ABSTRACT: Trace minerals have critical roles in the key interrelated systems of immune function, oxidative metabolism, and energy metabolism in ruminants. To date, the primary trace elements of interest in diets for dairy cattle have included Zn, Cu, Mn, and Se although data also support potentially important roles of Cr, Co, and Fe in diets. Trace minerals such as Zn, Cu, Mn, and Se are essential with classically defined roles as components of key antioxidant enzymes and proteins. Available evidence indicates that these trace minerals can modulate aspects of oxidative metabolism and immune function in dairy cattle, particularly during the transition period and early lactation. Chromium has been shown to influence both immune function and energy metabolism of cattle; dairy cows fed Cr during the transition period and early lactation have evidence of improved immune function, increased milk production, and decreased cytological endometritis. Factors that complicate trace mineral nutrition at the farm level include the existence of a large number of antagonisms affecting bioavailability of individual trace minerals and uncertainty in terms of requirements under all physiological and management conditions; therefore, determining the optimum level and source of trace minerals under each specific situation continues to be a challenge. Typical factorial approaches to determine requirements for dairy cattle do not account for nuances in biological function observed with supplementation with various forms and amounts of trace minerals. Trace mineral nutrition modulates production, health, and reproduction in cattle although both formal meta-analysis and informal survey of the literature reveal substantial heterogeneity of response in these outcome variables. The industry has largely moved away from oxide-based programs toward sulfate-based programs; however, some evidence favors shifting supplementation strategies further toward more bioavailable forms of inorganic and organic trace minerals. Furthermore, opportunities for specific modulation of aspects of health, milk production, and reproduction through supplementation strategies for diets of transition dairy cows are attractive because of the known dynamics of energy metabolism, immune function, and oxidative metabolism during this timeframe.

Key words: dairy cattle, immune function, oxidative metabolism, trace minerals

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INTRODUCTION

The modern dairy cow is a model of remarkable productive efficiency. In high-producing dairy herds with annual average milk production levels in excess of 15,000 kg/cow, 75% or more of the energy consumed is translated into milk production and other functions beyond maintenance. These levels of production require exquisite control of metabolism and interplay between key physiological systems to support simultaneously high production, health, and reproductive capacity.

Excellent nutritional management of dairy cows, particularly during the transition period from late pregnancy to early lactation, is critically important to meet-
ing overall demands for milk yield while maintaining health and reproductive capacity (Roche et al., 2013). Although systems for nutrient requirements (e.g., NRC, 2001) typically use classically defined accounting approaches to determine requirements for maintenance, growth, pregnancy, and lactation in dairy cattle, it is becoming increasingly clear that nutrients have roles in regulation of gene expression and modulating other aspects of metabolic and immunological systems. In this paper, we will first overview aspects of energy metabolism, immune function, and oxidative metabolism with particular focus on the transition cow, outline specific roles of trace elements in immune function and metabolism, and critique NRC approaches to determine nutrient requirements. We will then present our current state of knowledge regarding responses of dairy cows to varying strategies for trace mineral nutrition during the transition period and early lactation. Finally, we will outline several research priorities and considerations for the practicing nutritionist regarding trace mineral supplementation strategies.

Overview of Immune Function, Oxidative Metabolism, and Energy Metabolism in Transition Dairy Cows

The most critical timeframe of the lactation cycle in dairy cattle is the transition period because it represents the period of most dramatic and dynamic daily change in nutrient demands and partitioning to various physiological functions. Dairy cows adapt to sharply increased nutritional demands to support milk production through exquisite homeorhetic control mechanisms (Bauman and Currie, 1980; Bell, 1995). Concurrent with these changes in nutrient demand, transition dairy cows undergo a period of reduced immunological capacity during the last several weeks before parturition and the first several weeks postpartum (Goff and Horst, 1997; Overton and Waldron, 2004); as an example, decreased neutrophil function during the periparturient period has been associated with disorders including retained placent, metritis, and mastitis (Cai et al., 1994). Therefore, the time of greatest potential risk for infectious and metabolic diseases overlaps the time of greatest acceleration of milk production (Ingvarstsen et al., 2003). Furthermore, Sordillo and Aitken (2009) described the changes in oxidative metabolism that occur and the implications of increased production of reactive oxygen species (ROS) during this transition period.

It has become increasingly clear that there is substantial interplay between energy metabolism, immune function, and oxidative metabolism in the transition cow and that these systems cannot be considered independently of one another. As an example, oxidative stress can be a significant underlying factor to dysfunctional host immune responses because the production of ROS is increased due to these dramatic metabolic and physiological adaptations (Sordillo and Aitken, 2009). Indeed, it was reported that total antioxidant potential and thioredoxin reductase activity were decreased in peripheral blood mononuclear cells and plasma lipid hydroperoxide concentrations were increased during the first 21 d postpartum compared with the prepartum period (Sordillo et al., 2007). Also, antioxidants, such as vitamin E and Se, are associated with resistance to mastitis when supplemented during the transition period (Sordillo and Aitken, 2009). These results indicate that oxidative status can be related to compromised functionality of the immune system, and supplemental antioxidants can contribute to decreased incidence of certain diseases during the transition period.

Negative energy balance (EB), which results from the lag between DMI and the demands of milk yield during early lactation (Bell, 1995), also has been implicated in periparturient immunodysfunction. Kimura et al. (1999) reported that polymorphonuclear neutrophils (PMN) myeloperoxidase activity decreased from baseline prepartum values as parturition approached in both intact and mastectomized cows; however, they also reported that myeloperoxidase activity recovered to prepartum values within 1 wk following parturition in mastectomized cows but remained decreased in PMN obtained from intact cows throughout the first 20 d postpartum (Kimura et al., 1999). This indicates that the high energy demands and negative EB in intact cows modulated the immune system. Moyes et al. (2010a,b) reported that experimental negative EB during intramammary challenge conducted at about 100 d postcalving downregulated genes encoding for major histocompatibility complex type II on PMN and other mammary genes involved in enhancing cell development, proliferation, and growth, indicating that negative EB may be related not only to the impairment of PMN expression of major histocompatibility complex type II but also to the mechanism of increased susceptibility to mastitis during the transition period. Revelo and Waldron (2010) determined that neutrophils isolated from cows at d 7 postpartum produced less ROS following in vitro stimulation than those isolated from cows during the prepartum period or mid lactation. Metabolites associated with negative EB have been associated with negative effects on immune function. Hoeben et al. (1997) demonstrated that β-hydroxybutyric acid (BHBA) added in vitro had inhibitory effects on the respiratory burst activity of PMN isolated from blood of multiparous cows and Hammon et al. (2006) reported that plasma NEFA concentrations during the first week after calving were correlated negatively with PMN myeloperoxidase activity.
As discussed previously, the immune system is associated not only with oxidative status but also with metabolism; however, relationships between oxidative status and metabolism also exist. Bernabucci et al. (2005) reported that cows with greater plasma BHBA and NEFA had greater concentrations of reactive oxygen metabolites and thiobarbituric acid reactive substances (TBARS) and lesser levels of antioxidants in plasma during the transition period. Castillo et al. (2006) showed that plasma malondialdehyde was associated positively with serum NEFA during the close-up dry period. These results indicate that cows with accentuated negative EB and increased lipid mobilization have greater oxidative stress although cause and effect relationships have not been elucidated.

Clearly, energy metabolism, immune function, and oxidative metabolism are interrelated and interdependent. However, we think that a key concept relates to the importance of balance within and among these systems. Sordillo et al. (2009) described the “delicate balance” that must be maintained in the immune system such that there is sufficient activity needed to eliminate the insult, yet the response must be controlled to avoid damage to bystander tissues. The same concept applies to the other systems as well in that homeorhetic adaptations in energy metabolism that are important for the onset of copious milk production result in negative EB and increased blood NEFA and BHBA levels even in healthy cows (Bauman and Currie, 1980; Bell, 1995); however, excessive negative EB is problematic in terms of compromised health and productivity (Ospina et al., 2010a,b). Reactive oxygen species are necessary for normal metabolic processes, such as energy metabolism, cell growth and differentiation, and killing of pathogens (Miller et al., 1993; Valko et al., 2007); however, production of ROS in excess of antioxidant defense mechanisms results in oxidative stress (Spears and Weiss, 2008; Sordillo et al., 2009).

Overview of Specific Roles of Trace Elements in Aspects of Immune Function and Metabolism in Dairy Cattle

Trace minerals have critical roles in a variety of physiological processes in all systems in Fig. 1. Trace minerals are involved in the antioxidant defense system and a deficiency of any of them may depress immunity in transition cows (Spears and Weiss, 2008). To date, the primary trace elements of interest in diets for dairy cattle have included Zn, Cu, Mn, and Se although data also support potentially important roles of Cr, Co, and Fe in diets. In this section, we will outline specific roles of each of these in the dairy cow and highlight results from both formal meta-analyses when available and the scientific literature at large. It is beyond the scope of this paper to include results from all studies conducted, and it is important to note that there is heterogeneity of response for outcome variables for all minerals listed.

Zinc

The immune system has a large turnover of cells, which necessitates a high rate of proliferation, differentiation, and apoptosis; Zn is involved in all of these processes (Haase et al., 2006). Another role of Zn includes structural integrity of tissues (Andrieu, 2008). In dairy cows, Zn is essential for production of keratin, which has an important role in the mechanism of defense against pathogens in mammary gland (Andrieu, 2008). Zinc is also a component of superoxide dismutase (SOD), which scavenges superoxide, one of the components of ROS, in immune cells (Saker, 2006). Despite these known functions of Zn within the immune system, the specific effects of supplementation of different amounts and forms of dietary Zn on immune function in dairy cows have received little attention (Spears and Weiss, 2008). Recently, Wang et al. (2013) reported that serum antibody titers after vaccination for foot and mouth disease were increased in lactating cows fed either a Zn–AA chelate or zinc proteinate chelate compared with cows fed either an unsupplemented diet or a diet supplemented with zinc sulfate, indicating that humoral immunity can be affected by supplemental Zn. Kellogg et al. (2004) summarized 12 trials that evaluated the effects of feeding zinc methionine complex on lactation performance and udder health and reported that supplementing zinc methionine complex reduced somatic...
cell count (SCC). Cope et al. (2009) reported that cows fed zinc proteinate at NRC (2001) recommended levels had greater milk yield than those fed zinc oxide at the recommended level or zinc proteinate at a lesser level (66% of NRC) but was not different from those fed zinc oxide at the lesser level; furthermore, cows fed either form of Zn at NRC recommended levels had decreased SCC and milk amyloid A concentrations compared with cows fed lesser levels of Zn. Dairy cows that were fed zinc methionine tended to produce more milk with a lesser SCC compared with cows without supplemental Zn (Sobhanirad et al., 2010); however, Whitaker et al. (1997) reported that cows and heifers fed zinc proteinate beginning prepartum and continuing through 100 d postcalving had similar SCC and rates of clinical mastitis compared with cows fed an unspecified form of inorganic Zn. Although responses in the literature are heterogeneous, the results overall support generally favorable responses to organic forms of Zn.

**Copper**

Copper status alters functions of neutrophils, monocytes, and T cells (Wintergerst et al., 2007) and antibody production (Smith et al., 2008a). Marginal Cu deficiency in dairy heifers reduced PMN killing of *Staphylococcus aureus* (Torre et al., 1996a). Mononuclear cells isolated from lactating dairy cows that had received a marginal level of Cu produced less interferon-γ when stimulated with concanavalin A than cells isolated from cows fed adequate levels of Cu (Torre et al., 1996b). Milk bacterial count and clinical scores assessed for mammary gland health for were decreased after *Escherichia coli* intramammary challenge in cows fed supplemental copper sulfate compared with cows without Cu supplementation (Scaletti et al., 2003) and in primiparous cows fed copper proteinate compared with cows fed copper sulfate or an unsupplemented control (Scaletti and Harmon, 2012).

Copper status can affect oxidant–antioxidant balance in dairy cows because Cu is a component of SOD and ceruloplasmin (Underwood and Suttle, 1999) and may be involved in metabolism of vitamin A and E. Decreased values of vitamins A and E content in heifers without Cu supplementation were observed throughout the period of study when compared with heifers provided with a mineral mixture containing copper sulfate (Sharma et al., 2005); however, few studies have been conducted to evaluate effect of Cu on oxidative status.

Copper plays an important biological role as a cofactor for several Cu-dependent enzymes (Sharma et al., 2008) and can influence circulating concentrations of thyroid hormones (Sharma et al., 2005), indicating further links with energy metabolism. A recent study showed that milk yield and 4% fat-corrected milk yield of cows fed a mix of copper sulfate and copper methionine tended to be increased compared with those fed copper sulfate only or copper methionine only (Wang et al., 2012). However, the lack of a negative control treatment (no supplementation of Cu) limits interpretability with regard to the form or amount of Cu supplementation.

**Manganese**

There have been few studies that have specifically assessed the effect of Mn on immune function and metabolism in dairy cows although Mn is an essential part of a range of enzymes that are involved in 1) immune and nervous function, 2) antioxidant protection as an integral part of SOD, and 3) carbohydrate and lipid metabolism (Andrieu, 2008). Work conducted since the publication of the 2001 dairy NRC indicates that the NRC underestimates the requirement for Mn and that requirements for dry and lactating cows are 1.6 and 1.7 times those estimated by the 2001 dairy NRC (Weiss and Socha, 2005).

**Selenium**

Selenium is essential for an optimum immune response and influences both innate and acquired immune systems through a key role in redox regulation and antioxidant function and contributes to membrane integrity and protection against DNA damage (Wintergerst et al., 2007). Selenium-deficient bovine mammary artery endothelial cells had significantly enhanced PMN adherence when stimulated with tumor necrosis factor-α, which may lead to dysfunction exhibiting as either hyperresponsiveness to nominal stimuli or as tight adherence to endothelial cells, with possible hindrance of leukocyte extravasation to the affected tissue (Maddox et al., 1999). Supplementation of Se also affects innate immunity. Hogan et al. (1990) reported that intracellular killing of *S. aureus* was increased in PMN isolated from Se-supplemented cows than in PMN from cows not fed supplemental Se. Acquired immunity in ruminants also is influenced by Se status. Concanavalin A–stimulated lymphocyte proliferation was decreased in Se-deficient cows compared with cows fed diets supplemented with Se (Cao et al., 1992). Selenium status appears to be directly related to disease resistance. When Se injections were administered 21 d before expected calving, the duration of clinical mastitis symptoms was reduced by 46% compared with controls (Smith et al., 1984). Weiss et al. (1997) reported that rates of clinical mastitis and bulk-tank SCC were inversely related to plasma Se concentrations. Dietary supplementation of 0.14 mg/kg Se resulted in less severity and shorter duration for experimental *E. coli* mastitis than 0.04 mg/kg (Erskine et al., 1989). Several studies have also indicated...
that prepartum Se supplementation along with vitamin E can reduce the incidence of retained placenta in dairy cows (Spears and Weiss, 2008).

It can be speculated that Se directly affects oxidative status as a component of glutathione peroxidase. However, the effects of Se supplementation on oxidative status have not been consistent. Selenium supplementation (3 mg/d) during the 6-wk prepartum period in dairy cows did not affect TBARS in red blood cells whereas vitamin E supplementation decreased TBARS (Brzezinska-Slebdzinska et al., 1994). In another study, injection of 1,100 IU of vitamin E and 30 mg of sodium selenite 3 wk before calving reduced erythrocyte lipid peroxide concentrations and plasma cortisol (Gupta et al., 2005). These inconsistent results may be due to the different level of Se and other antioxidants in the basal diet used in each study.

Because dairy cows could experience increased oxidative stress during the periparturient period (Sordillo and Aitken, 2009), Se may help to control oxidants during this timeframe. Calamari et al. (2011) examined the effects of either dietary Se source or amount on metabolic profiles in heat-stressed lactating dairy cows. They reported increased NEFA levels and decreased BHBA along with a trend for decreased urea plasma levels in Se supplemented cows compared with control cows during the heat stress period (Calamari et al., 2011), suggesting that Se supplementation may modulate the impact of physiological stressors on energy metabolism.

**Chromium**

Although the primary role of Cr appears to relate to its ability to enhance the action of insulin (Spears and Weiss, 2008), studies have indicated that Cr supplementation may affect health and immune response in ruminants (Spears, 2000). Relative to innate immunity, a study showed that supplemental Cr did not affect PMN phagocytic function of dairy cows from 6 wk prepartum to 6 wk postpartum (Chang et al., 1996). However, other studies with periparturient dairy cows indicate that Cr supplementation may affect cell-mediated and humoral immune responses (Spears and Weiss, 2008). Cell-mediated immunity was assessed in vitro using antigen (ovalbumin)- and mitogen-stimulated blastogenesis of peripheral blood mononuclear cells collected from prepartum period through early lactation, with Cr supplementation leading to increased blastogenic responses of peripheral blood mononuclear cells to mitogen stimulation (Burton et al., 1993). Burton et al. (1996) also demonstrated that in vitro concentrations of interferon-γ, interleukin-2, and tumor necrosis factor-α of mitogen-stimulated mononuclear cells collected during the peripartum period were decreased in cows supplemented with Cr compared with controls. As for humoral immune response in peripartum and early lactation dairy cows, Burton et al. (1993) showed that anti-ovalbumin antibody responses were increased when supplemental Cr was fed and Faldyna et al. (2003) reported that tetanus toxoid–specific antibody titers were increased in cows fed 5 mg Cr per head per day compared with nonsupplemented cows. Recently, Kafizadeh et al. (2012) reported decreased circulating cortisol concentrations in cows fed Cr, which may relate to the effects on aspects of immune function described previously.

Although studies examining the effects of dietary Cr on health in ruminants are limited, 1 study showed that supplemental Cr had no beneficial effect on health status and mastitis-related parameters in dairy cows (Chang et al., 1996) and another study showed that Cr supplementation during the last 9 wk of pregnancy decreased the incidence of retained placenta from 56% in unsupplemented controls to 16% (Villalobos et al., 1997); however, it should be noted that this incidence in control cows was exceptionally high.

It is possible that supplemental Cr influences metabolic status through enhancing insulin function. In fact, several studies have shown effects of Cr on metabolic parameters in dairy cows during the transition period through early lactation period. Supplemental Cr resulted in reduced serum BHBA levels (Yang et al., 1996) and decreased serum insulin concentrations without affecting blood metabolites or liver triglyceride (Hayirli et al., 2001). Mcnamara and Valdez (2005) reported that cows fed Cr had greater lipogenic rates in adipose tissue during the postpartum period than controls. Smith et al. (2008b) determined that cows fed Cr had increased plasma glucose and glucagon and decreased NEFA during the prepartum period but no effect on postpartum metabolites and liver composition. Sadri et al. (2012) reported that cows fed Cr had trends for decreased BHBA and increased cholesterol on day of calving and increased glucagon on d 21 postpartum without effects on other plasma metabolites or hormones related to metabolism. Providing transition cows with Cr under heat stress did not affect serum glucose and Ca and P concentrations; however, NEFA were decreased during both the prepartum and postpartum periods whereas concentrations of serum insulin were increased and cortisol decreased throughout the whole experimental period compared with controls (Soltan, 2010).

**Cobalt and Iron**

Cobalt is an essential component of vitamin B$_{12}$. Vitamin B$_{12}$ is a cofactor for 2 major enzymes that are involved in gluconeogenesis and fat metabolism; therefore, Co status can affect metabolism in dairy cows. Supplemental copper glucoheptonate increased milk yield in multiparous cows (Kincaid et al., 2003). How-
ever, neither source (i.e., carbonate vs. glucoheptonate) nor level of Co affected lactation performance and metabolism in primiparous and multiparous cows (Akins et al., 2013).

Iron is related to both oxygen metabolism and energy metabolism. Also, Fe-dependent enzymes, such as catalase and peroxidase, are critical to controlling ROS (King, 2000). Hence, Fe deficiency can affect immune function and metabolism in dairy cows. However, Fe deficiency in adult cattle is very rare because requirements for maintenance and lactation are generally less than those for growth and because amounts of Fe consumed as a result of soil contamination of forages generally exceed requirements (NRC, 2001). Free Fe, which may increase as a result of an excess of dietary Fe, is reactive and a strong pro-oxidant, resulting in interfering absorption of other trace minerals and generating ROS (NRC, 2001). Weiss et al. (2010) fed cows 30 mg/kg of supplemental Fe from an Fe–AA complexed source for the last 60 d prepartum and continuing through 63 d postpartum. Differences in indices of Fe status and performance were not significant, except for a small decrease in SCC for cows fed the supplement.

**Determination of NRC (2001) “Requirements” For Trace Minerals**

The dairy NRC (2001) contains an excellent chapter on minerals, including a well-organized and extensive discussion of functional aspects, characteristics associated with deficiencies of trace minerals, approaches used to define requirements, special properties of each trace mineral if applicable, toxicity considerations, and known antagonisms affecting absorption of trace minerals. For most trace minerals, the classical factorial approach was used to determine “requirements” for maintenance, growth, pregnancy, and lactation. The absorbed supply for each trace mineral was calculated based on dietary supply and absorption coefficients. The reality is that use of this approach to accurately predict optimum status on a herd basis under widely varying field conditions is difficult, at best. One reason for this is that the approach to determine “requirements” for trace minerals does not fit our classical factorial approach very well. The quantities needed to optimize functions within the immune system and oxidative metabolism (as well as the myriad of other biological functions that involve trace minerals) are nearly impossible to measure directly. Second, the absorption antagonisms for many trace minerals are well known (Spears, 2003) but are going to vary from farm to farm based on variations in other feedstuffs and their composition and the micronutrient content of water. More sophisticated bioavailability models that better account for these antagonisms would help to predict supply. The extensive discussion provided by the authors of the dairy NRC (2001) with regard to various aspects of trace mineral requirements and supply is indicative of these challenges. Therefore, we must increase our reliance on phenotypic results to supplementation with varying amounts and sources of trace minerals with measured biological responses in key systems that are known to be dependent on trace mineral status.

**Recent Research Evaluating Supplementation of Trace Mineral Blends for Dairy Cows during the Transition Period and Early Lactation**

The predominant forms of trace minerals available for supplementation typically are categorized as either inorganic or organic, with primary reference to their chemical structure. Common inorganic sources are metals with ionic bonds and fed as oxides or sulfates. More recently, inorganic forms that contain covalent bonds of the metals with chloride (e.g., trisubic copper chloride; Spears et al., 2004) have become available and are commonly referred to as “hydroxy” forms. Various forms of organic trace minerals are available in which metals are chelated, complexed, or covalently bonded to AA, AA analogues, proteins, or organic acids, among other organic compounds. Most research conducted using these various forms has focused on either partial or full replacement of either sulfates or oxides with one of the other forms with potentially greater bioavailability (Spears, 2003). Although some research (described previously within the discussion for individual trace minerals) has been conducted using these various forms as single sources, a substantial amount of research, including several recent studies, have been conducted in which supplementation of blends of Zn, Cu, and Mn were evaluated in dairy cows during the transition period and/or early lactation.

The most commonly reported positive responses to feeding trace minerals in greater amounts and/or in more bioavailable forms to dairy cattle include increased milk yield, improved reproductive performance, decreased SCC, decreased lameness and improved foot health, and decreased disease incidence although there are many individual studies that have reported no differences between control and treatment groups for one or more of these outcome variables. Rabiee et al. (2010) conducted meta-analysis of production and reproductive outcomes from 24 studies in which cows were supplemented with AA complexes of Zn, Cu, and Mn plus copper glucoheptonate produced by the same company. Supplementation with these forms of trace minerals increased milk yield by a weighted mean difference of 0.93 kg/d although results were highly heterogeneous. Supplementation also
decreased days open (weighted mean difference = 13.5 d) and conception rate and increased risk of pregnancy by 150 d in milk (risk ratio = 1.07). Results in this meta-analysis for reproductive outcomes were not heterogeneous.

Nemec et al. (2012) fed cohorts of cows in 2 stages of lactation Zn, Cu, and Mn either as sulfates or chelated to 2-hydroxy-4-methylthiobutanoic acid (HMTBA) with 100% of predicted NRC (2001) requirements coming from the supplements, resulting in total dietary concentrations of 73 and 94 mg/kg of Zn, 21 and 23 mg/kg Cu, and 42 and 46 mg/kg Mn for the cows fed sulfate- or HMTBA-based treatments, respectively. Cows fed the HMTBA chelates had substantially increased (2.8x controls) titer to rabies vaccine, indicative of effects of trace mineral source on adaptive immunity. Phagocytosis from neutrophils was increased by HMTBA supplementation only in the cohort of cows starting treatment at 77 d postpartum.

Yasui et al. (2009) sought to determine whether amount and source of dietary trace minerals affected oxidative stress markers and aspects of immune function in cows during early to mid lactation. Cows were supplemented with Zn, Cu, and Mn as either sulfates or HMTBA chelates at either NRC (2001) levels or approximately 2x NRC levels. Actual concentrations for the NRC level averaged 55, 8, and 21 mg/kg for Zn, Cu, and Mn, respectively, and 75, 16, and 36 mg/kg for cows fed 2x NRC. Supplementation with these treatments followed a 4-wk preliminary period in which cows were fed no supplemental Zn, Cu, and Mn and also fed 0.37% dietary sulfur and top dressed once daily with iron sulfate (target of 250 mg/kg of Fe) and Mo from sodium molybdate (target of 5 mg/kg of Mo). After the preliminary period, dietary sulfur was reduced to 0.30% and the top dress of Fe and Mn stopped. During wk 1 of treatment, cows fed the HMTBA chelated forms tended (P = 0.13) to have decreased plasma TBARS and a trend for an interaction of level and source existed (P = 0.15) such that cows fed sulfate-based sources had decreased total antioxidant capacity in plasma when fed at the NRC level compared with either cows fed the HMTBA chelates at NRC levels or both forms at the greater levels. During wk 6 of treatment (i.e., 1 wk after intramammary challenge with lipopolysaccharide), cows fed the HMBTA chelates had decreased plasma TBARS (P = 0.05). Furthermore, cows fed the 2x NRC level tended (P = 0.09) to have increased total antioxidant capacity in plasma. Cows fed HMTBA chelates had increased total IgG concentrations in plasma over the entire treatment period.

Recently, we determined the effects of supplementation of 3 forms of Zn, Cu, and Mn to dairy cows during the transition period and early lactation on performance, indices of oxidative metabolism, and cytological endometritis (Yasui et al., 2012b). We fed Holstein cows (n = 60) entering second or greater lactation trace mineral supplementation strategies based on sulfates alone, a blend (75:25) of sulfates and AA complexes, or hydroxy forms of Zn, Cu, and Mn from 21 d before expected calving through 84 d postpartum. The resulting dietary concentrations of supplemental Zn, Cu, and Mn were similar among treatments and averaged 40, 10, and 27 mg/kg, respectively, before calving and 59, 15, and 40 mg/kg, respectively, after calving. The basal rations contained 39 and 43 mg/kg Zn, 6 and 8 mg/kg Cu, and 35 and 35 mg/kg Mn during the prepartum and postpartum periods, respectively. Cows fed hydroxy forms tended (P < 0.10) to have decreased plasma TBARS and total antioxidant capacity than the sulfate-based controls throughout the experiment; values for cows fed the blend were intermediate. Furthermore, cows fed hydroxy forms tended to have decreased plasma haptoglobin than cows fed the blend during wk 1 postpartum, which is indicative of less systemic inflammation (Huzzey et al., 2009). Cows fed hydroxy forms had faster increases in milk yield during early lactation (treatment × time interaction, P = 0.02).

In this experiment, we were interested in evaluating dietary effects on cytological endometritis as an intriguing model of phenotypically relevant (Gilbert et al., 2005) outcomes of improved immune function. We evaluated the proportions of neutrophils in uterine lavage samples collected in cows at both 7 d postpartum and on 1 d between d 40 and 60 postpartum. Our interpretation (Gilbert et al., 2007) is that “normal” cows (Fig. 1, panel A) will have greater proportions of neutrophils in uterine lavage samples collected during the early postpartum period but very low proportions by 40 to 60 d postpartum whereas cows with cytological endometritis (Fig. 1, panel B) would have a lesser proportion of neutrophils during the early postpartum period but greater proportions when characterized between d 40 and 60 postpartum. Effects of treatment in this experiment on both the proportion of neutrophils in uterine lavage at d 7 postpartum and the incidence of cytological endometritis evaluated between d 40 and 60 postpartum were not significant; incidence of cytological endometritis averaged 36% across treatments in this experiment, which is comparable to that observed in 2 other studies conducted by our research group (data not shown). These results indicated that trace mineral source modulated aspects of oxidative metabolism and early lactational performance but did not affect uterine health.

Chromium Supplementation during the Transition Period and Early Lactation

As described above, Cr has been shown to have a variety of effects on indices of immune function and metabolism. More recently, larger-scale studies have been
conducted with different forms of Cr to determine effects on lactational performance, metabolism, and reproductive function. Results from 6 of these experiments are summarized in Table 1. Of these experiments, 5 reported increased or trends for increased milk yield during the postpartum period, 5 reported increased or trends toward increased DMI during at least part of the study period, and 4 out of 6 studies reported either decreased circulating NEFA concentrations or trends toward decreased NEFA concentrations, particularly during the prepartum period. Although reproductive outcomes typically were not measured in these studies, Bryan et al. (2004) reported trends toward a greater percentage (50 vs. 39.2%) of cows pregnant during the first 28 d of the mating season and Kafilzadeh et al. (2012) reported decreased days to first ovulation in cows supplemented with Cr but no other effects on reproduction. Furthermore, although we did not find differences in milk yield in a recent study (Yasui et al., 2012a), cows fed Cr during the prepartum and postpartum periods had trends (P < 0.10) toward increased prepartum days in milk and decreased NEFA and had decreased incidence of cytological endometritis diagnosed, as discussed previously, supporting potential effects of Cr on reproductive function that warrant further investigation.

**Supplementation of Diets for Dairy Cattle with Various Forms of Selenium**

Selenium is a trace mineral with classical deficiency signs (NRC, 2001) and is limited by law in the United States to 0.3 mg/kg added Se (Food and Drug Administration, 1997). Inorganic Se typically is supplemented as either selenium or selenate. During the past 15 yr, organic forms of Se have become available for use in diets for dairy cattle and other species. Supplementation with Se reliably increases the Se concentration of milk; increases were greater when Se was supplemented as selenium yeast compared with inorganic forms (Ceballos et al., 2009). Waldron et al. (2004) determined that supplementation with 2 forms of selenomethionine substantially increased milk Se concentrations. One caveat in interpreting milk and tissue Se concentrations following supplementation of organic forms is that the organic molecule (e.g., methionine) will be incorporated into proteins as methionine; therefore, tissue or milk concentrations may not be a reliable indicator of status in these situations.

Despite apparently better Se status, results available to date indicate that feeding putatively more bioavailable forms of Se does not seem to have translated into improved phenotypic outcomes. Weiss and Hogan (2005) determined that replacement of selenite with selenium yeast during the dry period and early lactation doubled colostral and milk concentrations of Se and increased serum concentrations of Se in both cows and calves but did not affect neutrophil function, performance, or responses to intramammary endotoxin challenge. Furthermore, Rutigliano et al. (2008) fed cows Se as either selenite or selenium yeast from 25 d before expected calving until 80 d postpartum with 2 different reproductive synchronization protocols. Source of Se did not affect postpartum uterine health, resumption of cyclicity, ovulatory responses, conception rate, or pregnancy loss. In contrast to these results, Calamari et al. (2011) reported that early- to mid-lactation cows subjected to mild heat stress had decreased plasma TBARS when fed selenium yeast compared with cows fed either selenite or an unsupplemented control diet; furthermore, cows fed selenium yeast during mild heat stress had decreased plasma total antioxidants compared with cows fed supplemental selenite. These results indicate that physiological stressors may influence the responses of cows to different forms of Se.

**Research Priorities and Implications for the Practicing Nutritionist**

Our knowledge of the specific roles of trace minerals in metabolism and immunity has grown substantially during the past 15 yr, but it is clear that we still have much

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**Table 1.** Summary of recent experiments evaluating effects of chromium (Cr) supplementation as either chromium methionine (Cr-Met) or chromium propionate (Cr-Prop) on milk yield, DMI, circulating nonesterified fatty acids (NEFA), and reproductive performance (Repro)\(^1\)

<table>
<thead>
<tr>
<th>Study</th>
<th>Form and amount</th>
<th>Milk</th>
<th>DMI</th>
<th>NEFA</th>
<th>Repro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayirli et al., 2001</td>
<td>Cr-Met, approximately 8 mg/d Cr</td>
<td>↑</td>
<td>↑</td>
<td>Trend ↓</td>
<td>Not eval(^2)</td>
</tr>
<tr>
<td>Smith et al., 2005</td>
<td>Cr-Met, approximately 8 mg/d Cr</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td>Not eval(^2)</td>
</tr>
<tr>
<td>Bryan et al., 2004</td>
<td>Cr-Met, 6.25 mg/d Cr</td>
<td>↔</td>
<td>Grazing</td>
<td>↓ pre</td>
<td>Trend ↑ pregnancy rate</td>
</tr>
<tr>
<td>McNamara and Valdez, 2005</td>
<td>Cr-Prop, 10 mg/d Cr</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
<td>Not eval(^2)</td>
</tr>
<tr>
<td>Sadri et al., 2009</td>
<td>Cr-Met, approximately 10 mg/d Cr</td>
<td>Trend ↑</td>
<td>↑</td>
<td>Not eval</td>
<td>Not eval(^2)</td>
</tr>
<tr>
<td>Kafilzadeh et al., 2012</td>
<td>Cr-Met, 8 mg/d Cr</td>
<td>↑</td>
<td>Not eval</td>
<td>↓</td>
<td>Trend ↓ days to first ovulation</td>
</tr>
<tr>
<td>Yasui et al., 2012a</td>
<td>Cr-Prop, 8 mg/d Cr</td>
<td>↔</td>
<td>Trend ↑ pre</td>
<td>Trend ↓ pre</td>
<td>↓ CE(^3)</td>
</tr>
</tbody>
</table>

\(^1\)↑, ↔, and ↓ denote significant increase of Cr, no significant effect of Cr, and significant decrease of Cr, respectively. pre = prepartum.

\(^2\)Not evaluated.

\(^3\)CE = cytological endometritis.
to learn regarding trace mineral nutrition in dairy cattle. Although research is starting to unravel effects of level and source of supplemental trace minerals, we need to continue to refine our understanding of these interactions of source and level and how they in turn influence both the underlying metabolic and immunological systems and also phenotypic outcomes of economic importance. We need to further refine our dietary formulation models to better account for antagonisms for absorption and also requirements. Chromium is a relatively new trace mineral for supplementation in diets for dairy cattle. Results to date indicate positive effects on both production and reproduction; larger scale research needs to be conducted to evaluate if these reproductive benefits truly exist.

For the practicing nutritionist, it is important to recognize that responses to trace mineral nutrition are likely to be subtle yet important modulations in biological function that likely translate to improved health, performance, and reproductive performance over the longer term. Although there is heterogeneity of response to many components of animal responses to supplementation with more bioavailable trace minerals, generally favorable responses to replacement of sulfates or oxides with more bioavailable forms indicates the need to evaluate trace mineral supplementation strategies and become more strategic in terms of approach (i.e., feed less total mineral from more bioavailable sources).

**SUMMARY AND CONCLUSIONS**

Trace minerals play critical roles in aspects of energy metabolism, immune function, and oxidative metabolism of dairy cattle, particularly during the transition period and early lactation. These systems are not independent; rather, they must be considered in integrative ways and maintained in delicate balance both within and among each other. Collective results from studies conducted in dairy cattle support key roles for trace minerals in both immune function and oxidative metabolism; these likely underpin generally positive responses in production and immune function to supplementation with more bioavailable forms of common trace minerals (e.g., Zn, Cu, Mn). Chromium supplementation appears to enhance milk yield, improve metabolism, and potentially reproduction through a role in decreasing cytokological endometritis. More bioavailable forms of Se are available; however, results published to date indicate limited effects on aspects of immunity and reproductive function compared with supplementation with traditional inorganic forms. Trace minerals do not fit our traditional approaches for determining nutrient requirements; research going forward must continue to unravel the nuanced ways in which they affect immunity, oxidative metabolism, health, productivity, and reproductive performance in dairy cattle.

**LITERATURE CITED**


Trace minerals for dairy cattle


