofer, 2002; Kleczkowski et al., 2003a; Turk el al., 2017).

There are numerous antioxidants within the body. Each plays a role in ridding the body of excessive free radicals. Antioxidants may be classified into three forms: primary antioxidants, i.e. SOD, GSH-PX and metal binding proteins (e.g. ceruloplasmin); secondary antioxidants, i.e. vitamins E and C, and beta carotene; and tertiary antioxidants, i.e. methionine sulphoxide reductase.

Primary antioxidants

The activity of many antioxidative mechanisms depends on the presence of trace elements such as copper, zinc, molybdenum, and many others. Micronutrients are essential for various biochemical pathways (Kleczkowski et al., 2001; 2002; 2003b). The primary

antioxidants are SOD, GSH-PX, CP, and catalase (CAT) (Boulanger et al., 2002).

In the past, the name of the key antioxidant protein was erythrocuprein (haemocuprein). This was later renamed superoxide dismutase (SOD), isolated from erythrocytes as the Cu-Zn form of SOD, which was identified in mitochondria (McCord and Feridovich, 1969). SOD activity is important in the diagnosis of many diseases, e.g. the differences in SOD activity between normal and malignant cells may form the basis for a diagnostic test for cancer. Moreover, SOD activity can have a relationship with several diseases, such as mastitis, rheumatoid arthritis, respiratory infection, hypocuprosis, cardio-vascular diseases, and hepatitis (Markiewicz, 2004). Some studies have found that SOD acts in concert with two other antioxidant enzymes, catalase and glutathione peroxidase. The combined action of these enzymes forms one metabolic pathway for protection against excessive oxidation (Hayden et al., 1990).

Glutathione peroxidase is an Se-dependent enzyme, and has been found in most animal and human tissues. This enzyme may protect against oxidative tissue damage (Anderson et al., 1978). The concentration of glutathione peroxidase is less easily affected by sudden dietary changes, and therefore this enzyme may be used to assess selenium levels and to give a more precise reflection of selenium status than measuring the level of selenium itself. GSH-PX plays an important role in the aging process, which is associated with the decrease of GSH-PX activity. This role of GSH-PX is also related to several diseases. For instance, some forms of cancer are associated with GSH-PX activity and selenium concentrations (Harman, 1988). Selenium has a protective effect against several diseases, including inflammation, though this effect does not appear when the selenium concentration is low. Low activity of GSH-PX and low selenium concentrations are observed in mastitis and rheumatoid arthritis (Ali-Vehmas et al., 1997), while a high concentration of selenium and GSH-PX activity may reduce the incidence of certain cardio-vascular diseases (Young et al., 1984). Dicarbonyl compounds, such as methylglyoxal and 3-deoxyglucosone, the major products of the glycation reaction, induce heparin-binding growth factor (HB-EGF) by increasing intracellular peroxide concentration. This mechanism, in which dicarbonyls increase peroxide concentrations, can inactivate GSH-PX (Taniguchi, 2002).

The major antioxidant protein of serum is ceruloplasmin. Ceruloplasmin levels have a protective effect against iron-catalysed radical reaction due to its ferroxidase activity (Williams et al., 1980). Ceruloplasmin has been widely used as an indicator of inflammation, especially of acute phase reactivity. It is increased, as are antiproteases, due to their increased synthesis during inflammation (Milanino et al., 1989). Some studies have shown that during inflammation, the injury site is protected from the attack of free radical reaction by antioxidants such as ceruloplasmin. However, the anti-inflammatory effect of this copper-containing enzyme leads to a therapeutic intervention in many diseases associated with inflammatory reactions (Nathan and Jonathan, 2002).

Therefore, this phenomenon is very important both for recognition and clinical practice. However, further experiments on antioxidative activity in the blood of cows during inflammation of the mammary gland are recommended.

Secondary antioxidants

Antioxidative non-enzymatic defence mechanisms consist of molecules of various chemical properties that are able to neutralise ROS.

Vitamin E (tocopherol) is a well known antioxidant that protects the cell membranes of all tissues from microstructural destruction in the aging process and several diseases, e.g. inflammation, arthritis, and cancer (Esterbauer et al., 1991). Vitamin E also plays a protective role against the harmful effects of pollution.

Vitamin C (ascorbic acid) acts as an antioxidant in large doses (Chaiyotwittayakun et al., 2002; Vitetta et al., 2002). Vitamin C may also indirectly improve immune responses by helping to maintain tissue levels of vitamin E. On the other hand, ascorbic acid becomes a dehydroascorbate (prooxidant) during the free radical neutralization process and requires glutathione and vitamin E acting together to regenerate it back to its antioxidant status (Weiss et al., 2004).

Beta-carotene, the most nutritionally active carotenoid, comprises 15–30% of the total serum carotenoids (Jukola et al., 1996). Recent studies have shown an inverse relationship between the concentrations of beta-carotene and reactive singlet oxygen in cells. They also found that large doses of beta-carotene can block free radical-mediated reactions. Beta-carotene has a protective effect against the isolation of lipid membranes from peroxidation, whereas unsaturated fatty acids in cell membranes are prime targets for free radical reactions (Sies et al., 1992). Some studies have found that tocopherols effectively protect beta-carotene against free radical auto-oxidation; in the presence of tocopherol, carotene acts synergistically in preventing oxidation (Esterbauer et al., 1991). Other studies have correlated beta-carotene with protection against certain forms of mastitis and cancer, particularly lung cancer, where an excess of beta-carotene increases lung cancer (Mayne et al., 1996). Administration of carotenoids such as beta-carotene to animals suffering from skin cancer may lead to decreased tumour growth and a lower tumour burden (Nishono, 1995). Moreover, apart from the secondary antioxidants described above such, other components such as glutathione, urate, bilirubin, ubiquinones, albumins, carnosine, carotenoids, and riboflavin also belong to this group (Fenner and Schiesser, 2004).

Tertiary antioxidants

Methionine sulphoxide reductase is an enzyme that repairs DNA. Recent studies have found a group of enzymes known as the final line of antioxidant defence (Fabiani et al., 2001). One such enzyme is methionine sulfoxide reductase, which regenerates methionine residues within oxidized proteins and restores their function (Harper, 2000).

Conclusions

Changes in the serum activity of inflammatory mediators in the acute phase response have been observed in inflammation of the mammary gland in dairy cattle. The acute phase response can be responsible for changes in concentrations of a wide range of serum or plasma proteins associated with the host response, including antioxidative indices. Inflammation of the mammary gland might play an important role in increasing the risk of vascular events associated with increased oxidative stress. Transition metal ions play an important role in the antioxidative and prooxidative activity accompanying an inflammatory response. Interaction of oxidative stress and inflammatory response is possible in the inflammation of mammary gland.

Abstract

There are many studies exploring the topic of acute phase response and oxidative status in inflammation of the mammary gland of cows. However, many phenomena are relatively

poorly understood. Mastitis is associated with significantly higher concentrations of inflammatory and oxidative mediators in the cells and blood. Results of experiments have shown that there are evident changes in serum interleukins (IL), acidglycoprotein (alpha1AG), tumour necrosis factor (TNF), and haptoglobin (Hp). Thus, local and systemic inflammation might play important roles in increased mammary oxidative stress. Reactive oxygen species (ROS) have been implicated in the pathogenesis of a variety of diseases, including mastitis and in transgenic technology leading to the production of new bacterial proteins, which is very important in the prevention of mastitis. An interaction has also been observed between inflammatory and oxidative mediators. These results suggest an important role played by the acute phase response and oxidative status in inflammation of the mammary gland.

Key words: *acute phase response, oxidative status, mastitis, cow*

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Povezanost između akutno faznog odgovora, oksidativnog statusa i mastitisa u krava

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Postoje brojna istraživanja u području akutno faznog odgovora i oksidativnog statusa u upalnom procesu mliječne žlijezde krava. Međutim, mnoge pojave su manje poznate. Mastitis je povezan sa značajnim porastom koncentracije upalnih i okidativnih medijatora u tkivima uključujući i krv. Rezultati istraživanja su pokazali da ne postoje značajne promjene u serumskim koncentracijama interleukina (IL), acidoglikoprotein (alpha1AG), faktor nekrotiziranja tumora (TNF) i haptoglobina (Hp). Slijedom toga, lokalni kao i sistemski upalni odgovor mogu imati važne uloge u porastu oksidativnog stresa mliječne žlijezde. Reaktivni kisikovi spojevi (ROS) uključeni su u patogenezu različitih bolesti, uključujući i mastitis kao i u transgeničnu tehnologiju kojom se proizvode novi bakterijski proteini, od velikog značenja u prevenciji mastitisa. Isto se tako može uvrditit interackcije između upalnih i oksidativnih medijatora. Ti rezultati upućuju na važnu ulogu koju ima akutno fazni odgovor i oksidativni odgovor tijekom upale mliječne žlijezde.

Ključne riječi: *akutno fazni odgovor, oksidativni status, mastitis, krava*