Journal of Elementology ISSN 1644-2296

Żarczyńska K., Żarczyński P., Sobiech P., Snarska A, Stopyra A, Wieteska M., Płaczek A. 2017. *The effect of micronutrient deficiencies on the health status of transition cows*. J. Elem., 22(4): 1223-1234. DOI: 10.5601/jelem.2017.22.2.1447

**REVIEW PAPER**

# THE EFFECT OF MICRONUTRIENT DEFICIENCIES ON THE HEALTH STATUS OF TRANSITION COWS

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## Abstract

Minerals, in particular micronutrients such as copper, manganese, zinc, selenium and chromium, deliver health benefits for high-yielding dairy cows. The availability of nutrients is particularly important during the transition period (between 3 weeks prepartum to 3-4 weeks postpartum), which is a highly vulnerable time in the production cycle that determines the health status of lactating cows, their milk yield and reproductive performance. In cows, prolonged mineral deficiency can produce subclinical symptoms of nutrient deficiency and, if untreated, can lead to a clinical presentation of the disorder. Less severe deficiencies, in particular micronutrient deficiencies, are more difficult to identify because they induce only minor functional and structural changes in the body, but together with energy metabolism disorders, they can lead to oxidative stress and immunosuppression, in particular in the transition period. Micronutrient supplements reduce the harmful effects of free radicals because the majority of micronutrients form the active centers of antioxidant enzymes. Dietary supplementation promotes reproductive performance, healthy fetal development and immunity. Micronutrients alleviate bacterial infections associated with mastitis, lower somatic cell counts in milk and minimize the risk of metabolic diseases caused by a negative energy balance. This study discusses the role of the major micronutrients in cattle nutrition and their effect on the health status of transition cows.

**Keywords:** dairy cows, transition period, micronutrients, oxidative stress, immune system.

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# INTRODUCTION

The transition period – which begins 3 weeks before parturition and ends 3-4 weeks after calving - is the most vulnerable time in the production cycle of dairy cows, in particular high-yielding cows. The intercalving interval is estimated at 420 days, i.e. around 60 weeks, whereas the transition period lasts 6 weeks and accounts for 10% of the production cycle. The transition period determines the health status of lactating cows, their milk yield and reproductive performance (Machado et al. 2013). During that time, cows are particularly susceptible to numerous factors that exert adverse effects on their health status. The transition period is characterized by dynamic changes in the hormonal profile, loss of appetite, negative energy balance, nutrient and vitamin deficiencies, compromised immunity and oxidative stress, which can be exacerbated by an unbalanced diet. These factors can disrupt homeostasis not only in bodily organs, but also at the tissue, cellular and molecular level, thus contributing to diseases that commonly affect cows during the transition period, including postpartum paraplegia, slow uterine involution, placenta retention, mastitis, ketosis, fatty liver disease and displacement of abomasum (Sobiech et al. 2015).

Mineral nutrients can be classified into two groups based on their concentration in the body. Macronutrients are elements whose concentration exceeds 50 mg $kg^{-1}$ BW, whereas the concentration of micronutrients, also known as trace elements, is below the above limit. There are more than 20 nutrients that are essential for animal health, including 7 macronutrients (calcium, phosphorus, potassium, sodium, chloride, magnesium and sulfur) and 15 micronutrients (iron, iodine, zinc, copper, manganese, cobalt, molybdenum, selenium, chromium, zinc, vanadium, fluoride, silicon, nickel and arsenic) (Radwińska, Żarczyńska 2014).

Mineral concentrations in bodily or interstitial fluids can decrease below the reference level for various reasons. The utilization of bio-elements is largely determined by their bioavailability, namely the extent to which a given mineral nutrient is transformed during digestion and absorption into a form that can be metabolized or stored in the body. In cattle, the availability of mineral nutrients is influenced by various factors, including gastrointestinal health, ruminal pH and microbiota, overall health and nutritional status, chemical form of a mineral (organic minerals are generally more bioavailable), accumulation of mineral nutrients in the body and exposure to stress (urinary excretion of iron, zinc, copper and selenium increases significantly under stress) (Fairweather-Tait, Hurrell 1996). Minerals are assimilated differently in the body. The most assimilable mineral nutrients are iodine and cobalt, followed by zinc, copper, molybdenum and selenium, whereas iron and manganese are least readily assimilated. In living organisms, mineral nutrients do not occur independently, and they enter into mutual interactions (synergistic or antagonistic) that influence life processes, including nutrient assimilation. In some cases, enhanced bioavailability of one nutrient can inhibit the assimilation of another element. For example, increased levels of copper and iron limit zinc absorption (Jankowski et al. 2014). Diets with adequate calcium, magnesium and phosphorus levels also promote the assimilation of other mineral nutrients (Spears 2003, Kurek et al. 2010). The dietary micronutrient levels recommended for dry and lactating cows by the National Research Council (NRC, 2001) and the Federal Drug Administration (FDA, 2009) are presented in Table 1.

Table 1

Dietary micronutrient levels recommended for dry and lactating cows by the National Research Council (NRC, 2001) and the Federal Drug Administration (FDA, 2009)

| Micronutrient | Demand for micronutrients according to NRC/FDA (mg $kg^{-1}$ DM of feed) | |
|---|---|---|
| | dry period | lactation |
| Iron | 13 | 15 |
| Zinc | 21 | 48 |
| Manganese | 16 | 14 |
| Copper | 12 | 11 |
| Iodine | 0.4 | 0.5 |
| Selenium | 0.3 | 0.3 |
| Cobalt | 0.11 | 0.11 |
| Chromium | 0.5 | 0.5 |

## Oxidative stress

Reactive oxygen species (ROS), including oxygen free radicals, play an important role in living organisms. During homeostasis, ROS control metabolism and act as mediators (Valko et al. 2007). They influence cell proliferation and differentiation, activate genes, influence the synthesis, release or deactivation of the vascular endothelial growth factor, stimulate glucose transport to cells, and induce apoptosis. One of the most important roles played by ROS is the control of signal transmission between and within cells. Phagocytic cells use free radicals to eliminate pathogens (Dröge 2002). When present at low concentrations, ROS are not harmful to the body, and may even be essential in some situations. However, excessive ROS levels contribute to disease and inflammation. In healthy animals, there is a balance between the amount of formed and scavenged free radicals. Free radicals are not effectively eliminated by the body's defense mechanisms when they are produced in excessive quantities, when the concentration of ROS scavengers is low, when the activity of enzymatic systems responsible for ROS scavenging decreases and when antioxidant activity levels decrease. The above leads to an imbalance and a shift towards increased oxidation, leading to oxidative stress (czajka 2006). When cell repair mechanisms are impaired, ROS interact with cellular macromolecules such as lipids, proteins and DNA, which damages cell membranes, impairs the activation of or deactivates enzymes and contributes to mutations (Lykkesfeldt, Svendsen 2007).

Fresh cows are particularly susceptible to the harmful effects of free radicals. Heat stress, periparturient diseases, premature lactation and high milk yield contribute to excessive ROS production (Linn et al. 2011). Living organisms have developed various defense mechanisms, known as antioxidant capacity, to minimize the harmful effects of free radicals. These mechanisms rely on non-enzymatic and enzymatic factors. The non-enzymatic system is composed of protective substances that are transformed to oxidized forms by donating their electrons to free radicals. Oxidized substances are characterized by low reactivity, and they prevent the oxidation of other components. These compounds are known as free radical scavengers. The most popular scavengers include vitamins C and E, vitamin and provitamin A, coenzyme Q10, flavonoids and reduced glutathione (GSH). The enzymatic system is composed of specialized enzymes that eliminate free radicals and prevent their formation. The key enzymes that prevent ROS synthesis are superoxide dismutase, catalases, glutathione peroxidase (GSH-Px) and reductases, including glutathione reductase (Sordillo, Aitken 2009). Micronutrients such as copper, zinc, iron, manganese and selenium form the active centers of protective enzymes and are required for enzyme function. Trace elements, in particular selenium, copper, chromium and zinc, play a key role in bovine immune responses which are significantly impaired by physiological and metabolic processes during the transition period. Immunosuppression is manifested mainly by decreased neutrophil activity, lower lymphocyte proliferative responses to mitogen, decreased production of antibodies and cytokines, which leads to endometritis, placenta retention and mastitis (Spears, Weiss 2008). For this reason, the micronutrient content of cow diets has to be adequately balanced during the transition period.

### Selenium (Se)

Selenium is an essential micronutrient for metabolic processes, and it is a cofactor of many enzymes and selenoproteins such as glutathione peroxidase, iodothyronine deiodinases, selenoprotein P (the main serum selenoprotein, a glycoprotein that contains around 10 selenocysteine residues, binds up to 60% of micronutrients and acts as selenium transport protein, SeP) and selenoprotein W, found in skeletal muscles, heart and brain (Whanger 2000, Żarczyńska et al. 2013). Selenium enters into synergistic interactions with vitamin E and other elements with antioxidant properties, such as copper and zinc, to protect cell membranes and DNA against the adverse effects of free radicals (Jalilian et al. 2012). A selenium deficiency can affect all animal species, but ruminants seem to be most susceptible. Ruminants assimilate selenium less efficiently than monogastric animals, and the low pH of ruminant contents, which is characteristic of high-yielding cows fed large amounts of concentrates, further decreases selenium absorption (Gerloff 1992). According to Abdelrahman and Kincaid (1995), selenium concentration decreases significantly in the last 60 days of pregnancy, which is why selenium supplements should be administered to dry cows. The above authors also noted that prepartum supplementation of selenium increases selenium levels in the blood and liver of calves. Sordillo and Aitken (2009) demonstrated that the administration of selenium supplements to transition cows significantly inhibits the production of free radicals and stimulates neutrophil activity, which has a positive effect on the immune system.

Placenta retention is one of the most common reproductive disorders that accompany hyposelenosis during the transition period. Selenium-dependent GSH-Px exerts protective effects on the placenta, and a selenium deficiency can impair this function. Glutathione peroxidase metabolizes peroxides by transforming them into less biologically active forms, thus protecting cell membranes against the negative effects of oxidation that can lead to physicochemical changes (Kankofer et al. 1996, Allison, Laven 2000). Placenta can also be retained due to neutrophil damage by ROS whose synthesis is intensified in hyposelenosis (Rutigliano et al. 2008). According to Harrison et al. (1984), a single selenium dose of 0.1 mg $kg^{-1}$ BW administered 21 days before the expected parturition date decreases the incidence of endometritis by 70% and the incidence of ovarian cysts by 40%. Many authors have suggested that mastitis is closely linked with a selenium deficiency and lower GSH-Px activity in the blood (Smith et al. 1997, Moeini et al. 2009). Selenium and vitamin E mobilize the transfer of neutrophils to mammary glands and increase their ability to eliminate bacterial pathogens, which shortens the duration of clinical mastitis and alleviates its symptoms, but only in infections caused by *E. coli, S. uberis, S. faecalis* and *Klebsiella* spp. (Moeini et al. 2009). Selenium supplementation did not influence the progression or duration of mastitis caused by *Staphylococcus aureus* (Smith at al. 1997). Moeini et al. (2011) administered selenium and vitamin E supplements to heifers before parturition and reported an increase in colostrum and milk production in the first 8 weeks of lactation relative to animals whose diets were not supplemented. According to the cited authors, inflammatory processes that accompany mastitis cause damage to mammary epithelium and decrease milk production.

In recent years, attempts have been made to evaluate the effects of single doses of selenium supplements at concentrations that significantly exceed the selenium requirements of cows. Research results indicate that high-selenium supplements stimulate the activity of mammary tissue, increase milk yield and the selenium content of milk. The observed effects could be attributed to the stimulation of blood vessels in mammary glands (O'Rourke 2009)

The influence of selenium on glucose metabolism in ruminants has been extensively investigated. Nayyar et al. (2003) reported significantly higher blood glucose levels in heifers receiving selenium and vitamin E than in control group animals. In contrast, SOLIMAN et al. (2012) did not observe a significant increase in blood glucose levels of ruminants whose diets were supplemented with selenium and vitamin E in the last weeks of pregnancy and during lactation. STAPLETON (2000) demonstrated that selenium participates *in vivo* and *in vitro* in various processes that are mediated by insulin, such as stimulation of glucose uptake and regulation of metabolic processes, including glycolysis, gluconeogenesis, fatty acid synthesis and stimulation of the pentose phosphate pathway.

## Copper (Cu)

Copper is a cofactor of ceruloplasmin and superoxide dismutase (SOD), and it participates in redox reactions. This element plays a key role in iron absorption and metabolism, and it participates in the myelination of nerve fibers, hematopoiesis and bone growth. Copper regulates the synthesis of collagen, elastin, melanin and catecholamines, and it is responsible for the pigmentation and crimp of animal hair (SPEARS, WEISS 2008). Copper influences neutrophil, monocyte and T cell functions, antibody production, and transformation of vitamins A and E. Copper deficiencies are most frequently observed in regions with copper-deficient soils and pastures where animals are not supplied with the required amounts of this micronutrient from green fodder. Secondary copper deficiencies are noted when the availability of copper is low in feeds that contain copper antagonists, including molybdenum, sulfur, cadmium, lead, zinc, calcium and iron. Gastrointestinal diseases inhibit copper absorption from feed and can also contribute to secondary copper deficiencies (RADWIŃSKA, ŻARCZYŃSKA 2014).

The influence of copper on the health status of transition cows has been poorly researched. Several authors reported that copper alleviates the severity of mastitis caused by *Staphylococcus aureus* as well as experimentally-induced and clinical colomastitis (SCALETTI et al. 2003, SPEARS, WEISS 2008). Copper deficiencies can lead to embryo death, fetal resorption, higher risk of placenta retention and low fertility due to delayed postpartum estrus (YASOTHAI 2014). TORRE et al. (1995) demonstrated that that in heifers fed a copper-deficient diet, mononuclear cells produced significantly less interferon-γ after stimulation with concanavalin A than in heifers whose diets were rich in copper.

## Manganese (Mn)

Similarly to copper, the effects of manganese on the health status of transition cows have not been fully explored. Manganese plays an important role in immune and nervous systems, and together with zinc and copper, it is a cofactor of SOD. Manganese also influences carbohydrate and lipid metabolism (ANDRIEU 2008). There is limited evidence to indicate that manganese supplementation has a positive influence on bovine mammary glands by increasing the ability of macrophages to eliminate bacteria responsible for mastitis (Linn et al. 2011). Manganese also plays an important role in the synthesis of cholesterol which is essential for the production of hormones such as progesterone and estrogen. A manganese deficiency can inhibit the synthesis of the above hormones and lead to reproductive disorders. According to Krolak (1968), manganese supplementation can shorten postpartum anestrus and increase fertilization success during the first estrus after calving.

**Chromium (Cr)**

Until recently, chromium was not included in the nutritional requirements for dairy cattle (NRC, 2001). At present, the daily dietary intake of chromium as chromium propionate for dairy cattle is set at 0.5 mg $kg^{-1}$ DM (FDA 2009). The main health benefits of chromium are associated with its positive influence on the immune system (both cellular and humoral immune responses) and glucose and lipid metabolism (Spears, Weiss 2008). Burton et al. (1996) observed an increase lymphocyte blastogenic responses and decreased cytokine (IL-2, interferon, TNF-α) production by mononuclear cells in cows receiving 0.5 mg of chromium/kg of feed after stimulation with concanavalin A. Chromium supplementation did not influence neutrophil activity (Faldyna et al. 2003). It is believed that chromium indirectly influences the immune system by exerting antagonistic effects on cortisol (Soltan 2010, Kafilzadeh et al. 2012). Chromium supplementation can potentially inhibit the release of cortisol from the adrenal glands, thus preventing the cortisol-mediated destruction of lymphocytes in lymphoid tissue and protecting immune system cells.

Low feed intake in early lactation has prompted breeders and producers to search for new methods of improving performance and minimizing health problems in the postpartum period. Chromium influences insulin metabolism and glucose utilization, and chromium supplementation can reduce the risk of ketosis. Chromium propionate stimulates lipid synthesis in adipose tissue and reduces lipolysis by binding with insulin receptors and improving glucose transport (McNamara, Valdez 2005). A decrease in serum β-hydroxybutyrate levels and triglyceride concentrations in the serum and liver was observed in cows whose diets were supplemented with chromium amino acid chelate in the form of chromium picolinate (Besong et al. 2001). When administered to transition cows, chromium propionate decreased the serum levels of non-esterified fatty acids, NEFAs (Soltan 2010, Yasui et al. 2014). According to other authors, chromium supplementation one month before to two months after calving can significantly increase feed intake during early lactation as well as milk production (McNamara, Valdez 2005, Smith et al. 2005). Villalobos et al. (1997) reported a four-fold reduction in the incidence of placenta retention in cows supplemented with 3.5 mg of chromium picolinate/kg of feed in the last 9 weeks of pregnancy relative to non-supplemented animals. Chromium supplementation before parturition and in the first two months of lactation had no influence on mammary gland health (Chang et al. 1996).

## Zinc (Zn)

Zinc is a cofactor in nearly 300 enzymes, and it plays diverse roles in living organisms. Zinc is required for catalytic functions in SOD, alkaline phosphatase, carbonic anhydrase, lactate dehydrogenase, RNA and DNA polymerases. It is essential for the synthesis of metallothionein, a metal-binding protein that scavenges free radicals and is required for the function of the growth hormone, thyrotropic hormone, glucagon, insulin, folliculotropin, luteinizing hormone and adrenocorticotropic hormone (Spears, Weiss 2008). In ruminants, zinc is essential for fermentation processes in the rumen, mainly cellulose digestion and synthesis of volatile fatty acids, and it also participates in the metabolism of vitamins A and E (Alsaad et al. 2011). Zinc is involved in the proliferation, differentiation and apoptosis of immune system cells, and it is required for healthy immune function (Haase et al. 2006). It also influences the growth and activity of neutrophils and NK cells, and it regulates lymphocyte gene expression.

Similarly to other micronutrients, zinc deficiencies can be both primary and secondary. A primary deficiency is attributed to a zinc-deficient feed, whereas a secondary deficiency is noted when zinc cannot be effectively assimilated from zinc-rich diets that contain zinc antagonists (copper, magnesium, calcium, phosphates, bivalent iron compounds) or diets deficient in amino acids. In cows, significant fluctuations in blood zinc levels are observed in the transition period. In a study by Goff and Stabel (1990), the lowest plasma zinc concentration was reported one day after parturition when it reached 67% of pre-calving levels. Normal zinc levels were restored already three days after calving. Significantly lower zinc concentrations were also reported in cows with hypocalcemia (Wang et al. 2014) and subclinical ketosis (Zhang et al. 2010) in comparison with healthy animals.

Dietary supplementation with zinc and methionine decreases somatic cell counts in milk and lowers the incidence of mastitis by stimulating keratin synthesis in the teat canal (O'Rourke 2009). Chelated zinc can improve milk yield by increasing zinc levels in the body, stimulating immunity and fermentation processes in the rumen (Ahmed et al. 2002). According to Chandra et al. (2013), the supplementation of cow diets with zinc and vitamin E for two months before parturition can minimize a negative energy balance and improve milk yield. A study evaluating the influence of an experimentally induced mammary gland infection with *Staphylococcus aureus* on serum zinc levels revealed that zinc concentration reached 83% of baseline value 24 h after the infection (Middleton et al. 2004). A much greater decrease in serum zinc levels was noted in response to an infection with *Escherichia coli* (Erskine, Bartlett 1993). According to Ranjan et al. (2005), a decrease in blood zinc concentration in mastitis is probably associated with higher oxidative stress.

Ahmed et al. (2002) observed that lower zinc levels and higher copper concentration in the blood of pregnant cows increase the risk of miscarriage. According to Greene et al. (1998), zinc contributes to the regeneration of uterine mucosa and speeds up estrus after parturition.

It can be concluded that trace elements significantly contribute to the maintenance of homeostasis in living organisms, in particular in high-yielding dairy cows. Proper micronutrient levels in tissues and bodily fluids promote healthy growth and development, whereas micronutrient deficiencies lower animal performance and cause economic losses.

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