Effect of trace mineral amount and source on aspects of immune function in dairy cows

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Abstract

Previous studies have shown a relationship between trace mineral supplementation and improved immune function in dairy cattle. In this experiment, 48 multiparous Holstein cows were utilized to determine if trace mineral supplementation of inorganic or chelated organic sources at National Research Council (NRC) or higher commercial levels would produce improved immune function after vaccination with an environmental mastitis vaccine (J-5) and during a LPS challenge. Cows were fed a typical postpartum diet for ad libitum intake formulated to meet or exceed NRC (2001) requirements for all nutrients except the trace minerals of interest: Zn, Cu, and Mn. Cows were then assigned to four treatments containing organic or inorganic trace mineral sources at commercial or NRC levels. At the end of week two of treatment, cows were administered a J-5 vaccine and blood samples were collected to measure level of IgG production. At the end of week six of treatment cows were subjected to an intramammary LPS challenge. Heart rate and rectal temperature were measured and blood samples were drawn throughout the eight-hour challenge at thirty-minute intervals and again at 24 and 48 hours post challenge. Plasma IgG levels from samples collected on a weekly basis were highest for cows supplemented trace minerals in an organic form regardless of amount. Heart rate and rectal temperatures following LPS challenge did not vary between treatments. Data for plasma concentrations of TNFα, IL-1, and cortisol levels during LPS challenge were not available at the time of thesis presentation. Overall, the results imply that supplementation of trace minerals in chelated organic form improves immune function and may be an effective tool in enhancing cattle health through optimizing nutrition. However, further study is warranted to determine the

optimal level of chelated trace mineral supplementation and the mechanism of trace minerals on immune function.

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List of Abbreviations

APP – Acute phase proteins

APR – Acute phase reaction

BPM – Beats per minute

Cu – Copper

DIM – Days in milk

GI – Gastrointestinal

IFN – Interferons

IgG – Immunoglobulin G

IL – Interleukin

LPS – Lipopolysaccharide

Mn – Manganese

NEL – Net energy for lactation

NRC – National Research Council

PMN – Polymorphonuclear neutrophils

TNF – Tumor necrosis factor

Zn – Zinc

Introduction

One of the mostly costly diseases affecting profits for producers within the dairy industry is mastitis (Rajala-Schultz et al., 1999). Mastitis during the periparturient and early lactation periods is often caused by Gram-negative bacteria, with *E. coli* as the most prevalent causal pathogen (Eberhart, 1977; Smith et al., 1985). Cases of mastitis decrease profits through increased treatment costs and reduced milk yield and impact well-being through increased rates of death and culling (Shpigel et al., 1997). Despite these serious outcomes, the severity of mastitis can be mitigated through efforts to improve immune system function.

One method of improving immune system function in cattle is ensuring that the diet fed contains all of the necessary nutrients needed for cell metabolism in proper amounts and sources. Trace minerals are those needed by the body in minute amounts and are typically included in the diet in parts per million quantities. Several of the trace minerals such as zinc, manganese, copper, cobalt, and selenium are required for the functionality of numerous structural proteins, enzymes, and cellular proteins (National Research Council, 2001; Nocek et al., 2006). Several studies have shown that increasing the level of trace mineral supplementation in the diet of dairy cattle has resulted in improved health and reproductive outcomes (Kellogg et al., 2004; Nocek et al., 2000; Ballantine et al., 2002). These improvements in animal performance appear to be related to increased availability of trace minerals for metabolism (Wedekind et al., 1992; Peripatananont and Lovell 1994).

In this study, we sought to determine if trace mineral supplementation at either National Research Council (NRC) level or higher commercial level used within the dairy

industry and from either inorganic or chelated organic sources have an impact on immune system function and health. Immune system function was evaluated through the following methods: the administration of a vaccine for environment mastitis (J- 5 bacterin, Pfizer) and the administration of an intramammary LPS challenge. Parameters planned for measurement after vaccination were plasma IgG levels, and after LPS challenge heart rate, rectal temperature, and plasma levels of TNFα, cortisol, and IL-1. The following review of the literature discusses a broad overview of the immune system and known effects of trace minerals on immune function and cell metabolism. Furthermore, I discuss the process of the LPS challenge and its connection to immune response and the J-5 vaccine and its connection to immune response.

Literature Review

The immune system is part of the host's defense against destructive forces from outside the body, such as bacteria, viruses, and parasites, or from within, such as malignant cells or those that produce autoantibodies (Bower, 1990). This system is composed of two branches: the innate or non-specific immune system, and the adaptive or specific immune system (Saker, 2006).

Innate Immunity

Innate immunity comprises physical barriers, soluble factors, and phagocytic cells that provide an immediate first line of defense against invading microorganisms (Medzhitov and Janeway, 1997). Physical barriers such as skin and mucous or gastrointestinal (GI) mucosa are reached first by pathogenic microorganisms and are the true first lines of defense. Once the host is compromised by microorganisms, endotoxins, or any substrate considered foreign, the complement system may be activated. The complement system is a complex cascade of proteins that promote functions such as phagocytosis, viral neutralization, and destruction of virus-infected cells (Saker, 2006). Innate immunity is similar among normal individuals, and does not contain a memory effect. Thus, the focus of innate immunity action is targeted against molecular structures of microorganisms that are essential for microbial survival, are present in many microorganisms, and are unique to pathogenic microorganisms. Examples of these include chemicals such as bacterial lipopolysaccharides and teichoic acid (Medzhitov and Janeway, 1997).

The major cells of innate immunity are phagocytic macrophages and neutrophils that possess surface receptors specific for common bacterial surface molecules. When the receptors of these cells are triggered, phagocytosis and destruction of the microorganism is initiated (Medzhitov and Janeway, 1997). Initially, neutrophils bind to pathogenic microorganisms, phagocytose them, and kill them. Phagocytosis is facilitated by opsonization. Opsonins activate neutrophils, resulting in an oxidative burst that includes production of hydrogen peroxide (H2O2) and O2- free radicals. These substances kill the bacteria and the neutrophil with the release of hypochlorus acid (Saker, 2006).

The innate immune system provides a rapid first line of first defense until the more powerful and flexible adaptive immune response can be activated. Although different, the innate and adaptive immune systems are not completely independent (Medzhitov and Janeway, 1997). The innate immune response influences the action of the adaptive response, and the active process of the adaptive response harnesses innate effector mechanisms such as phagocytes (Fearon and Locksley, 1996).

Humoral Immunity

The adaptive immune system is both extremely powerful and also highly regulated and flexible. Lymphocytes originate in the bone marrow from common lymphoid stem cells. Further development and maturation of B- and T-cells occurs in the bone marrow and thymus, respectively. Mature B- and T-cells then enter the blood stream. Specific receptors on the cell's surface enable adherence to capillary endothelial cells and migration into peripheral lymphoid tissues such as the lymph nodes and the

spleen. Thus, this ability to remain both in the blood stream and in lymphoid tissues allows continuous circulation of T- and B-cells throughout all of the body tissues ensuring constant immunological surveillance for invading pathogens (Huston, 1997).

Each T- and B-cell bears surface receptors with specificity for a single antigen; however, the specificity of each individual lymphocyte is different. An enormous number of different receptor recognition sites are generated during lymphocyte development through the random rearrangement of a limited number of receptor genes (Fanning et al., 1996). Although the immune system is able to recognize a large number of antigens, any single antigen is recognized by relatively few lymphocytes, typically 1 in 1,000,000. Consequentially, there are not enough lymphocytes to immediately eradicate an invading microorganism. When a lymphocyte antigen receptor engages its complementary antigen, the lymphocyte ceases migration, enlarges, and rapidly proliferates, so that within 3 to 5 days there are a huge number of effector cells, each specific for the initiating antigen. B-cells react to stimulation by antigens through differentiating into plasma cells that synthesize and secret antibodies commonly termed immunoglobulins (Huston, 1997). Cell-mediated immunity also relies on T-cells. Monocytes and macrophages phagocytize antigens, process them through an oxidative burst reaction, and then present antigen particles to T cells (Abbas et al., 1991). Once triggered, helper T-cells divide rapidly and secrete cytokines which help to regulate and increase the immune response. Certain T cells persist after an infection is resolved, and are responsible for immunological memory. These effector T-cells expand to large numbers after re-exposure to their cognate antigen, thus providing the immune system with memory against past infections (Saker, 2006).

This antigen-driven clonal expansion accounts for the characteristic delay of several days before adaptive immune responses become effective. Some effector cells generated by clonal expansion are very long lived, and are the basis for the immunological memory characteristic of adaptive immunity. Functionally, immunological memory enables a more rapid and effective immune response upon re-exposure to microorganisms. Additionally, the antigen specificities of adaptive immunity reflect the individual's lifetime exposure to infective agents and thus will vary between individuals (Huston, 1997).

Trace Minerals Cu, Zn, Mn and Their Effect on Immune Function

Trace minerals are those needed by the body in minute amounts (generally included in the diet in parts per million quantities). Several of these trace minerals such as zinc, manganese, copper, and cobalt are required for the functionality of numerous structural proteins, enzymes, and cellular proteins (Nocek et al., 2006). Trace minerals may function as cofactors, activators of enzymes, or stabilizers of secondary molecular structure and serve essential functions in cell metabolism (Valee and Wacker, 1976). Numerous studies have shown that feeding amino acid complexes of Zn, Mn, and Cu have improved the performance of dairy cattle through improving fertility rates and reducing the incidence of disease (Kellogg et al., 2004; Nocek et al., 2000; Ballantine et al., 2002). These improvements in animal performance appear to be related to increased availability of trace minerals for metabolism (Wedekind et al., 1992; Peripatananont and Lovell 1994).

Ruminants are frequently subjected to severe dietary deficiencies of trace elements such as copper, cobalt, selenium, iodine, manganese, and zinc (Hidiroglou, 1979). These deficiencies have been linked to a decline in fertility from enzymatic dysfunctions. Hypocuprosis in dairy cattle and sheep has been linked to female reproductive disorders such as prevention of embryo implantation and high prenatal mortality, particularly early embryonic loss (Hidiroglou, 1979). Several studies in rats and mice have shown that both cell-mediated and humoral immunity are greatly depressed by copper deficiency (Prohaska and Faila, 1993, Hidiroglou 1979). Torre et al. (1996) showed that marginal copper deficiency in dairy heifers reduced the capacity of neutrophils to kill *S. aureus*. Animals deficient in copper also show an increased susceptibility to bacterial pathogens. This has been attributed to the role of copper in superoxide dismutase and cytochrome c oxidase enzyme systems (Chandra, 1977). Babu and Failla (1990) reported that copper deficiency impaired the ability of macrophages to kill yeast cells. Jones and Suttle (1981) demonstrated that copper-depleted calves exhibited impaired phagocytic killing activity that was reversed by copper supplementation. In another study, low copper status was associated with a reduced response of peripheral-blood lymphocytes to stimulation with T-cell mitogens (Wright et al., 2000). Despite these studies, the overall effect of copper deficiency on macrophage function in cattle has not been studied extensively (Spears, 2000).

Extensive research conducted on human subjects and laboratory animals suggests that zinc deficiency reduces immune responses and disease resistance (Chesters, 1997). In children, zinc deficiency has been shown to affect T- lymphocyte and neutrophil function along with reduced proliferation of lymphocytes in the presence of mitogens and

slower neutrophil chemotaxis (Chandra, 1980). Zinc deficiency also produces atrophy of the lymphoid tissues such as the thymus. Zinc deficiency also negatively impacts phagocyte function resulting in decreased ingestion and phagocytosis (Chandra, 1977). A study conducted in laboratory animals fed a moderately zinc deficient diet showed that the differentiation and function of B-cells may be impaired (Vyas and Chandra, 1983). In cattle, surprisingly little research has been conducted to examine the relationship between dietary zinc and immune function. Marginal zinc deficiency appears to have marginal effects on immune function in ruminants, but research also suggests that the addition of zinc to practical diets may affect disease resistance (Spears, 2000). Zinc deficiency is most deleterious to the reproductive function of male animals; however, the administration of a zinc supplement to cattle was shown to increase conception rate by 23% compared to controls, and discontinuation of this supplement resulted in decreased conception rate (Nedyjlkov and Krustev, 1969).

Manganese deficiency has been linked to suppression of estrus, reduction in conception rates, increased incidence of abortions, and low birth weights. In dairy cattle, the main clinical sign of restricted manganese intake is anestrus or irregular return to estrus sometimes with extended periods of anestrus (Wilson, 1966). This leads to depressed conception rates (Hidiroglou, 1979). Experimental animals fed a manganese deficient diet have been shown to have deficient antibody synthesis and secretion (Keen et al, 1984). After adding manganese to the diet, antibody production improved. The mechanism(s) by which manganese affects antibody synthesis or release have not been clearly elucidated and further study in this area is needed (Keen et al, 1984).

Lipopolysaccharide (LPS) Challenge and Immune Function

Mastitis caused by environmental coliform bacteria is an increasing problem for the dairy industries in many countries (Dingwell et al., 2004; Kossaibati et al., 1998). In dairy cattle, mastitis has a sustained effect on milk yield resulting in 110 to 525 kg of lost milk per lactation. Additionally, cows with mastitis fail to reach their pre-mastitic milk yields for the remainder of lactation after the onset of infection (Rajala-Schultz et al., 1999). Mastitis is also an important risk factor for culling (Grohn et al., 1998). Bovine coliform mastitis is regarded as a serious problem because it is severe, sometimes fatal, and often difficult to control with antibiotics formulated for intramammary use. Mastitis during the periparturient and early lactating periods is often caused by Gram-negative bacteria, with *E. coli* as the most prevalent causal pathogen (Eberhart, 1977; Smith et al., 1985).

Coliform mastitis is often associated with severe clinical signs, extensive tissue damage, and considerable losses in milk yield (Golodetz and White, 1983; Shpigel et al., 1997). Periparturient cows and those in early lactation most often show severe clinical signs and fatal outcomes (Wang et al., 2003; Wenz et al., 2001). In *E.coli* mastitis, the response of the host mainly determines the severity of the disease (Burvenvich et al., 2003). The observed systemic and local clinical reactions result from the acute phase response (APR) which is the response of the host to any tissue injury caused by trauma or inflammation (Van Miert, 1995). The APR is characterized by fever, changes in vascular permeability, along with changes in the biosynthetic, metabolic, and catabolic profiles of many organs (Paape et al., 2002). The acute symptoms most often associated with

coliform mastitis are due to the rapid and unrestricted growth of the organism, the release of endotoxin, and the subsequent development of an unlimited inflammatory reaction (Hill, 1981). During *E.coli* mastitis, lipopolysaccharide released from bacteria is thought to be the initiating factor of APR (Burvenvich et al., 2003; Olson et al., 1995). Purified LPS administered to animals mimics the clinical symptoms caused by bacterial infection (Memon et al., 1992). These symptoms include lethargy, respiratory distress, fever, hypotension, tachycardia, increased cardiac output, diarrhea, changes in blood cell counts, and alteration in the blood coagulation system (Cullor, 1992).

Inflammatory changes occur in bovine mammary glands in response to infectious, mastitis-causing pathogens (Sordillo and Peel, 1992). When cells or tissues are injured, the host attempts to protect and heal itself by initiating the inflammatory process. However, an exaggerated, acute or protracted chronic inflammatory response can cause extensive necrosis of mammary gland parenchyma tissue, loss of milk production, and mortality in dairy cows (Hill, 1981; Lohius et al., 1990). The release of LPS from gram-negative bacteria initiates the nonspecific acute phase response by evoking the synthesis and release of cytokines at the foci of infection (Dinarello, 1983). Cytokines regulate local inflammatory reactions by cell to cell communication, but also have systemic effects through their presence in the circulation. Cytokines produced during the early stages of infection include tumor necrosis factor (TNF), interleukins (IL), and interferons (IFN) (Van Miert, 1991). These cytokines then act locally and systemically to attract polymorphonuclear neutrophils (PMN) from the circulation to the infection site and induce the production of acute phase proteins (APP) in the liver (Lehtolainen et al., 2004). In mastitis caused by Gram-negative bateria, pathophysiological responses to

endotoxins are thought to occur indirectly through the release and subsequent absorption of endogenous mediators from infected mammary glands and into the circulation (Lohuis et al., 1988). Cytokines including TNF, IL-1, and IFN are among these mediators which are induced by endotoxin, and they have an important role in the development of the acute inflammatory response (Dinarello, 1983).

TNF-α is thought to play a pivotal role in the pathophysiology associated with endotoxemia (Mathison et al., 1988; Tracey et al., 1986). TNF-α response to LPS is local and regulated at the level of the mammary gland (Paape et al., 2002). It is not responsible for the rise of body temperature or other systemic signs (Hoeben et al., 2000; Paape et al., 2002). TNF-α is produced rapidly by LPS-stimulated macrophages during initial bacterial colonization and appears to be responsible for the induction of other endogenous mediators such as IL-1β, IL-6, IL-8 and IFN (Van Miert, 1995). TNF-α is not a potential chemoattractant, but it can prime neutrophils to express adhesion molecules and thus support PMN migration (Lee et al., 2003). Changes in TNF concentrations coincide with the development of clinical signs of disease (Sordillo and Peel, 1992). Several studies showed that when TNF-α was administered intravenously, it induced shock and tissue injury of a magnitude similar to that observed after endotoxin administration (Mathison et al., 1988; Tracey et al., 1986). Dramatic increases in TNF concentrations appear to have an important role in the development of an exaggerated inflammatory response leading to death during acute E*.coli* mastitis (Sordillo and Peel, 1992). Circulating concentration of TNF-α were correlated with the severity of inflammation (Calandra et al., 1990; Tracey et.al., 1987). Results from a study conducted by Sordillo and Peel (1992) suggested that local and systemic clinical changes in severe *E.coli* mastitis are at least partially mediated

by the production of TNF by cell populations in the mammary gland and by the subsequent absorption of this cytokine into the circulation. The cytokines produced during the acute phase of immune system activation interact with one another to stimulate the production of acute phase proteins which may inactivate bacteria, reduce the amount of available zinc and iron required for bacterial growth, and induce a febrile response that can inhibit the growth of a number of gram negative bacteria (Lohuis et al., 1988; Van Miert 1991). Reducing TNF activity can effectively moderate the pathology and lethality associated with endotoxemia (Sordillo and Peel, 1992).

Differences in the production, function, and kinetics of cytokines and APR may explain the varying local and systemic signs of individual cows infected with *E.coli* mastitis. (Ohtsuka et al., 2001; Blum et al., 2000). Higher concentrations of TNF-α in milk in early lactation may not be connected with the severity of response but rather with differences in the immune system, including the cell populations in early and late lactation (Sordillo and Peel, 1992).

J-5 Vaccine and Immune Response

Due to the serious nature of coliform mastitis, several vaccines have been developed to counteract its deleterious effects. A mutant strain of *Escherichia coli* 0111:B4 (J5) has a unique characteristic in which the cell is unable to synthesize the exogenous layer of the outermost cell wall leaving the core and lipid A antigens of LPS exposed (Blum et al., 2000). Thus, immunization with *E. coli* J5 produces antibodies that are cross reactive with other coliform pathogens, most importantly LPS without severely

damaging the host (Ziegler, 1988; Blum et al., 2000). This generation of antibodies after injection with *E. coli* J5 forms the basis of the J-5 vaccine developed and marketed by Pfizer (Daigneault et al, 1991).

Results from challenge and field trials have shown that immunization with an *E. coli* J5 bacterin increases antibody titers to *E. coli* J5 core antigens in serum and mammary secretions (Cullor and Smith, 1996; Rings, 1985). Enhanced antibody titers to *E. coli* J5 are associated with a decreased risk of developing clinical coliform mastitis (Tyler et al, 1991) and immunization with *E. coli* J5 reduced the severity and lowered the rates of clinical coliform mastitis (Hogan et al, 1992; Hogan et al, 1995).

The endotoxin challenge (LPS challenge) model has been widely used to simulate mastitis caused by Gram-negative bacteria in adult cows (Ziegler, 1998; Blum et al., 2000). Administration of LPS post vaccination as a simulated *E. coli* infection allows the study of the efficacy of vaccination with *E. coli* J5 bacterin in reducing the severity of clinical disease caused by *E. coli* infection (Deluyker et al., 2004). In a recent study performed on dairy calves vaccination with J5 bacterin resulted in increased whole-cell J5 IgG titers at the time of LPS challenge when compared to an unvaccinated control group. Calves that were previously vaccinated with J5 bacterin were better protected from *E. coli* infection as demonstrated by less dramatic changes from pre-challenge baseline values for clinical variables, physiological parameters, and hematology (Deluyker et al., 2004).

Despite the described connections between trace mineral status and immune function, relatively few studies have been done to determine the ideal level or source of trace mineral supplementation in the diet of lactating dairy cows. Thus, research is

required to further clarify this area. We hypothesized that supplementation of trace minerals in a chelated organic form at commercial levels would foster the highest level of immune system health. This would be exhibited by increased levels of IgG production after J-5 vaccination and lower heart rates, body temperatures, cortisol, TNFα, and IL-1 levels during LPS challenge.

Materials and Methods

Animals, Treatments, and Sampling

All treatments involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. Multiparous Holstein-Friesan cows (n = 48) were housed at the Cornell Teaching and Research Center and utilized in this experiment. Cows were housed in individual tiestalls beginning at 60 to 140 days in milk (DIM) and allotted to the experiment in five groups (blocks). Cows were fed a postpartum diet typical of the Northeast for ad libitum intake which was formulated to meet or exceed NRC (2001) nutrient requirements for all nutrients except for the trace minerals of interest – zinc, copper, and manganese (Table 1). These trace minerals were supplied from basal ration ingredients only. In addition, sulfur level in the diet was elevated in an effort to further decrease status of trace minerals. Cows were fed this low trace mineral level diet for four weeks (weeks -4 to 0) along with an iron and molybdenum topdress of 250 ppm of Fe and 5 ppm of Mo. Copper, Molybdenum, and Sulfur from organic or inorganic sources can combine in the rumen to form an unabsorbable triple complex, Copper tetrathiomolybdate ($CuMoS_4$) (Suttle, 1991). Thus, supplementation of sulfur and molybdenum was given in order to decrease status of the trace minerals of interest.

After the four-week pretreatment depletion period, cows were fed a different basal treatment diet formulated to meet or exceed NRC (2001) requirements for all nutrients except the trace minerals of interest. Ingredient and nutrient compositions of the pretreatment and basal treatment diets are described in Table 1. Cows were then divided into four treatment groups of trace mineral supplementation to the basal diet. Treatment

one consisted of trace mineral supplementation at NRC (2001) levels in an inorganic form. Treatment two consisted of trace mineral supplementation at NRC (2001) levels in a chelated organic form. Treatment three consisted of trace mineral supplementation at commercial levels using an inorganic source. Treatment four consisted of trace mineral supplementation at commercial levels using a chelated organic source. All trace mineral supplements were obtained as Mintrex™ chelated minerals (Novus International (St. Louis, Missouri) and the organic treatment was composed of the trace minerals of interest chelated to a methionine analog. All treatments were administered to cows once daily immediately after feeding through topdress and were given for six weeks. Levels of trace mineral supplementation are described in Table 2.

Blood (10 ml) was collected from each cow by coccygeal venipuncture into heparinized evacuated tubes once weekly at approximately 1100 h beginning during the last week of the pretreatment period and continuing through 6 wk of treatment. Blood was then centrifuged to collect plasma (2,800 x g, 15 min at 4°C), snap frozen in liquid nitrogen, and then stored at -20°C until analysis. After the blood sample collected at the end of wk 2 of treatment, all cows were administered a commercially available vaccine for environmental mastitis (J-5 bacterin, Pfizer). Plasma IgG levels were determined on all weekly blood samples using a commercial ELISA kit (Bovine IgG ELISA Quantitation Kit, Bethyl TX). All spectrophotometric measurements were done using a $Versa_{max}$ tunable microplate reader (Molecular Devices, Sunnyvale, CA).

At the end of week five of treatment all cows were fitted with a single indwelling jugular catheter. The following day all cows were subjected to an intramammary lipopolysaccharide (LPS) challenge. Using sterile technique, 100 μg of LPS from *E. coli*

suspended in sterile saline was injected into two homolateral quarters. Each quarter was manually massaged for one minute post infusion to enhance LPS distribution. Following LPS introduction, 20 ml of blood was sampled from the jugular catheter at 30-minute intervals for an 8-h period following challenge and again at 24 and 48 h post challenge. Rectal temperatures and heart rates were measured and recorded at the same intervals as blood collection. Blood samples were kept on ice until centrifugation (2,800 x g, 15 min at 4°C) after which plasma was harvested and snap frozen at -20°C until analysis for TNFα, IL-1, and cortisol (results pending for all of these).

Statistical analysis

Data for weekly IgG concentrations in plasma and clinical measurements following LPS challenge were subjected to analysis of variance for a randomized complete block design with repeated measures using the MIXED procedure of SAS (version 9.1; SAS Institute, Cary, NC). Terms in the model included block, treatment, time, and the interaction of treatment and time. Cow within treatment was a random effect. Four different covariance structures (autoregressive order one with and without heterogeneity and compound symmetry with and without heterogeneity) were tested and the model having the smallest Akaike's information criterion was chosen. During analysis of plasma IgG, values from samples collected at the end of the pretreatment period were used as covariates. Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$. Least squares means are reported throughout.

Table 1. Ingredient and nutrient composition on a dry matter basis diets of the pretreatment and basal treatment diets.

Ingredient	Pretreatment Diet	Basal Treatment Diet
Corn silage	44.19	44.19
Hay crop silage	14.73	14.73
Corn meal	12.85	7.15
Soybean meal	9.24	5.09
Citrus pulp	3.59	2.08
Soybean hulls	5.74	3.07
Corn germ meal	1.82	1.02
Corn gluten meal	0.92	0.48
Expeller soybean meal	2.30	1.29
Blood meal	1.29	0.69
Minerals and vitamins	3.31	1.01
Chemical Composition		
Crude Protein, %	16.80	17.00
Soluble Protein, % of CP	37.75	39.00
Acid Detergent Fiber, %	20.90	21.36
Neutral Detergent Fiber, %	33.10	32.75
NEL, Mcal/kg	1.68	1.68
Ca, %	0.89	1.08
P, %	0.33	0.32
Mg, %	0.29	0.28
K, %	1.09	1.02
Na, %	0.26	0.29
Fe, ppm	252.00	305.80
Zn, ppm	32.00	51.67
Cu, ppm	5.75	10.00
Mn, ppm	25.50	29.00
Mo, ppm	0.77	0.83
S %	0.34	0.26

Table 2. Trace mineral content of top dress supplement given to cows at start of treatment period.

			Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
	Basal Diet	NRC Recommendation	NRC Inorganic	NRC Chelated	Commercial Inorganic	Commercial Chelated
	ppm	ppm	ppm	ppm	ppm	ppm
Copper	10	11	13.3	13.3	21.3	21.3
Zinc	52	48	80	80	100	100
Manganese	29	14	29	29	43.5	43.5

-The diet was originally formulated to contain 7.7 ppm Cu, 20.3 ppm Zn, and 18.5 ppm Mn. However the basal diet actually contained higher values for all trace minerals as indicated above such that actual levels for both the NRC and commercial levels were elevated compared to the formulated levels.

Results

Weekly plasma concentrations of IgG during the treatment period are described in Figure 1. After administration of the vaccine for environmental mastitis (J-5 bacterin, Pfizer) at the end of wk 2, concentrations of plasma IgG generally increased for cows assigned to all treatments during wk 3 and 4 and then decreased during wk 5 and 6. Concentrations of IgG in plasma for cows fed either of the chelated trace mineral treatments were greater than that for inorganic supplementation.

Immediately after LPS infusion, rectal temperature and heart rate was measured to assess clinical reactions to LPS infusion (Figures 2 and 3). In all cows rectal temperature began to rise two hours after LPS infusion, with the maximum temperature around five to six hours post infusion. Rectal temperatures then declined after six hours post infusion but did not return to basal levels. There was no significant difference in the temperature increase observed among treatments. Heart rate measurements followed a similar pattern with elevated heart rates observed one hour post LPS infusion with maximum heart rates reached three to five hours post infusion. Heart rates remained elevated after reaching a maximum and did not return to basal levels by the end of observation. There was no significant difference in the increase in heart rate observed among treatments.

Figure 1. Plasma IgG concentration in dairy cows after administration of J-5 vaccine on week two of treatment. Data was adjusted for pretreatment values using analysis of covariates; SEM averaged 181 mg/dl.

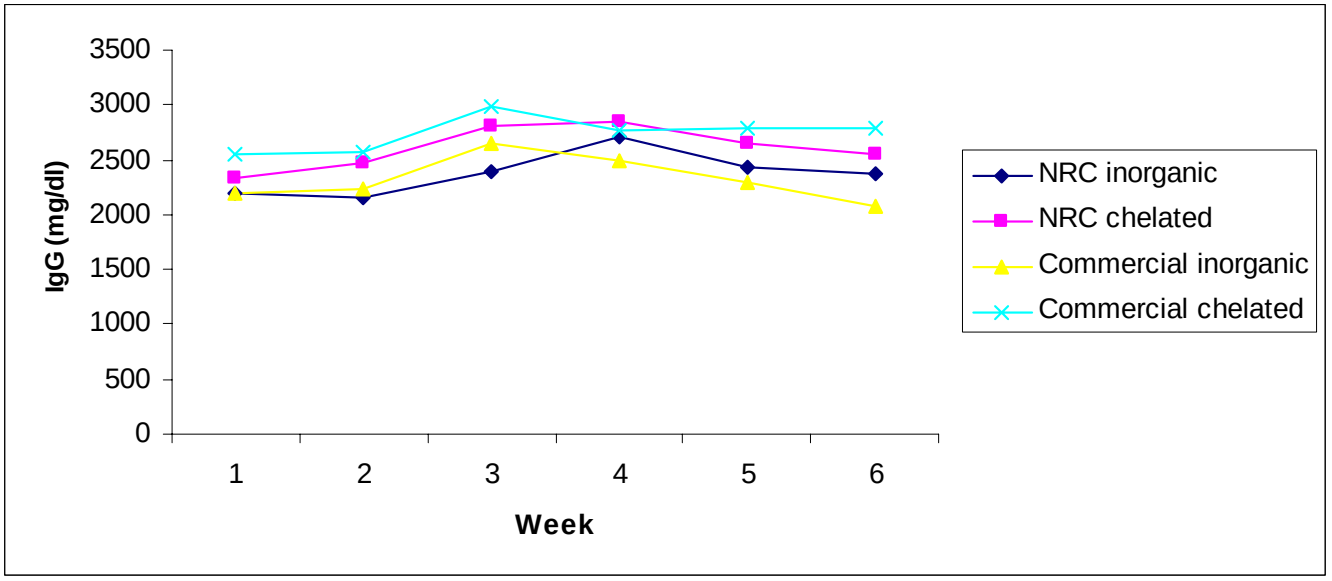


Figure 2. Rectal temperature in dairy cows following intramammary LPS administered on week five of trace mineral supplement treatment. The interaction of treatment and time were shown to not be significant; SEM averaged 0.20°C.

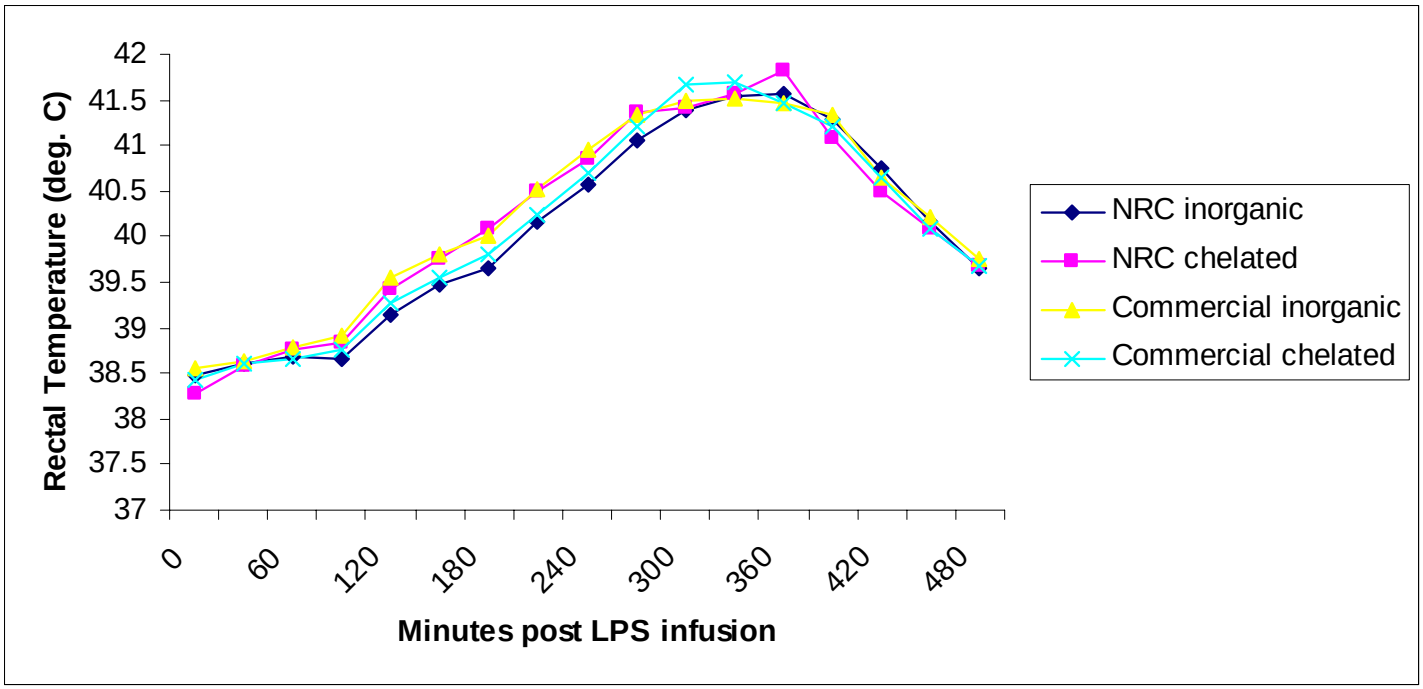
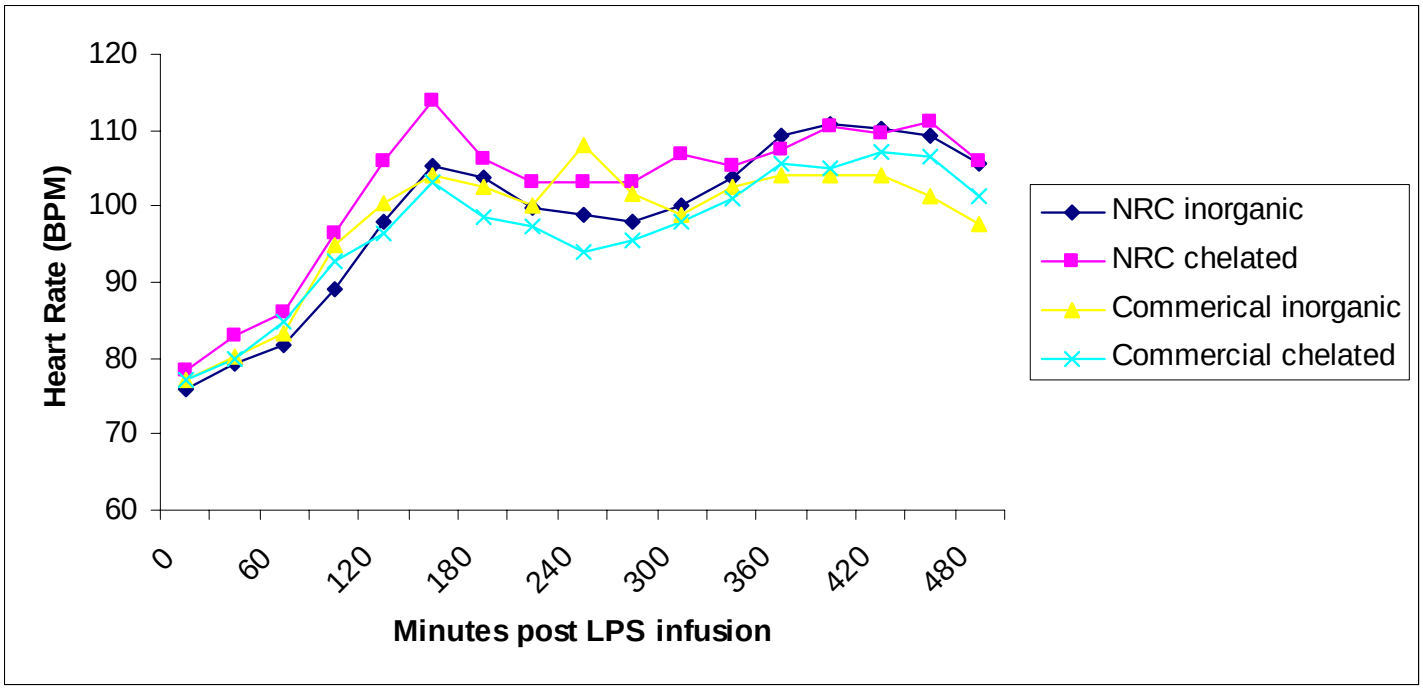


Figure 3. Heart rate in dairy cows following intramammary LPS challenge administered on week five of trace mineral supplement treatment. The interaction of treatment and time was not significant; SEM averaged 30 BPM.



Discussion

After the administration of J-5 vaccine, all treatment groups exhibited an initial rise in IgG titers (Figure 1). This result is consistent with previous findings (Deluyker et al., 2004; and Tomita et al., 2000). Overall, cows fed chelated organic sources of trace minerals, regardless of amount, had greater concentrations of IgG in plasma (Figure 1). This difference in IgG production may be attributable to the greater absorption of trace minerals from the chelated treatments due to their organic nature (Kincaid and Socha, 2004). A study conducted by Nocek et al. (2006) determined that feeding chelated trace minerals at 75% of NRC recommended levels still produced more favorable health, reproductive, and performance outcomes than supplementation of trace minerals at NRC levels using inorganic sources. This suggests that chelated trace minerals are more bio-available than inorganic sources, and are possibly an important factor in the cow's production of antibodies and maintenance of immune health. The increased level of trace mineral absorption could then be responsible for greater production of IgG through some mechanism of increased trace mineral level affecting cell metabolism resulting in better immune function performance (Wedekind et al., 1992; Peripatananont and Lovell 1994). Further research is required in this area to elucidate the exact mechanism of these effects of trace minerals on immune function.

The amount of LPS administered was sufficient to create an acute immune response as indicated by elevated rectal temperatures and heart rates among all treatments following infusion (Figures 2 and 3). However, no significant differences were observed among treatments. This may be attributable to the macroscopic nature of these physiologic responses. Heart rate is variable and can be affected by movement of the

animal, level of excitement, and many other factors that are difficult to control during data collection. Rectal temperature can be affected by ventilation or presence of fans in cattle housing facilities. Our findings for rectal temperature and heart rate are consistent with several other experiments in both their general pattern of increase followed by gradual decrease during LPS challenge and absence of significant differences among treatments (Waldron et al., 2003; Deluyker et al., 2004). Thus, these parameters are not the most definitive for determining level of immune response following an LPS challenge.

Unfortunately, the effect trace mineral supplementation level and source on level of immune response following LPS challenge was unable to be completely evaluated due to the unavailability of TNFα, Il-1, and cortisol measurements at the time of preparation of this thesis. However, some predictions on general trends of these parameters can be made in accordance with previously published findings and our preliminary data indicating increased IgG production in animals supplemented with chelated forms of trace minerals. We speculate that cows provided with chelated trace minerals resulting in improved immune function will exhibit a lower overall increase in TNFα levels with a quicker return to basal levels during LPS challenge (Sordillo and Peel, 1992). Treatments indicating less favorable immune response would exhibit higher peaks in TNFα levels which would be sustained throughout LPS challenge (Sordillo and Peel, 1992). Levels of cortisol and IL-1 would be expected to follow a similar pattern with treatments exhibiting improved immune function showing a lower overall cortisol and IL-1 level peak with a quicker return to baseline levels (Waldron et al., 2003; Van Miert, 1995).

Conclusion and Implications

Data from this experiment suggest that administration of trace minerals in chelated form may improve immune function in dairy cows. Preliminary results indicate that supplementation of chelated forms, at both amounts studied, increased circulating IgG concentrations compared to supplementation with inorganic forms. Results of clinical measurements indicated no significant difference between supplementation at NRC or commercial levels; however, future data on TNFα, cortisol, and IL-1 levels may allow differentiation among treatments. These findings have substantial potential in influencing the composition of rations fed to dairy cattle in order to improve immune health and reduce the incidence of disease.

Further research is necessary to confirm these results using larger numbers of cows, and to identify the ideal level of trace mineral supplementation. Additionally, further studies must be performed to illuminate the precise mechanisms by which trace minerals act within the immune system.

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