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Role of Vitamin and Minerals Supplementation in Periparturient Dairy Cows

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Abstract

The transition stage is characterized by reduced dry matter intake, impaired liver function, and increased inflammation and oxidative stress. Which may increase the need for trace minerals and vitamin E. Trace elements are important for many important biological functions in dairy cows, including immunity, oxidative metabolism, nutrient, energy metabolism, and reproductive function. Selenium (Se) plays a role in the antioxidant system as a key component of the glutathione peroxidase enzyme, which can remove hydrogen peroxide (H₂O₂) and lipid hydroperoxides. Vitamin E has an immunity booster effect in dairy cow. Copper (Cu) and zinc (Zn) are important components of several enzymes, including Cu-Zn superoxide dismutase (SOD) and redox processes. Manganese (Mn) is an important cofactor in enzymatic reactions related to metabolic regulation. Moreover, there is strong evidence that trace elements such as Se, Zn, Cu, and Mn can minimize the adverse effects of oxidative stress. Cows in subtropical and tropical regions are exposed to many stressors, including seasonal malnutrition, parasites and blood-sucking insects, high temperature, and high humidity. The maintenance of efficient milk production is of utmost importance and high reproductive performance is absolutely crucial. Biological adjustments are necessary to support dramatic production increase during post-partum in cow.

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Introduction

Dairy cows are an essential component of agriculture worldwide, providing income source to farmers in the form of milk and milk products. Revenue generated by dairy cows comes from the production of milk and the sale of their products (Grohn *et al.*, 2003). Efficient production of these products is paramount, for which high reproductive performance is essential. Cows have demonstrated biological adaptations that support significant increases in production. Factors that affect each of these revenue streams (e.g., cow genetics, nutrition, environmental management, herd health, etc.) can affect the overall profitability of a dairy herd. The

transitional period is defined as the stage of the lactation cycle when dairy cows move from pregnancy and non-lactation stage to the onset of lactation stage (Drackley, 1999). The duration of the dry period varies depending on the management strategy and is divided into the "distant" period (usually the first 4-6 weeks after the dry period) and the "proximal" period (usually the last 3 weeks before calving) (Dunn *et al.*, 2006). The length of the transitional period includes the last 3 weeks of the dry period (from the proximal period) through 3 weeks after birth (Drackley, 1999; Grummer, 1995). Nutrient requirements to support fetal growth and initiate milk synthesis increase during the transitional period (Grummer, 1995). In contrast, this period is

characterized by a 30% decrease in dry matter intake between prenatal days 5 and 7, followed by a steady increase between postnatal days 0 and 21 (and beyond) (Bertics *et al.*, 1992). Significant changes in endocrine status occur during preparation for parturition and lactogenesis (Grummer, 1995). Progesterone concentrations remain elevated throughout gestation to maintain pregnancy but decline rapidly approximately 2 days before parturition. Similarly, plasma estrogen levels increase late in gestation but decline rapidly at parturition (Chew *et al.*, 1979). Growth hormone levels increase gradually as the cow transitions from late gestation to early lactation; however, a peak is reached at parturition (Kunz *et al.*, 1985). Plasma NEFA concentrations increase slightly approximately 2 weeks before parturition, with concentrations increasing dramatically around and after parturition (Grummer, 1995). Plasma thyroxine and triiodothyronine concentrations increase gradually during late gestation, decline significantly at parturition, and then increase again in the first few weeks after birth (Kunz *et al.*, 1985). Prolactin levels increase just before birth (Farmer and Petitclerc, 2003), and glucocorticoid and prolactin concentrations increase on the day of parturition, returning to levels close to prepartum concentrations shortly after birth (Edgerton and Hafs, 1973). The increased nutritional requirements, dramatic changes in endocrine status and reduced DMI during late gestation affect metabolism, particularly lipid metabolism (Grummer, 1995). Adipose tissue, liver, intestine and mammary gland are important components of the adaptations that dairy cows undergo to achieve the balance necessary to adapt to the onset of lactation (Drackley, 1999).

The transition period is considered to be the most critical stage of the lactation cycle, as successful transition is essential for successful lactation (Drackley, 1999). However, immunosuppression during this period results in increased susceptibility to invading pathogens (Mallard *et al.*, 1998), and the incidence of health problems during this period is significantly higher compared to the rest of the lactation cycle (Drackley, 1999). Furthermore, the risk of udder infection, abomasal luxation, milk fever, ketosis, retained amniotic fluid, and metritis is highest during the transition period (Shaver, 1997; Smith *et al.*, 1985). It is important to mention that in addition to infections, this period also results in increased susceptibility to metabolic disorders (Drackley, 1999), which may be responsible for the increase in proinflammatory cytokines and the resulting adverse effects (Bertoni and Trevisi, 2008).

Metabolic and physiological changes during the transition period: inflammation, energy balance, endocrine changes and immunosuppression

A major challenge for cows is the sudden increase in nutrients required for milk production at a time when dry matter intake (DMI) and nutrient supply are decreasing. High nutritional demands for lactogenesis are accompanied by mobilization of body reserves to support milk production. This places cows in a physiologically unavoidable state of negative energy balance (Grummer *et al.*, 2004; Ingvarsen, 2006). This is further exacerbated by additional factors such as stress and management, which further reduce DMI (West, 2003; Dobson *et al.*, 2007; Rhoads *et al.*, 2009). Dysregulation of inflammation and immune responses is thought to be the missing link in the pathology of metabolic disorders in transition cows. There is strong evidence that inflammatory mediators directly cause metabolic disorders (Trevisi *et al.*, 2010). Concentrations of many metabolic hormones and their receptors also change during the peripartum period. In particular, interdependent changes occur in the GH-insulin-IGF-I-glucose signaling pathway (Lucy *et al.*, 2001). Nutritional requirements during late pregnancy are met in part by increased insulin resistance and sensitivity of adipose tissue and muscle to lipolytic agents (Bell, 1995). This reduces peripheral glucose uptake and promotes nutrient flow from maternal stores to the placenta. Continuing glucose demand after parturition often leads to reduced circulating insulin levels during early lactation. Genetic selection for milk production has also been linked to reduced circulating insulin levels (Taylor *et al.*, 2004). However, during NEB, the GH-IGF axis becomes uncoupled due to downregulation of GHR in the liver, which is accompanied by a decrease in circulating IGF-I and an increase in GH concentrations (Lucy *et al.*, 2001). This, combined with generally low insulin levels, provides an endocrine milieu that favors the direct action of GH on lipolysis and gluconeogenesis during early lactation.

Leptin is another metabolic hormone of interest in terms of energy balance. Circulating concentrations of leptin are highly correlated with body condition score (BCS) and decrease during late pregnancy. However, leptin concentrations remain low after parturition, even if energy balance improves. Leptin may affect voluntary feed intake and may also contribute to the peripheral insulin resistance that develops in ruminants during parturition. Plasma progesterone levels, which are high during pregnancy, decrease rapidly at parturition,

accompanied by a transient increase in estrogen and glucocorticoid levels. These hormonal changes not only contribute to a decrease in DMI, but also promote metabolic changes that promote mobilization of body fat stores from adipocytes (Drackley *et al.*, 2005; Ingvarstsen, 2006).

Transient hyperthermia (>39.5 °C) during the first few days after calving in transitional cows is mainly due to inflammation-related generalized malaise and release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α) and interleukins 1 and 6 (IL-1 and IL-6). These cytokines stimulate hepatic synthesis of positive acute phase proteins (pAPPs), such as haptoglobin and ceruloplasmin. At the same time, cytokines impede the synthesis of negative acute phase proteins (nAPPs), some of which are important for normal liver function.

Energy balance in periparturient cows

In general, regardless of physiological and environmental conditions, animals attempt to achieve energy balance by utilizing available dietary energy and tissue reserves (Baile and Forbes, 1974). As already mentioned, during the transition period, dairy cows experience a significant decrease in DMI, which results in limited dietary energy intake and negatively impacts energy balance. At the same time, fetal nutritional requirements, mammary gland development, and the onset of milk synthesis increase, leading to a worsening of energy balance (Grummer, 1995). After birth, as milk production increases, so does the energy required for milk production, leading to a phase of negative energy balance (NEB). To meet the energy requirements during this phase, dairy cows rely on the mobilization of fat and muscle tissue.

This NEB phase continues until milk production begins to decline (6-10 weeks postpartum) and energy from DMI is sufficient to meet the cow's needs. The level of NEB that dairy cows are most likely to experience is dependent on milk production, as higher milk-producing cows require more energy for lactation. Periods of severe NEB, with extensive mobilization of adipose tissue, can lead to metabolic disorders such as ketosis and fatty liver disease (Drackley, 1999).

A central area of transition cow biology is related to fat metabolism. In the process of energy mobilization, adipose, udder and liver tissues are key components to which dairy cows adapt. Briefly, high levels of NEFA in the bloodstream are the result of hydrolysis of

triacylglycerol in adipose tissue by the action of hormone-sensitive lipase (Zammit, 1984). At this point, NEFA may be used as an energy source by other tissues. However, the liver is the primary site of removal of NEFA from the circulation. Once in the liver, NEFA are extracted in a concentration-dependent manner and converted to acyl-CoA by acyl-CoA synthetase. Carnitine plays a key role in the transport of acyl-CoA to the mitochondrial matrix where β -oxidation occurs. In mitochondria, acyl-CoA is oxidized to acetyl-CoA by β -oxidation, which is then further oxidized to obtain energy in the citric acid cycle (TCA). However, when the amount of acetyl-CoA produced by the β -oxidation of fatty acids exceeds the capacity of the TCA cycle or when the activity of the TCA cycle is reduced due to the presence of small amounts of intermediates such as oxaloacetate, acetyl-CoA is used for the biosynthesis of ketone bodies (Drackley, 1999). Partially oxidized acetyl-CoA is converted to the ketone bodies acetoacetate (ACAC), BHBA, and acetone, which are secreted into the blood by the liver (Bell, 1979). Ketones can be utilized by many tissues (e.g., heart and skeletal muscle) as an alternative water-soluble energy source when glucose levels are limited (Leslie *et al.*, 2000). However, when the rate of lipid mobilization exceeds the rate of ketone body utilization, ketone bodies can accumulate and have adverse effects on cow health and productivity. NEFAs and acyl-CoAs can increase ROS production by slowing down the flow of electrons in the mitochondrial electron transport chain, and fatty acids, when used as energy substrates, increase the production of ROS during oxidation. Saturated NEFAs, including palmitic acid (C16:0) and stearic acid (C18:0) or their corresponding acyl-CoAs, can directly activate the NF- κ B signaling pathway. NF- κ B induces the transcription of several adhesion molecules, including endothelial leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM1), intercellular adhesion molecule (ICAM1), and E-selectin (Collins *et al.*, 1995; Ahn *et al.*, 2005). NF- κ B also induces the expression of numerous cytokines, chemokines and their receptors, thereby enhancing the cellular inflammatory response. As already mentioned, saturated fatty acids can activate TLR-4, thereby initiating the NF- κ B signaling pathway.

2.3

Immune Cells and Defense Mechanisms

The immune system is composed of a large number of cells and molecules that are able to recognize and eliminate invading foreign microorganisms in a specific way (Baumann and Gauldie, 1994). There are two

defense mechanisms: innate and specific. Both mechanisms interact to recognize and distinguish between foreign bodies and the host's own molecules (Kehrli and Harp, 2001).

Macrophages are one of the most common immune cells. They are the first line of defense against invading microorganisms and play a key role in the innate immune response. Macrophages arise from monocytes. They arise in the bone marrow during hematopoiesis and then enter the blood where they differentiate into mature monocytes. Within 8 hours, circulating monocytes expand and migrate to tissues where they further differentiate into tissue-specific macrophages. They sense and recognize non-specific foreign pathogens locally and produce cytokines that trigger an immediate response called the acute phase response. They also recruit other immune cells to the site of infection and act as a bridge between innate and specific immune responses through antigen presentation to primary T cells (Rainard and Riollet, 2006).

Lymphocytes are produced in the bone marrow by leukocyte hematopoiesis and are activated in response to local antigenic stimulation. They replicate and recognize foreign antigens via membrane receptors. Lymphocytes consist of T lymphocytes and B lymphocytes. T cells can be divided into helper T lymphocytes and cytotoxic T (CTL) lymphocytes. Helper T cells produce cytokines such as interleukin 2 (IL-2) and interferons (IFNs) that are essential for an effective cell-mediated immune response. B lymphocytes differentiate to produce proteins called antibodies or immunoglobulins (Ig), and effector B cells or plasma cells. Plasma cells are important for specific immunity; one of their main functions is the production of antibodies. Immunoglobulins such as IgG1, IgG2, and IgM are the main defense mechanism of specific immunity (Paganelli *et al.*, 1984).

During transition period metabolic stress increase the production of reactive oxygen species (ROS). ROS can cause lipid peroxidation, resulting in cellular damage in tissues. Immune cells are particularly sensitive to oxidative stress because their membranes contain high concentrations of polyunsaturated fatty acids that are susceptible to peroxidation and generate large amounts of reactive oxygen species when stimulated (Spears and Weiss, 2008). During the periparturient period, neutrophil function is impaired and neutrophilia and eosinopenia occur. In addition, sick cows have lower paraoxonase activity (PON) (Bionaz *et al.*, 2007). This is

thought to be due to lipid mobilization and triglyceride deposition in hepatocytes, causing liver damage and dysfunction and leading to decreases in total cholesterol and high-density lipoprotein (HDL) increased oxidative stress (Turk *et al.*, 2004, 2005), or in association with a combination of these factors, leading to PON binding in the blood.

Phagocytosis

Some immune cells of the innate and specific immune systems perform phagocytosis. During this process, microorganisms are ingested by phagocytes

Phagocytosis first requires macrophages or PMNs to attach and label the bacterial cell wall for uptake and destruction (opsonization). Cell surface receptors on phagocytes specific for certain opsonins, such as certain classes of antibodies and complement components, can also enhance attachment and phagocytosis. Once the microbe is completely surrounded and enclosed by pseudopodia, it enters the cytosol as a membrane-bound structure called a phagosome. The phagosome then fuses with a lysosome that contains enzymes that digest the ingested material, forming a phagolysosome. During this process, a burst of oxidative metabolism, called the respiratory burst, occurs in activated phagocytes through activation of membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH)-linked oxidase, which catalyzes the reduction of oxygen to superoxide anion (O₂⁻). After the respiratory burst, the digested contents of the microorganisms are expelled by exocytosis (Burvenich *et al.*, 2004).

Mastitis

Mastitis is defined as an inflammation of the mammary gland (MG). It is often associated with the presence of pathogens (Bradley, 2002). Loss of profit due to mastitis results in reduced milk yield, costs of treatment, removal of milk from the milk tank after treatment, veterinary costs, increased labor, early culling, and sometimes animal death. Depending on the severity and duration of the inflammation, mastitis can be classified into subclinical and clinical mastitis. In subclinical mastitis, there are no visible signs of infection and there is usually an increase in somatic cell count (SCC) in the milk and a decrease in milk production (Sordillo *et al.*, 1997). Clinical mastitis is characterized by an increase in the SCC content in milk, and visible signs of infection such as lumpy, watery, bloody, and/or yellowish colored milk may be observed. Furthermore, clinical mastitis can lead

to reduced milk yield and feed intake, udder swelling, and in extreme cases, death due to mastitis-induced sepsis. Milk composition can also be affected, including changes in salinity, conductivity, sourness, appearance, and taste, increased SCC, and reduced casein and fat content.

Changes in the expression of glucose transporters in monocytes

Phagocytosis, production of reactive oxygen species, and secretion of proinflammatory cytokines are important functions of monocytes and macrophages that require high energy input during inflammatory responses. Glucose is the primary energy source for most mammalian cells, including immune cells. In bovine mammary epithelial cells, GLUT1 expression increases during lactation and decreases during the non-lactation period. In humans, GLUT3 is a high-affinity transporter and is therefore thought to be restricted to tissues that are highly dependent on glucose as an energy source. Insulin-responsive GLUT4 is widely expressed in adipose tissue and muscle of several species.

Activation of human monocytes and lymphocytes increased the expression of GLUT1 and GLUT3 on the plasma membrane and promoted glucose transport. Taken together, these previous studies emphasize that glucose transport via GLUT isomers is the rate-limiting step for glucose utilization in both resting and activated monocytes and macrophages. However, to date, the presence of GLUT isomers in bovine leukocytes has not been described.

When energy sources such as glucose are imbalanced, changes in GLUT levels can alter the magnitude and duration of inflammatory and immune responses. Bovine blood monocytes are known to exhibit a reduced inflammatory response around the time of parturition, but the possible effect of reduced glucose intake on monocyte function is unknown.

Role of leptin in the peripartum period

Plasma concentrations of leptin were highest during late pregnancy and declined until age 50 (Ingvarsen and Boisclair, 2001). The decline in plasma leptin after parturition is due to a negative energy balance, as plasma leptin levels remain high in cows that were not milked after birth. In periparturient cows, many immune cell functions are impaired, including chemotaxis, locomotion, phagocytosis, production of superoxide

anion and respiratory burst, and secretion of immune proteins such as conglutinin. Leptin therapy reverses the immunosuppressive effects of acute starvation in mice by acting directly on immune cells (Lord *et al.*, 1998). This suggests that leptin plays a role in linking nutritional status and cellular immune function.

Role of vitamin and mineral supplementation in the peripartum period

Oxidative stress during the transitional period also probably contributes to the increased risk of disease. The change in metabolic profile associated with the transition from dry period to parturition and early lactation may increase the production of reactive oxygen radicals that cause oxidative stress. Oxidative stress occurs when reactive oxygen radicals overwhelm antioxidant defense mechanisms. Immune cells are highly sensitive to oxidative stress because their cell membranes contain polyunsaturated fatty acids, which are oxidized by reactive oxygen radicals and generate even more reactive oxygen radicals. Several trace elements and vitamins are essential for an effective antioxidant defense system (Spears and Weiss, 2008).

Selenium and Vitamin E

Selenium and Vitamin E complement each other to help cows overcome immune system challenges. Both selenium and Vitamin E are required to optimize the effectiveness of neutrophils in attacking and destroying invading bacteria. Neutrophil chemotactic migration towards invading organisms was reduced by selenium deficiency in goats (Aziz *et al.*, 1984). Similarly, peripheral blood lymphocytes isolated from selenium-deficient cows showed reduced response to mitogen stimulation. The complementary nature of selenium and Vitamin E in reducing the frequency and severity of mastitis was clearly demonstrated by Smith *et al.*, (1997). Some studies have shown that supplementing a low-selenium diet with selenium reduces the incidence of retained placenta in dairy cows (Allison and Laven, 2000).

Copper

Copper is important to the antioxidant system because it is part of the copper-zinc superoxide dismutase enzyme. This enzyme helps convert superoxide radicals to hydrogen peroxide within cells. Copper deficiency can occur even in diets that are normally considered adequate, because high concentrations of antagonists

such as sulfur, iron, and molybdenum reduce bioavailability. Harmon (1998) reported that heifers fed a slightly copper-deficient diet (6-7 mg Cu/kg diet) had 60% of mammary glands infected at calving, compared with 36% of heifers fed a diet containing sufficient copper (20 mg Cu/kg diet).

Zinc

Zinc is an essential cofactor for over 80 enzymes, many of which are required for DNA or RNA synthesis. Zinc therefore plays a key role in cell replication and proliferation and may therefore affect immune function. Zinc is also required for the synthesis of metallothionein, a metal-binding protein capable of scavenging hydroxyl radicals. Severe zinc deficiency in calves has been shown to impair immunity. Plasma zinc concentrations in dairy cows typically decline at parturition but usually normalize within 3 days (Goff and Stable, 1997). What role this may play in the increased incidence of postpartum disease remains to be investigated.

Conclusion

In transitional crossbred cows, metabolic adaptation to negative energy balance has a significant impact on energy availability as well as inflammatory responses and immune function. Supplementation with selenium, vitamin E, copper, zinc, and additional concentrates during the postpartum period may improve animal performance and/or reduce immunosuppressant and negative energy balance. Adequate intake of Se, Mn, Cu and Zn in the cow's diet is important for optimizing the health of lactating and parturient dairy cows. Adequate presence of trace elements can protect cows from the adverse effects of acute inflammation caused by mastitis pathogens. Furthermore, supplementation with organic Cu, Zn and Se improved udder health and significantly reduced SCC, reduced mastitis and reduced SCC provide economic benefits and improve the health and welfare of dairy cows.

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