

THE EFFECT OF ANTIOXIDANTS ON PREVENTING THE RETAINED PLACENTA IN DAIRY COWS

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Abstract: Retaining of placenta is one of the most common ailments in dairy cows and it occurs if placenta is not removed in the period of 12-14 hour after calving. The ailment is of multifactorial etiology and a primary cause is often unknown. During periparturient period in cows the metabolic, hormonal and biochemical changes are observed. Immunosuppression also characterizes this period and it is associated with endocrinal changes and decreased feed intake. Reduced function of neutrophils before parturition is in correlation with increased frequency of incidence of postparturition ailments. Selenium and vitamin E are the most important antioxidants which have a positive effect on neutrophils function protecting them from oxidative damage.

Key words: dairy cows, placenta retaining, selenium, vitamin E

Introduction

Frequency of retained placenta (RP) in cows after calving is 4-18% (*Han and Kim, 2005*). Aetiology of this ailment is not completely confirmed but numerous factors are included: mechanical, nutritive, inadequate management and infectious diseases (*Gunay et al., 2011*). Some studies show that 30-50% dairy cows have some metabolic and infectious diseases in the period about parturition. Certain physiological changes that happen during a transitional period may affect a nutritive status and contribute to higher sensitivity to some ailments. In this period the needs for energy are increased and they are caused by the beginning of lactation and insufficient feed intake, what brings the individual into the state of negative energy balance (NEB). The consequences of negative energy balance are mobilisation of fat from body reserves and entering of non-esterified fatty acids (NEFA) into blood. Abundant fat mobilisation and excessive accumulation of NEFA leads to the occurrence of metabolic and inflammatory ailments. Negative energy balance may occur at the end of gestation (*Brydl et al., 2008*) and contribute to the development of ailments such as fat liver and ketosis (*Bertics et al., 1992*) or provoke immunosuppression (*Goff, 2003*).

Disturbances in energy balance and acidobased balance are key factors for the incidence of placenta retaining. Increased levels of NEFA and ketonuria observed two weeks before parturition are important indicators for possible occurrence of this ailment. Besides these factors an excessive weight of animal must not be neglected (*Hayirli et al., 2002*) along with numerous management oversights – uncomfortable conditions (*Grummer et al., 2004*) and heat stress (*De Rensis and Scaramuzzi, 2003*). Oxidative stress may also contribute to placenta retaining. A great number of studies show that addequate supplement of selenium, zinc, cooper, iron and vitamins A, C and E playing a role of antioxidant, can reduce the percent of individuals with retained placenta.

Hormonal and biochemical changes in cows with retained placenta

Biochemical, hormonal and electrolyte profiles can be used as prognosis criteria for the incidence of placenta retaining in dairy cows. In cows with retained placenta a substantially higher level of progesterone and cortisol, and low level of estrogene is observed in relation to healthy cows (*Kornmatitsuk et al., 2000*). The increased level of cortisol is associated with the stress in dairy cows. The process of liberation between cotyledons and caruncle depends on hystological changes that happen inside them. In cows with retained placenta the chemotactic activity and leukocytes migration are reduced. On the contrary, increased hemotactic activity and number of lekocytes in placenta enable the proces of ejection of placenta so that in such cases the percent of placenta retaining in cows is low (about 1.4%). Cortisol reduces the function of neutrophiles and it can completely prevent their activity and thus lead to development of retained placenta. Increased content of cytotoxic aldehyde (malonil aldehyde) in erythrocytes and increased concentration of cortisol are deemed as major indicators of the incidence of retaining placenta. Decreased activity of mieloperoxidasis in cotyledones indicates to a reduced function of neutrophiles while a high activity of lysosomes and acid phosphatasis in retained placenta indicate to an acute inflammatory reaction of fetomaternal combination (*Gupta et al., 2005*). It is believed that in the week prior parturition the level of estradiol reaches its maximum what helps uterus to get free from the remains of fetal membrane and thus prevent the incidence of endometritis. However, reduced level of estrogene is one of the main factors which increases the risk of the occurrence of retained placenta.

In individuals with this ailment the occurrence of fat liver has also been found. As a consequence thereof, increased concentration of some enzymes in blood (alkaline phosphatase – ALP, aspartate aminotransferase – AST and gamma-glutamyl transferase – GGT) (*Semacan and Sevinc, 2005*) has been observed as well. Infiltration of fat in the liver is associated with the increase in the level of liver

enzymes and the fall in the level of glucosis, total lipids, cholesterol, triglycerides and electrolytes in the serum of dairy cows. A fat liver leads to its disfunction with no distruction to hepatocytes and the consequence is the increase in the activity of the liver enzymes (*Bülent et al., 2006*). In cows with retained placenta it can be combined with accumulation of lipids in hepatocytes. Liberated endotoxines in infectious diseases, such as endometritis, may cause the destruction and necrosis of liver what results in different level of its disfunction (*Semacan and Sevinc, 2005*). In preparturition cows a hypoglycemia has been observed what was caused by an increased needs of fetus and production of colostrum. Hypoglycemia can be also correlated with a high level of cortisol which is connected with the incidence of placenta retention. It has been determined that hypoglycemia in the last month of gravidity is one of the important indicators of the incidence of retained placenta and metritis (*Markiewicz et al., 2001*). A total level of lipids, cholesterol and triglyceride in serum is lower in cows with RP than in healthy ones. A low level of cholesteroles is a consequence of the increased synthesis of progesterone. The fall in total lipids and triglycerides is in correlation with disturbances in the metabolism of lipids and/or increase in the level of tissual lipolytical enzymes (*Michal et al., 2006*). Disturbances in energy balance and acid-based balance are key factors for the incidence of RP. Dairy cows, during a longer time period, become adapted to the state in energy balance by way of physiological, metabolic and endocrine changes (*Ingvartsen, 2006*). This process of adaptation can be monitored by help of indicators such as NEFA, hormonal status and net acido-based excretion (*Jorritsma et al., 2003*). Due to reduced feed intake in periparturition period there occurs an excessive mobilisation of fat and releasing of NEFA from fat tissue. Metabolism of NEFA in liver is followed by a partial oxidation and creation of ketone bodies or reesterification into triglycerides (lipogenesis). Accumulation of ketone bodies in blood unables the maintaining of homeostasis glucosis. These alternations change the functions of immuno cells, what results in higher frequency of the incidence of RP. The level of proteins in the serum of cows with RP is mostly unchanged but hiperglobulinemia may occur as a consequence of bacterial infection.

The cows with RP are anaemic, the number of erythrocytes concentration of hemoglobine and the percent of hematocrit are significantly reduced. In addition, the leukocytosis associated with lymphopenia and monocytosis is observed.

Immunosuppression and postparturition ailments

Metabolical and infectious diseases during periparturition period are caused by diminished immuno function in cows (*Sharma et al., 2011*). Immunosuppression is a consequence of endocrine changes which may happen in this period and reduced feed intake. Insufficient intake of nutritive matters and

reduced level of vitamins E are one of many factors which lead to the fall in the activity of neutrophils, and therefore to their possibility to take active part in the phagocytosis process. Vitamin E and selenium are the most important antioxidants which enhance the activity of neutrophils protecting them from oxidative damage after intracellular killing of bacteria (Joksimović Todorović and Davidović, 2013). In postparturition period the response of lymphocytes to stimulation by mitogens is reduced as well as the synthesis of antibodies and production of cytokines by immune cells.

Reduced function of neutrophils before parturition increases the bovine sensitivity to various postparturition diseases. Neutrophils are found in greatest number at places with high concentration of interleukin-8 (IL-8) (Elliott et al., 2000). This interleukin is a strong chemoattractant and the activator of neutrophils. IL-8 secreted by cotyledon and/or uterus enters systemic circulation, attracts neutrophils and sets them to action. It is known that it increases the secretion of collagenase which accelerates separating of fetal cotyledons from maternal caruncles (Luo et al., 2000). In the period of two weeks before and two weeks after calving the concentration of IL-8 is lower in cows with RP, so that on the day of calving in cows without RP it was 134 ± 11 pg/ml, and in cows with RP 51 ± 12 pg/ml (Kimura et al., 2002). After calving, the concentration of IL-8 is considerably higher in cows with RP than in cows without RP but all the same its level remains lower in a considerable degree.

Numerous studies confirm a stimulative effect of Se and vitamin E on the immunological status, including the activity of neutrophil granulocytes. Their antioxidative capability is manifested in the protection of non-esterified fatty acids, other cellular macromolecules and membrane against peroxidation. Bovine neutrophil granulocytes contain very little catalase, but the activity of selenoenzyme GSH-Px has an important role in protecting cytosol. Reduced function of neutrophil granulocytes is associated with a high level of superoxide (O_2^-). Inadequate protection against autooxidants leads to reduction of the function of neutrophil granulocytes what may cause frequent ailments. A primary role of these nutrients is to ensure immunological defence, increase the migration of neutrophil granulocytes into mammary gland where they phagocytose and destroy present bacteria (Hogan et al., 1993).

The importance of selenium and vitamin E in preventing the retained placenta in dairy cows. The relationship between antioxidative nutrition, oxidative stress and the incidence of retained placenta is well-known in dairy cows. Although pathogenesis of this ailment connected with Se and E vitamin deficiency is not clear enough the participation of oxidative stress in its etiology indicates a reduced incidence after selenium treatment. By adding the vitamin E and Se into feed, the level of this vitamin in erythrocytes, neutrophils and plasma is increased along with the activity of enzyme GSH-Px. Besides the level of selenium in blood plasma (Todorović et al., 1999a) the activity of glutathione peroxidase (GSH-Px) is also a

reliable indicator of biologically adoptable selenium. Optimal and suboptimal selenium levels in feed (0.1 and 0.15 mgSe/kg feed) result in linear increase of GSH-Px in plasma of studied individual animals. However, at certain concentrations of selenium in feed the level of GSH-Px reaches plateau so that further increase of the selenium level does not lead to the increase in the enzyme activity as well. High levels of Se (above 2 mgSe/kg feed) do not result in proportionate increase of this selenoenzyme whose activity increases at the start but fall significantly after 10th day (*Joksimović Todorović and Jokić, 2005; Joksimović Todorović et al., 2005*).

These nutrients reduce oxidative stress and lead to certain changes in placenta. Selenium and vitamin E possess immunomodulatory effects, in the sense of improving the function of neutrophils, increase their migration and chemotactic activity (*Gupta et al., 2005*). Se deficiency affects unfavourably the function of polymorphonuclear neutrophils and the changes in the level of GSH-Px (*Finch and Turner, 1996*). The absence of leukocytes in placenta leads to retention of placenta in 100% cows after calving. Se and vitamin E increase the number of leukocytes in placenta, leukocyte chemotaxis, assist the abating of the links between fetomaternal juncture and ejection of placenta.

Retained placenta can be prevented by the supplement of Se and vitamin E, unless it is provoked by mechanical and pathological factors (*Han and Kim, 2005; Joksimović Todorović and Davidović, 2007*). Dairy cows are fed by different forms of selenium: selenate, selenite (by adding the non-organic Se in feed) and Se-met and Se-cys (Se-yeast and basal food). Numerous studies show that the adding Se (usually in the inorganic form) ensures immunological function, soundness of mammary gland and prevents placenta retention. Inorganic selenium (selenite and selenate) and selenized yeast (Se-yeast) represent the sources of selenium used in bovine nutrition. Predominant form of selenium in Se-yeast is selenomethionine (Se-met). The mechanism of intestinal resorption differs in inorganic and in Se-met. Factors which reduce the resorption of inorganic selenium probably do not affect the absorption of Se-met. Metabolism of inorganic and organic selenium in the cell also differ. Inorganic Se is used exclusively for the synthesis of seleno specified enzymes while Se-met can be used for the synthesis of those proteins, but it can be built into any protein which contains methionine (*Weiss and Hogan, 2005*). Organic selenium is less toxic than inorganic (*Mihailović et al., 1996a,b, 1997*), can be more rapidly stored and retained in tissues (*Todorović et al., 1999b; Joksimović Todorović et al., 2006*).

Besides selenium and vitamin E other minerals and vitamins (zinc, copper, iron, vitamins A, C and E) have also been found to have important role in preventing RP. Low levels of these nutrients in dairy cows in preparturition and postparturition period predispose occurrence of RP (*Tillard et al., 2008*). Insufficient content of these minerals and vitamins in food can result in abortion in

dairy cows and as a consequence thereof in majority of cases the retained placenta is perceived.

Conclusion

Selenium and vitamin E as natural antioxidants have an important role in preventing the occurrence of retained placenta. These nutrients increase the activity of neutrophils, enhance their chemotactic effect and phagocytosis of opsonised pathogenic microorganisms. Adequately balanced rations with sufficient content of selenium, vitamin E and other antioxidants in food, appropriate housing of animals and good management lead to reducing the incidence of one of the most often ailments in dairy cows in postparturition period.

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Rezime

Zadržavanje posteljice je jedno od najčešćih oboljenja mlečnih krava, a nastaje ukoliko se posteljica ne izbací u periodu od 12-14h nakon teljenja. Oboljenje je multifaktorijalne etiologije, a primarni uzrok često ostaje nepoznat. U toku peripartalnog perioda kod krava dolazi do metaboličkih, hormonalnih i biohemijskih promena. Imunosupresija takođe karakteriše ovaj period, a u vezi je sa endokrinim promenama i smanjenim unosom hrane. Smanjena funkcija neutrofila pre porođaja u korelaciji je sa povećanom učestalošću nastanka postpartalnih oboljenja. Selen i vitamin E su najvažniji antioksidansi koji pozitivno utiču na funkciju neutrofila štiteći ih od oksidativnog oštećenja.

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