SOURCE AND DURATION OF TRACE MINERAL SUPPLEMENTATION AND THEIR EFFECTS ON EARLY POSTPARTUM REPRODUCTIVE PERFORMANCE IN MARES AND INNATE AND ACQUIRED IMMUNITY IN FOALS

By

JEROME GEORGE VICKERS IV

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To my mother and father, Terry and Mike Vickers, because they always told me I could, and to the two truest friends I have and will ever know, brothers Brandon and Chase.

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Jerome George Vickers IV

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A study was conducted to investigate the effects of source and duration of trace mineral supplementation on foal-heat ovulation and innate and acquired immunity in nursing foals. Mares were blocked by age, breed, and expected foaling date and randomly assigned to dietary treatments in a 2 x 2 factorial arrangement. Mares were supplemented with isoelemental amounts of Zn, Mn, Cu and Co in one of two forms: 1) inorganic (SULF; n=18); or 2) amino acid complexes (4PLEX; n=18), supplied in amounts to provide 1X Zn and Mn, 1.5X Cu and 44X Co requirements for mares in late gestation and lactation. Within each form of trace mineral supplementation, mares originated from one of two populations: 1) mares that had been maintained on a similar program of inorganic or amino acid complex trace mineral supplementation for 6 mo as part of a previous experiment (LONG; n=18); or 2) mares that had no prior exposure to amino acid complex trace minerals (SHORT; n=18). This arrangement resulted in four treatments: 1) SULF-LONG (n=9); 2) SULF-SHORT (n=9); 3) 4PLEX-LONG (n=9); and 4) 4PLEX-SHORT (n=9). Supplementation began 84 d before expected foaling and continued through 112 d post-foaling. Neutrophil function was not affected by trace mineral source or duration of supplementation in mares or their foals. Colostrum IgG and IgM were not

affected by trace mineral treatment. Colostrum IgA was higher ($P<0.05$) in mares receiving 4PLEX and serum IgA at 24 h was higher ($P<0.05$) in foals nursing 4PLEX mares. Foals received a series of three tetanus toxoid vaccinations beginning at 112 d of age. Tetanus-specific IgG titers did not differ in foals nursing 4PLEX or SULF mares. Improved transfer of trace minerals *in utero* was not evident based on similar trace mineral composition of umbilical cord blood among treatments. Trace mineral treatment had no effect on mare serum Zn, Mn, or Cu. Foals from SULF mares had higher Zn levels at 1-2 d of age ($P<0.05$) and higher Cu levels at 112 d of age ($P<0.05$). Serum vitamin B12 was higher in 4PLEX mares at 112 d post-foaling ($P<0.05$) and in foals from 4PLEX mares at 1-2 d of age ($P<0.05$). Dietary trace mineral source and duration of supplementation had no effect on growth rate of the six largest follicles, days to follicle deviation, days to ovulation, or the number of mares that ovulated within or after 10 d post-foaling. In addition, temporal changes in intrauterine luminal fluid score, endometrial edema score, uterine body diameter, and non-gravid horn diameter were not affected by trace mineral treatment. Growth rate of the largest follicle ($P<0.05$) and rate of gravid horn involution ($P<0.05$) were greater in 4PLEX-SHORT mares than 4PLEX-LONG mares, but did not differ among other treatments. Based on the results of this study, it appears supplementing mares with trace mineral amino acid complexes in late gestation may positively affect transfer of passive immunity in the foal. In addition, this study illustrates the potential for amino acid complexes supplemented in late gestation to more rapidly repare the uterus for rebreeding at foal-heat.

CHAPTER 1
INTRODUCTION

Trace minerals are important nutrients in the diets of all animal species, as they are essential components of certain enzymes and transport proteins, and they play fundamental roles in optimal immune and reproductive functions (Power and Horgan, 2000). While the efficacy of organic trace mineral supplementation has been studied in most farm animal species (Spears, 1996; Acda and Chae, 2002; Hostetler et al., 2003), research in horses is lacking. The small number of studies using horses have principally examined organic trace mineral supplementation in weanlings and yearlings, with little focus on supplementation of the broodmare and its effect on the foal.

The physiologic state of the broodmare is unique from other classes of horses, due to an increased systemic demand for nutrients during pregnancy, parturition, and lactation. Research has shown that deposition of the trace minerals copper, zinc, and manganese in foal tissues is greater at birth than during the first year of the foal's life (Meyer and Ahlswede, 1978), indicating accretion of trace minerals during gestation. Increasing the trace mineral content of the diet above NRC requirements during lactation has little effect on the trace mineral content of the mare's milk (Lewis, 1995), thus making *in utero* transfer of trace minerals important. Therefore, supplementing the broodmare with organic trace minerals during gestation may serve as an appropriate means to improve immune function and trace mineral status of the foal.

The reproductive success of the mare during foal-heat is critical due to the mare's lengthy gestation and the industry's goal to maintain a 12-month foaling interval. To the authors knowledge, only two studies (Ley et al., 1990; Ott and Asquith, 1994) have investigated the effects of organic trace mineral supplementation on reproduction in horses. By comparison, several studies in ruminants have shown enhanced reproductive function compared to inorganic

trace mineral supplementation. Organic trace mineral supplementation of broodmares in late gestation through the first postpartum estrous cycle may provide a method for enhancing reproductive performance during foal-heat.

The objectives of this research were to determine the effects of dietary trace mineral source and duration of supplementation in the mare on: 1) passive transfer of immunity to the foal; 2) innate and humoral immunity in nursing foals; 3) circulating trace mineral concentrations in mares and foals; 4) vitamin B12 status of mares and foals; and 5) reproductive performance of the mare during the first postpartum estrous.

CHAPTER 2
REVIEW OF LITERATURE

Trace Mineral Source, Absorption, and Metabolism

Source

Trace minerals are generally available in two molecular forms: inorganic or organic. Traditionally, trace mineral supplementation has been achieved through the use of inorganic trace minerals. The trace metal ion in the inorganic form is combined with an inorganic salt, largely oxide, sulfate, chloride, or carbonate. More recently, there has been interest in using organic trace minerals or a combination of inorganic and organic trace minerals in equine diets. The term organic is used to describe the molecule formed when a metal ion reacts with some organic ligand. The ligand in the metal-ligand complex can potentially be any one or more amino acids, proteins of varying size, polysaccharides, or propionate. As such, organic trace mineral sources encompass a wide variety of metal-ligand arrangements, which have been defined by the Association of American Feed Control Officials (AAFCO, 2005) (Table 2-1).

Table 2-1. AAFCO feed ingredient definitions for organic trace mineral products[1]

Product	Feed Ingredient Number	Description
Metal amino acid complex	57.150	Product resulting from complexing of a soluble metal salt with amino acid(s). Declared as a specific metal amino acid complex ("Zinc amino acid complex")
Metal (specific amino acid) complex	57.151	Product resulting from complexing a soluble metal salt with a specific amino acid. Declared as a specific metal, specific amino acid complex ("Zinc lysine complex")
Metal amino acid chelate	57.142	Product resulting from the reaction of a metal ion from a stable metal salt with amino acids with a mole ratio of one metal to one to three moles of amino acids to form coordinate covalent bonds and heterocyclic ring(s). Declared as a specific metal amino acid chelate ("Manganese amino acid chelate")
Metal polysaccharide complex	57.29	Product resulting from complexing of a soluble salt with a polysaccharide solution. Declared as specific metal complex ("Copper polysaccharide complex")
Metal proteinate	57.23	Product resulting from chelation of a soluble salt (mineral) with amino acids and/or partially hydrolyzed protein. Declared as specific metal proteinate ("Copper proteinate")

[1]Adapted from AAFCO, 2005

The terms chelates and complexes are often used interchangeably when referring to organic trace minerals, but in fact the terms identify different complexes. In general, a complex describes the product formed when a metal ion reacts with a ligand. The ligand is a molecule or ion that contains an atom which has a lone pair of electrons. Donor atoms such as oxygen, nitrogen, or sulfur serve to bond the metal ion to the ligand, forming a complex. Hynes and

Kelly (1995) describe ligands based on the number of donor atoms present. Ligands that contain a single donor atom are referred to as monodentate, while those containing two or more donor atoms capable of bonding to a metal ion are referred to as bi-, tri-, or tetradentate, collectively known as polydentate ligands. Amino acids are examples of bidentate ligands, whereby amino acid(s) are bound to metal ions via an oxygen of the carboxylic acid group and the nitrogen of the amino group (Hynes and Kelly, 1995). The complex resulting from the use of polydentate ligands and metal ions creates one or more heterocyclic rings containing the metal atom, and is referred to as a chelate. Therefore, all chelates are complexes, but not all complexes are chelates.

Multiple chelates may form the organic trace mineral referred to as a proteinate. Proteinates are formed by partially hydrolyzing a protein source through enzymatic or acid procedures. The hydrolyzing of the protein results in the formation of an amino acid/peptide hydrolysate consisting of varying chain lengths. The reaction of this hydrolysate with a metal salt under appropriate conditions yields a complex containing chelated metal ions, called a proteinate (Hynes and Kelly, 1995).

Inorganic and organic trace mineral supplementation strategies are both widely implemented, and important differences exist in their molecular forms. As such, important differences also exist in uptake mechanisms and the ultimate bioavailability of different sources.

Absorption

The absorption of ingested minerals is often the primary factor determining their ultimate utilization. Ingested minerals can be separated into two categories which aid in distinguishing their absorptive capacities. First are those minerals soluble throughout the varying pH of the gastrointestinal tract. Minerals such as magnesium, sodium, and calcium fall into this first category, in which hydroxy-polymerization is not a concern. Minerals that undergo hydroxy-

polymerization in the gastrointestinal tract fall into the second category, and include most trace minerals. Acid soluble cations like copper, manganese, and zinc are least soluble when exposed to the neutral pH of the small intestine (Whitehead et al., 1996). Because of their hydrolytic nature, water molecules tend to coordinate around the metal ion, subsequently losing protons in the neutral pH of the small intestine. This results in a hydroxy-metal species, ultimately forming insoluble precipitates in the absence of soluble binding ligands. For normal metal ion uptake to occur, it has been proposed that both endogenous soluble ligands and mucosally associated ligands are required (Ashmead, 1993; Whitehead et al., 1996). Gastroferrin, albumin, lactoferrin, citrate and soluble mucins have all been identified as possible secreted soluble ligands, preventing the hydroxy-polymerization of susceptible metal ions. The predominant mucosally associated ligand is thought to be mucin. Mucus is chiefly produced by goblet cells and released throughout the digestive tract, and constitutes two separate phases (Hunter et. al., 1989), the aforementioned soluble mucin and the insoluble mucous gel layer adherent to the mucosal surface.

Luminal nutrients must pass the mucosally adherent mucous layer before actual presentation to the enterocyte and subsequent absorption occurs. The soluble mucins present throughout the gastrointestinal tract have a strong affinity for metal binding, in which highly charged metals are bound more readily than more neutrally charged metals. The affinity of metal binding to mucus in the mucosally-adherent mucus layer follows the same pattern, and the pattern for mineral absorption is its inverse (i.e., the greater the binding of minerals to mucus, the less absorption) (Whitehead et al., 1996). In short, the mucous layer acts as a filter in regulating metal uptake and the strength of binding to and rate of passage across the mucosally-adherent mucus layer could be important in determining the overall absorption of a metal (Powell et al.,

1999a). Highly charged metals would be bound tightly by mucus, having kinetically slow rates of ligand exchange, rendering the metal unable to pass quickly through this layer. Metals that remain bound are shed with the mucus back into the lumen, and excreted (Powell et al., 1999b). This is illustrated by toxic Al^{3+}, in which the metal is so tightly bound by the mucus layer that it rarely manages to traverse it, and is sloughed off into the lumen during mucosal turnover. The tight binding of trivalent cations also serves to explain the decreased absorption of ferric iron (Fe^{3+}) compared to ferrous iron (Fe^{2+}) (Whitehead et al., 1996). Thus, metal ions must avoid hydroxy-polymerization and also penetrate two functional barriers before presentation to the enterocyte for absorption.

While hydroxy-polymerization accounts for much of the metal ion loss in the gastrointestinal tract, antagonistic relationships among minerals also occur that inhibit their absorption and utilization. Antagonism can occur between two different minerals, as well as among minerals and other nutrients present in the gastrointestinal tract. For example, in ruminants the formation of insoluble thiomolybdates can occur in the presence of sulfur and high intakes of molybdenum, resulting in a copper deficiency (McDowell, 2003). In the horse, an antagonistic relationship exists between zinc and copper, such that when high levels of zinc are supplemented, competition among minerals for binding sites can result in a copper deficiency (Bridges et al., 1984). In addition, phytic acid and dietary fibers have been shown to bind trace minerals, thus negatively influencing their absorption (Forbes and Erdman, 1983).

While inorganic trace minerals are subject to a host of physiochemical factors affecting absorption, organic trace minerals are proposed to avoid such factors. Although not fully elucidated, organic trace minerals are believed to utilize alternate uptake mechanisms in the gastrointestinal tract. Amino acid complexes and proteinates have been proposed to utilize

amino acid uptake mechanisms rather than normal metal ion uptake pathways. The theory, as described by Power and Horgan (2000), is that the metal in question is protected within the complex in a chemically inert form by covalent and ionic bonding by the amino acid ligand. Therefore, the metal is not susceptible to dissociation because of the metal-ligand high stability constant, preventing exposure to the physiochemical factors mentioned earlier. It is believed that the organic trace mineral is absorbed intact through the intestinal mucosa, effectively pulling the metal along with it. However, if organic trace minerals have strong stability constants such that dissociation fails to occur once absorbed, the metabolism of the mineral may be compromised (Miles and Henry, 2000). Regardless of uptake mechanism, ligand binding can reduce the charge on mineral ions. By reducing charge, metal ions may arrive at the mucosally-adherent mucus layer in a non-precipitated form, avoid negative interactions with dietary phytates and phenols, speed the passage of the metal through the mucus layer, and avoid competition with unprotected metal ions for binding sites on mucins (Power, 2006).

Bioavailability

Bioavailability, defined differently by many, can be described as the involvement of both the absorption and ultimate metabolic utilization of nutrients within the cell (Power and Horgan, 2000). The bioavailability of various inorganic trace mineral sources has been reviewed elsewhere (Ammerman et al., 1995; McDowell, 2003) and will not be described here. The alternate uptake mechanisms as well as protective mechanisms exhibited by organic trace minerals may result in increased absorption, ultimately enhancing their bioavailability. By increasing the availability of the trace mineral, it may be possible to elicit different and often optimal physiologic responses in the animal, which may subsequently redefine what constitutes normal performance. Evidence for greater bioavailability of organic trace minerals has been

sought from a variety of different measures, which will be discussed in relevant sections throughout this chapter.

Trace Mineral Supply to the Nursing Foal

Placental Transfer of Trace Minerals

The equine placenta is characterized as having an epitheliochorial design. The epitheliochorial design is such that the uterine epithelium, connective tissue and blood-vessel endothelium separate the maternal blood from the placental absorbing surface, similar to the ruminant placenta. This is unlike the hemochorial placenta of most higher primates, in which there is direct contact between the chorionic villi and a circulating pool of maternal blood. As a result, the equine fetus is completely dependent on the dam for its supply of nutrients, including trace minerals. The trace minerals copper, manganese, and zinc have all been proposed as limiting trace elements required by the fetus for normal growth (Abdelrahaman and Kincaid, 1993). Little research exists describing the placental transfer of trace minerals in the horse. In one of the few studies completed, Meyer and Ahlswede (1978) reported that almost half of the fetal copper, manganese, and zinc deposited in the developing equine fetus occurs during the last 2 months of gestation. In contrast, Abdelrahman and Kincaid (1993) evaluated copper concentrations in fetal tissue at different stages of pregnancy in cattle, and found that stage of gestation did not affect copper concentrations in the fetal liver or kidney. However, others have noted an accumulation of copper in the ovine fetal liver with the progression of gestation (Hostetler et al., 2003).

Trace Mineral Content of Mare Colostrum and Milk

In addition to placental transfer and subsequent storage of trace minerals occurring *in utero*, mare colostrum and milk serve as sources of trace minerals available to the nursing foal. Colostrum is considered the mare's first milk, and is more viscous than milk. During the last

trimester of gestation, the mare produces colostrum and it is secreted for a very short duration after parturition (Lavoie et al., 1989). Within 24 h after foaling, mammary secretions have transitioned from viscous colostrum to milk (Ullrey et al., 1966). Trace mineral concentrations are generally highest in colostrum and decrease as colostrum transitions to milk. Schryver et al. (1986) examined the trace mineral composition of mare milk from 1 to 17 wk postpartum. They found that the concentrations of total solids, ash and minerals were highest during the first week of lactation. Zinc concentration progressively declined by 41% from wk 1 to wk 5, and remained relatively unchanged through wk 17 of lactation. Copper displayed the greatest decrease in concentration, declining 35% from wk 1 to 4, further declining by 47% from wk 5 to 8, and remaining relatively constant from wk 9 to 17. Ullrey et al. (1974) reported similar declines in the trace mineral concentrations of mare milk with the progression of lactation. The authors obtained frequent samples from mares beginning at parturition and continuing through 16 wk of lactation. Zinc concentration was highest in colostrum obtained at parturition, but declined 44-56% between 12 and 24 h postpartum. Thereafter, zinc concentration progressively declined an additional 39% by wk 5 and remained relatively unchanged through 16 wk of lactation. Similar to zinc, copper concentration was highest in colostrum and declined 16-26% between 12 and 24 h postpartum. Copper concentration declined a further 60% in the first week of lactation and an additional 48% between wk 1 and 5 of lactation. Based on comparisons between zinc and copper concentrations in milk and estimates of foal requirements, the authors concluded that milk copper concentrations were low, but zinc from milk may be sufficient to meet the needs of the nursing foal. A study by Grace et al. (1999) in New Zealand confirmed that mare milk is low in trace minerals relative to that required by the foal. Milk was sampled from pasture-fed Thoroughbred mares at various times during early, mid, and late-lactation. Results showed that

milk provided adequate amounts of magnesium, sodium, potassium, sulfur, and zinc to the foal, but appeared to be inadequate in meeting the foal's daily calcium, phosphorus, iron, and copper requirements. Other than a study demonstrating manganese concentration in mare milk to be similar to that of cow milk (Anderson, 1992), little information exists regarding the manganese concentration in mare milk. It is unknown whether the stage of lactation affects the manganese concentration of mare milk, or whether the manganese supplied in mare milk is capable of meeting the requirements of the nursing foal.

Increasing the zinc and copper concentration of the mare's diet above NRC requirements has been reported to have little effect on the content of the mare's milk (Baucus et al., 1987; Breedveld et al., 1988; Kavazis et al., 2002). In contrast, there is some evidence that supplementation of the pregnant mare in late gestation may effectively increase fetal trace mineral stores, which may be of use after birth (Pearce et al., 1998). Therefore, natal reserves resulting from placental transfer of trace minerals *in utero* and consumption of solid feeds likely contribute significantly towards meeting the trace mineral requirements of the foal in addition to milk.

Overview of the Immune System

Innate Immunity

The innate immune system, unlike that of adaptive and acquired immunity, consists of all the immune defenses that lack immunologic memory. Thus, innate immunity is characterized by responses that remain unchanged however often the antigen is encountered (Mackay and Rosen, 2000). The nonspecific nature of the innate immune system provides a defense mechanism for antigens, as described by Goldsby et al. (2003), in anatomical, physiologic, phagocytic, and inflammatory ways. The skin and the surface of mucous membranes serve as anatomic defense mechanisms, preventing entry of most microorganisms. The physiologic barriers contributing to

innate immune function are, among others, temperature, pH, and various cell associated molecules that act as chemical mediators of innate immunity. Once pathogens gain access to the body, many are recognized, ingested, and killed by phagocytes. Macrophages are the first cells to encounter pathogens, but are quickly reinforced as neutrophils are recruited to the site of infection (Janeway et al., 2005). Functioning as phagocytic cells, macrophages and neutrophils act by phagocytosis, in which the bound pathogen is surrounded by the phagocyte membrane and then internalized. Upon phagocytosis, the neutrophils and macrophages of the innate immune system also produce toxic products like nitric oxide, superoxide anion, and hydrogen peroxide to aid in killing bacteria. Superoxide is generated by a multicomponent, membrane-associated NADPH oxidase process termed respiratory burst (Janeway et al., 2005). Unlike macrophages, neutrophils are short-lived cells, dying after the completion of oxidative burst, and comprise the majority of pus that forms with an infection. Lastly, inflammation represents a complex sequence of events that stimulates immune responses, known as the inflammatory response. The end result of inflammation is often the marshalling of specific immune responses to the invasion or clearance of the invader by components of the innate immune system (Goldsby et al., 2003).

Acquired Immunity

The acquired immune system involves a number of different cells and proteins that respond to a specific antigen. Acquired immunity, unlike innate immunity, is highly specific. The term acquired is used because the immune cells involved must be exposed to an antigen to develop memory of that antigen, known as a primary response. In a subsequent exposure to the antigen, immune cells will react with greater competence because specificity for the antigen has already been established (Goldsby et al., 2003). The responses elicited by the acquired immune system involve the proliferation of antigen-specific B and T lymphocytes (also referred to as B and T cells). For proliferation to occur, surface receptors of the B and T cells must bind to the

antigen (Mackay and Rosen, 2000). Specialized cells termed antigen-presenting cells display the antigen to lymphocytes, and work in concert with them during response to the antigen. Developing from pluripotent stem cells in the fetal liver and bone marrow, B and T cells will mature and eventually circulate throughout the extracellular fluid (Mackay and Rosen, 2000). B cells mature within bone marrow, while T cells migrate to the thymus where their development is completed. The B cells within the immune system function to secrete immunoglobulins, the antigen-specific antibodies responsible for eliminating extracellular microorganisms. T cells aid in the antibody production by B cells, and also function to eliminate intracellular pathogens by activating macrophages and by killing virally infected cells (Mackay and Rosen, 2000).

The acquired immune system is comprised of, among others, the humoral immune system. The humoral immune system is mediated by B cells, and describes the type of immunity associated with extracellular fluids, including plasma and lymph (Goldsby et al., 2003). The antigen-recognition molecules of B cells are the immunoglobulins, produced in a vast range of antigen specificities (Janeway et al., 2005). Antigen-specific immunoglobulins are secreted as antibodies by the differentiated B cells known as plasma cells. Antibodies are primarily found in blood but are also located in mucosal secretions and in fluids such as milk.

The immunoglobulin repertoire of the horse consists of IgG, IgM, IgA and IgE (Nezlin, 1998). Immunoglobulin G is the smallest of the immunoglobulin isotypes (Nezlin, 1998), moving easily from the blood to other tissues. Therefore, IgG is the major immunoglobulin constituent present in blood and milk (Tizard, 1996). In the horse, four IgG subclasses have been described, consisting of IgGa, IgGb, IgGc, an IgG[T] (Sheoran et al., 2000). The subclasses are differentiated by their γ-chain sequence (amino acid), with each IgG subclass having different antigenic properties (Goldsby et al., 2003). More recently, the nomenclature of equine

immunoglobulin subclasses has been changed to reflect their corresponding immunoglobulin heavy chain constant genes. Seven genes have been identified for encoding gamma heavy chains; thus, more IgG isotypes occur than the four described above (Wagner, 2006). The newer nomenclature designates the IgG isotypes as IgG1 through IgG7 (Wagner, 2006). Regardless of the subclass, IgG functions predominantly by binding to specific antigens like those found on the surfaces of bacteria (Tizard, 1996). While mainly present in the blood, IgG may also provide mucosal protection by paracellular passive transfer (Snoeck et al., 2006).

Immunoglobulin M is second to IgG in its concentration in the blood, and is the largest of the immunoglobulin isotypes (Nezlin, 1998). Immunoglobulin M is secreted by plasma cells as a pentamer in which five monomer units are held together by disulfide bonds. Thus, the valency of IgM is greater than any other immunoglobulin, which is capable of binding up to five molecules of large antigens simultaneously (Goldsby et al., 2003). During the primary immune response to antigen, IgM is the first immunoglobulin class produced, and is also the first immunoglobulin synthesized by the neonate. IgM also plays an important role in the first line of defense, having an accessory role to IgA as a secretory immunoglobulin (Goldsby et al., 2003). Unlike the mode of transport utilized by IgG, IgM and IgA are bound to polymeric-immunoglobulin receptor (pIgR), enabling the transport of the IgM and IgA to the mucosa (Snoeck et al., 2006).

Immunoglobulin A is chiefly known for its role in mucosal immunity. The quantity of IgA produced exceeds that of all other immunoglobulin isotypes combined (Nezlin, 1998). Although present in serum, IgA is the predominant antibody present in mucosal secretions, as well as in saliva, milk, and tears. The mucosal predominance of the antibody depends on cooperation between local plasma cells that produce IgA and mucosal epithelial cells that

express the pIgR receptor (Snoeck et al., 2006). Once released from the plasma cell, the IgA molecule diffuses through the stroma, and becomes bound by the pIgR receptor. This facilitates the transport of IgA across mucosal epithelial cells for extrusion into external secretions (Snoeck et al., 2006). Secretory IgA secreted on epithelial surfaces throughout the body serves in the first line of defense against pathogens, in large part by preventing adherence of bacteria and viruses to epithelial surfaces (Widmann and Itatani, 1998)

Immunoglobulin E is present in the blood in very minute concentrations, and is the immunoglobulin most associated with allergic reactions. Multivalent antigens bound by IgE antibodies triggers the release of histamine, leukotrienes, prostaglandins, and chemotatic factors from cells (Nezlin, 1998). Therefore, allergic and inflammatory reactions are initiated, such that serum IgE levels become elevated. This is exemplified when conditions like hay fever and chronic parasitic infections are present, which result in increased serum IgE concentrations (Nezlin, 1998).

Passive Immunity in the Foal

Importance of Mare Colostrum

The epitheliochorial placenta of the mare restricts transplacental passage of large molecules, including the large glycoproteins that are immunoglobulins (Jeffcott, 1974). Due to the absence of transfer of maternal antibodies *in utero*, the maternal supply of passive immunity occurs postnatally from immunoglobulins in colostrum and milk of the mare. Therefore, newborn foals, while immunocompetent, are immunologically naïve due to the lack of placental transfer of immunoglobulins.

For successful passive transfer of immunity, lactational secretions must contain adequate quantities of immunoglobulins, and the immunoglobulins must be delivered intact to the site of absorption and subsequently delivered to circulation (Rooke and Bland, 2002). Mares begin

synthesizing colostrum during the last trimester of gestation, and it is secreted for a very short duration after parturition (Lavoie et al., 1989).

Average immunoglobulin concentrations found in mare colostrum, milk, and serum are presented in Table 2-2. The immunoglobulin content of mare colostrum is characterized by high concentrations of IgG, and relatively lower concentrations of IgA and IgM (Lavoie et al., 1989). Sheoran et al. (2000) reported that IgGb was the dominant isotype in pre-suckle colostrum of mares, followed by IgGa, IgG(T), IgA, and IgGc in order of descending concentration; colostral IgM was not determined in their study. There is a rapid decline in all colostral immunoglobulins during the first 24 h after parturition (Pearson et al., 1984). Sheoran et al. (2000) observed a more than 40-fold decline in colostrum immunoglobulin concentration from birth to 1 d postpartum. Rouse and Ingram (1970) reported 20-fold decreases in IgG concentrations in mammary secretions of ponies between 0 to 3 hr and 9 to 24 hr after parturition. Such a rapid decline differs from other livestock, and suggests that transfer of immunoglobulins from serum to mammary secretions ceases at or possibly before parturition in the mare. Although IgG concentration continues to decline throughout lactation, IgA concentration remains relatively constant after about d 7 postpartum (Sheoran et al. 2000). As a result, IgA becomes the predominant immunoglobulin in mare milk (Norcross, 1982; Sheoran et al., 2000).

Table 2-2. Immunoglobulin concentrations in colostrum, milk, and serum of adult horses[1]

	Immunoglobulin, mg/dL		
Sample	IgG	IgM	IgA
Serum	1000-1500	100-200	60-350
Colostrum	1500-5000	100-350	500-1500
Milk	20-50	5-10	50-100

[1]Adapted from Tizard, 1996.

As described above, foals are born essentially agammaglobulinemic and, thus, depend on passive transfer of maternal immunoglobulins from colostrum. Foals can, however, synthesize their own immunoglobulins, although *de novo* immunoglobulin production fails to yield serum concentrations similar to those of adult horses until 3 to 6 months of age (Jeffcott, 1974, 1975). The onset of endogenous immunoglobulin production by the foal has been shown to vary by isotype. Foals were observed to produce mucosal IgA between 4 and 8 wk of age (Sheoran et al. 2000). Production of IgGa and IgG(T) did not begin to occur until 4 to 6 wk of age (Sheoran et al., 2000; Holznagel et al., 2003), whereas production of IgGb was delayed beyond 16 wk of age (Holznagel et al. 2003). Endogenous synthesis of immunoglobulins by the foal may be inhibited by the presence of maternal antibodies, which, prevent the foal from producing antigen-specific antibodies that maternal antibodies are already specific for (Wilson et al., 2001). Therefore, active immunization may be hindered in the presence of maternal antibodies. A series of studies have demonstrated that IgGb is a critically important immunoglobulin for resistance to viral and bacterial pathogens (Sheoran et al., 1997; Nelson et al., 1998). Therefore, although the uptake of all immunoglobulins in colostrum is crucial for successful passive transfer in the foal, the supply and uptake of large amounts of IgGb in colostrum are particularly critical, due to the late onset of endogenous IgGb production in the foal.

Because colostrum secretion is short lived and immunoglobulin concentrations decline rapidly, there is a narrow window of opportunity to ensure passive transfer of immunity to the foal. Immunoglobulins ingested shortly after birth are absorbed by the epithelial cells of the small intestine (Jeffcott, 1975). The epithelial cells of the newborn intestine are highly proliferating, but are replaced by mature cells within 38 h of life (Jeffcott, 1975). Thus, the

permeability of the neonatal foal's intestinal wall to immunoglobulins is a time-sensitive process. Known as gut closure, permeability of colostral immunoglobulins is terminated by approximately 24 h of age in the foal (Jeffcott, 1971).

Failure of Passive Immunity in the Foal

Under normal circumstances, colostrum-derived immunoglobulins provide protection for the foal until that time when the foal is capable of synthesizing its own. However, ingestion of insufficient amounts of immunoglobulins or a delayed ingestion may result in the failure of passive transfer of immunity in the foal. Defined by Kohn et al. (1989) as the failure of absorption of maternal immunoglobulins, failure of passive transfer of immunity often predisposes the foal to infection and death (Pearson et al., 1984). The causative factors of failure of passive transfer in foals are described as: 1) failure by the foal to ingest a sufficient volume of colostrum, 2) delayed access to colostrum resulting in an inability of the intestinal epithelial cells to absorb immunoglobulins, and 3) low colostral immunoglobulin concentrations (Pearson et al., 1984).

The concentrations of immunoglobulins that confer failure of passive transfer have been equivocal. Pearson et al. (1984) suggested that foals over 24 h of age having serum IgG concentrations of less than 200 mg/dL were classified as having failure of passive transfer, while others have suggested failure of passive transfer when serum IgG is less than 400 mg/dL (LeBlanc et al., 1986). Partial failure of passive transfer has been defined when foal serum IgG is between 200 and 400 mg/dL (McGuire et al., 1977). An increased risk of bacterial infection indicative of failure of passive transfer was associated with foals having serum IgG levels of less than 400 mg/dL, while 800 mg/dL was considered satisfactory (Tyler-McGowan et al., 1997). Regardless of values indicative of failure of passive transfer, the value of ensuring passive

transfer from mare to foal is realized as it is the most commonly recognized immune deficiency in neonatal foals, often resulting in septicemia and death (Raidal et al., 2005).

Effect of Trace Mineral Supplementation on Immune Function

The role of trace minerals in immune function has been well documented. Zinc affects the immune response by mediating T and B cell responses to antigens, and is also known to influence neutrophil and natural killer cell function (Calder et al., 2002; McDowell, 2003). In zinc deficiency, the aforementioned immune functions have all been suppressed (Calder et al., 2002). In addition, the immune system as a highly proliferating system depends on the availability of zinc, in which reduced circulating lymphocyte counts have been observed in zinc deficient livestock (McDowell, 2003). Furthermore, elevated zinc intake has been shown to potentiate immune function above basal levels. Salvin et al. (1987) found that mice fed a high zinc diet had increased numbers of splenic plaque forming cells in response to T lymphocyte dependent antigens. In a study by Singh et al. (1992), mice that received supplemental doses of zinc showed increased T lymphocyte and macrophage function. High zinc diets have also been shown to decrease the amount of lipid peroxidation in the livers of mice infected with *Plasmodium berghei* (Arif et al., 1976). During the acute phase in infection, decreasing plasma zinc levels are often observed in humans (Ibs and Rink, 2003). Although zinc is required by human beings for the proliferation of immune cells, the same is true for the necessity of zinc for the proliferation of pathogens. Thus, decreasing plasma zinc levels may serve as a defense mechanism to inhibit the proliferation of pathogens (Ibs and Rink, 2003). Even so, zinc supplementation has resulted in a reduced duration and severity of cold symptoms (Prasad et al., 2000), while other studies have shown that zinc has limited effectiveness for treatment of the common cold (Turner and Cetnarowski, 2000).

Similar to zinc, copper metabolism affects T and B cells, neutrophils, and macrophages, and in a copper deficient state, have been shown to be depressed (McDowell, 2003). In humans, copper deficiency results in neutropenia (Percival, 1995) and in animals has been shown to result in decreased phagocytic and killing function of neutrophils (Boyne and Arthur, 1981). However, Arthington et al. (1995) found no depression of neutrophil bactericidal function during copper depletion or repletion of heifers. In addition to copper, a deficiency in zinc has been shown to impair chemotaxis, phagocytosis, and generation of the oxidative burst by neutrophils in humans and primates (Allen et al., 1983; Keen and Gershwin, 1990). Although less is known about manganese and its role in immune function, the interaction of manganese with cells such as neutrophils and macrophages has been demonstrated (McDowell, 2003). In addition, abnormalities of cell function and ultrastructure occur in manganese deficiency (McDowell, 2003), having the potential to compromise cellular responses and activity in the immune system.

Although most of the known impact of trace minerals occurs when animals are deficient, organic trace minerals may perform better at meeting the animals' requirements. By avoiding the antagonistic relationships associated with inorganic trace mineral supplementation, organic trace minerals may meet animal requirements more efficiently, enabling further support of the immune function and reproductive performance of animals in high production and high stress situations. However, evidence for the benefits of organic trace minerals over inorganic sources for their effects on immune function has been somewhat equivocal. Dorton et al. (2003) observed an increased antibody response to vaccination with ovalbumin in feedlot steers supplemented with copper lysine compared to copper sulfate. Ferket and Qureshi (1992) reported greater antibody titers in young turkeys supplemented with zinc and manganese amino acid complexes compared with inorganic trace minerals. In recently transferred feedlot cattle, supplementation

with zinc methionine tended to result in a greater primary humoral response to bovine herpesvirus-1 vaccination compared to cattle fed zinc oxide or no zinc supplementation (Spears et al., 1991). Weanling horses supplemented with zinc, manganese, and copper amino acid complexes exhibited greater serum IgM concentrations in response to a single injection of pig red blood cells compared to weanlings fed isoelemental amounts of inorganic trace minerals (Siciliano et al., 2003). In contrast to the positive responses described above, an equal number of studies have found either no benefit to organic trace minerals over inorganic sources or greater responses to inorganic trace mineral supplementation. Dorton et al. (2003) reported that feedlot steers supplemented with copper sulfate had greater total IgM and IgG concentrations after vaccination with pig red blood cells compared to steers supplemented with copper lysine. Ward et al. (1993) observed no difference in humoral immune response between steers supplemented with copper sulfate or copper lysine in response to an injection of ovalbumin. Spears and Kegley (2002) vaccinated cattle with infectious bovine rhinotracheitis and found no difference in serum antibody titers between steers supplemented with zinc oxide or zinc proteinate. The lack of consistency between studies likely results from several factors, including the specific antigen used to elicit the humoral response, the animal's previous exposure to the antigen, the rate and duration of trace mineral supplementation, how closely the diet met animal requirements, and the different organic and inorganic trace mineral sources fed.

Several studies have also reported on other aspects of immune function. Working with calves, Saker et al. (1994a) observed an increased expression of histocompatability complex II for copper lysine fed cattle compared to those supplemented with copper sulfate. Copper lysine has also been found to increase monocyte phagocytic activity in calves compared to those supplemented with copper sulfate, although copper sulfate was supplemented at one half the rate

of copper lysine (Saker et al., 1994b). George et al. (1997) noted an increase in both cell-mediated and humoral immune response when heifers were supplemented with trace mineral amino acid chelates compared to heifers that underwent isoelemental supplementation with the same inorganic trace minerals. In converse, Stanton et al. (2000) found no difference in cell mediated immune response as measured by injection with phytohaemaglutinin (PHA) between calves fed trace minerals from organic or inorganic sources. In a study by Heugten et al. (2003), weanling pigs supplemented with zinc lysine had greater lymphocyte proliferation in response to PHA and pokeweed mitogen compared to pigs supplemented with zinc sulfate. In lactating dairy cattle, decreased somatic cell counts in milk have been observed when supplemented with zinc methionine. A pooled statistical analysis of eight studies using each experiment as a replication indicated a significant improvement in somatic cell count as well as milk production in dairy cattle supplemented with zinc methionine (Spears, 1996).

Foal-Heat Ovulation

The first postpartum estrous in mares is commonly referred to as foal heat. The majority of mares show signs of estrus within 5 to 12 d postpartum (Ginther, 1992). The reproductive state of the prepartum mare is governed by progesterone; however, once parturition has occurred, progesterone is undetectable in the early postpartum mare (Hillman and Loy, 1969). Concentrations of luteinizing hormone (LH) are low immediately after foaling due to the suppressive effects of fetoplacental-derived progestins (Ginther, 1979). A surge of follicle stimulating hormone (FSH) begins a few days before parturition and peaks just on or shortly after the day of parturition, accounting for the development of follicles during the first postpartum estrous cycle (Ginther, 1992). Serum LH concentrations increase during the periparturient period, usually occurring 72 h after foaling. Serum LH generally peaks near the day of ovulation; if LH does not peak on the day of ovulation, it will peak the day after.

Ovulation during foal heat estrous generally occurs between 9 and 12 d postpartum. Due to the mare's lengthy 11-mo gestation, conception would need to occur within 1 mo after parturition in order for the mare to foal on a yearly basis. Thus, the long gestation of the mare necessitates breeding during foal heat, particularly for mares foaling late in the season, in order to achieve maximum economic returns. However, pregnancy rates achieved during this time are reported to be 10 to 20% lower than rates achieved during subsequent estrous periods (Ginther, 1992). The decreased conception rates associated with breeding on foal heat are thought to be due to incomplete involution of the uterus (Gyax et al., 1979; Griffin and Ginther, 1991).

Uterine Involution

Uterine involution is the restoration of the endometrium to a condition where conception can take place again, ensuring an optimal climate for embryonic development. During involution, degenerating or detached epithelial cells are replaced and the uterine epithelium redifferentiates into ciliated and secretory cell types (Steven et al., 1979) as the endometrium returns to prepartum histological appearance by 14 d postpartum. Accompanying these morphological changes is a change in uterine size, in which the uterus returns to its pre-gravid size by 21 d postpartum (McKinnon et al., 1988). The return of the endometrium to its pre-gravid morphological state is critical for the support of the next pregnancy. This can be illustrated by the observation that pregnancy rates when bred at foal-heat are higher in mares that ovulate 10 or more days after foaling than in mares that ovulate on or before 10 d after foaling (Loy, 1980). On average, the equine embryo enters the uterus 5 d after ovulation. Therefore, ovulation 10 or more days after foaling ensures that the endometrium has returned to normal prior to arrival of the embryo (Arrott et al., 1994).

Uterine Fluid

The process of uterine involution is crucial for the expulsion of uterine fluids associated with foaling. Postpartum luminal fluids include a mucus-like material present 3 d after foaling, which is reported to be gone by 6 d postpartum if uterine involution progresses normally (Ginther, 1992). It has been suggested that some of the fluid represents an influx of fluid from the uterine wall into the lumen (Ginther, 1992). Ultrasonic monitoring of mares during the first postpartum ovulatory period has indicated that mares with detectable uterine fluid accumulations was associated with decreased pregnancy rates (McKinnon et al., 1988). Mares often exhibit a profound increase in the amount of uterine fluid immediately after foaling, representing both the fluid associated with foaling, as well as that resulting from the process of uterine involution. Intrauterine fluids decrease in temporal association with decreased uterine tone and diameter (i.e., uterine involution). Without successful uterine involution and subsequent elimination of the luminal fluid, the ability of the mare to achieve and sustain pregnancy is compromised.

Uterine Edema

The appearance and disappearance of endometrial edema is related to the onset of estrus and ovulation in mares. During estrous, the mare is under the influence of estrogen, and will have an increase of edema in the reproductive tract associated with the development and progression of follicles. Substantial edema occurs during estrus, and is identified by trans-rectal ultrasonography as dark (non-echogenic) centrifugal rays between the lumen and peritoneal surface. When ovulation is imminent, a sudden decline of uterine edema occurs with the already declining estrogen, decreasing sodium retention in the lamina propria. The presence of uterine edema has been shown to be a reliable indicator of the estrogenic competence of the dominant follicle (Samper, 1997). Thus, the rise and subsequent decline in uterine edema is often used as a tool to aid in timing of breeding. Once the mare has ovulated the reproductive tract is under the

influence of progesterone and uterine edema is no longer present under normal conditions (Samper, 1997; Watson et al., 2003).

Effect of Dietary Trace Mineral Supplementation on Reproductive Performance

The exact biological roles of trace minerals in reproduction are largely unknown. However, reports of compromised reproduction during dietary trace mineral deficiencies suggest their necessity for optimal reproductive performance. In copper deficient animals, estrous cycles and conception rates appear to remain unaffected, however, reproductive failure due to fetal death and resorption has been known to occur (Underwood, 1977). Copper is also necessary for the formation of connective tissue (McDowell, 2003), and as such would be necessary for proper fetal development. Zinc is known to be centrally involved in cell division, suggesting its importance during fetal growth and during the physiologic events such as uterine involution that occur in the postpartum female. In addition, zinc is intimately associated with several hormones; for example, the steroid hormones androgen and estrogen function by binding to transcription factors that contain zinc fingers (McDowell, 2003). Zinc deficiency in the female has been shown to impair the synthesis and or secretion of FSH and LH, as well as cause abnormal ovarian development and disruption of the estrous cycle (Bedwal and Bahuguna, 1994). Doisey (1974) proposed that insufficient manganese interrupts the synthesis of cholesterol and its precursors, which would ultimately inhibit the synthesis of sex hormones, adversely affecting reproduction. Hostetler et al. (2003) hypothesized that manganese may play a role in the secretion of progesterone based on the findings by Hidirolgou and Shearer (1976) that the manganese concentration in the corpus luteum of ewes increased during early pregnancy, and that inadequate progesterone concentrations are known to cause early embryonic loss.

While much is known regarding the effects of trace mineral deficiency on reproductive performance, little is known about what optimal levels of trace mineral supplementation achieve

optimum reproductive performance. Because organic trace minerals are generally considered to be more bioavailable to the animal, their supplementation may redefine what optimal reproductive performance is as it relates to optimal supplementation.

The majority of studies investigating the effects of organic trace mineral supplementation on reproductive performance have been conducted in species other than the horse. Boland et al. (1996) reported that dairy cattle receiving proteinated trace minerals had a non-significant reduction in days to follicle deviation, and 5 fewer days to first ovulation. Manspeaker et al. (1987) found that dairy cattle supplemented with chelated trace minerals had increased ovarian activity, greater postpartum uterine involution of the pregnant horn, and less embryonic mortality, although results were not significantly different from cattle receiving no trace mineral supplementation. Toni et al. (2007) replaced inorganic minerals with amino acid complexed trace minerals in dairy cows 60 d prior to calving and through 200 d of lactation, and observed that cows receiving the amino acid complexed trace minerals tended to have an increase in first service to conception compared to those cows receiving inorganic trace minerals. Campbell et al. (1999) found that dairy cattle supplemented with complexed trace minerals from parturition to 154 days in milk showed fewer days to first estrus than cattle receiving a control diet consisting of no organic trace minerals during the same time, although days to first service, days open, days from first service to conception, and services per conception were similar between control and supplemented cows. Siciliano-Jones et al. (2008) supplemented dairy cattle 3 wk prepartum through 35 wk postpartum with isoelemental amounts of trace minerals as sulfates or amino acid complexes and found no differences in days to first service, services per conception, or number of days open.

Reports addressing the effects of organic trace mineral supplementation on reproductive performance of mares are limited. Ott and Asquith (1994) observed a reduction in the number of cycles bred and services per mare when mares were provided proteinated vs. inorganic trace minerals. Similarly, Ley et al.(1990) found that barren mares supplemented with inorganic trace minerals experienced no first cycle pregnancies, two early embryonic losses, and higher number of services per 17-d conception compared to mares receiving chelated trace mineral supplementation. Neither the observations of Ott and Asquith (1994) nor Ley et al. (1990) were found to be significant after statistical analyses. Nonetheless, these studies, along with the positive responses seen in other species, provide impetus for further investigation of the effects of organic trace mineral supplementation on reproductive performance in the mare.

Conclusions

Evidence for improved bioavailability of organic trace minerals exists for many animal species (Spears, 1996; Acda and Chae, 2002; Hostetler et al., 2003). Although responses have varied, animals supplemented with organic trace minerals have often shown enhanced immune function when compared to animals supplemented with inorganic trace minerals. This improved response is particularly evident in animals stressed by transport or a high level of production, and in those placed in an environment with a high level of pathogen exposure. Similarly, reports on the effect of trace mineral source on reproductive performance have been conflicting, but several studies have described improvements in ovulation and conception when animals are supplemented with organic trace minerals. Most of these studies have considered conception rates or pregnancy rates as the ultimate end-points, but have not attempted to identify the factors that work to enhance conception rates in animals fed organic trace minerals.

Few studies have investigated the use of organic trace minerals in equine diets. In particular, it is unknown whether supplementation of the pregnant or lactating mare with organic

trace minerals will also benefit the health of her foal. Based on studies in other species, supplementing the mare with organic trace minerals may have potential to affect foal immunity. Milk is generally a poor source of trace minerals for the foal and the mare's diet has little effect on milk mineral composition; therefore, supplementing the pregnant mare with a more bioavailable form of trace mineral may promote greater placental transfer of trace minerals *in utero* and greater natal reserves. Because of the role of trace minerals in immune function, these reserves may act to enhance innate and humoral immunity in the foal. In addition, supplementing the pregnant mare with organic trace minerals may improve colostrum immunoglobulin composition, thereby augmenting passive transfer of immunity and disease resistance in the foal. Finally, organic trace mineral supplementation could improve early postpartum reproductive performance, permitting the mare to be rebred earlier to align with industry standards.

The study presented in this thesis was conducted to better elucidate the potential benefits of substituting inorganic zinc, manganese, copper and cobalt with amino acid complexes of these trace minerals in the diets of broodmares. The objectives of this research were to determine the effects of dietary trace mineral source and duration of supplementation in the mare on: 1) passive transfer of immunity to the foal; 2) innate and humoral immunity in nursing foals; 3) circulating trace mineral concentrations in mares and foals; 4) vitamin B12 status of mares and foals; and 5) reproductive performance of the mare during the first postpartum estrous.

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CHAPTER 3
MATERIALS AND METHODS

Animals

Thirty-six pregnant Thoroughbred (n=20), Quarter Horse (n=14), and Standardbred (n=2) mares and their resulting foals were used to determine the effects of organic or inorganic trace mineral sources on reproductive performance at foal-heat and innate and acquired immunity in nursing foals. The age of mares ranged from 5 to 22 yr with mean ± SE of 12.0 ± 0.7 yr.

Mares and foals were housed at the Institute of Food and Agricultural Sciences, Horse Research Center located in Ocala, FL. Other than 1 to 2 wk surrounding parturition, mares were housed on pasture. Within 1 wk of expected foaling, each mare was moved to a small, dry-lot paddock. Milk testing for calcium concentration was performed during this time to better gauge time of foaling. As signs of impending parturition were evident, the mare was moved into a large 4.3 x 4.9 m foaling stall. After foaling, the mare and foal were kept in the stall for 24 h and then turned out into a small paddock for approximately 1 wk before returning to pasture. Throughout the experiment, horses received routine anthelmintic treatment and vaccinations according to the protocols established for pregnant and lactating mares at the Horse Research Center. In addition, all foals were evaluated at 12 h postpartum for failure of passive transfer by using a semi-quantitative enzyme immunoassay to detect immunoglobulin G in whole blood (SNAP® Foal IgG Test kit; IDEXX Laboratories, Inc., Westbrook, ME). The experiment was performed in accordance with the regulations and approval of the Institutional Animal Care and Use Committee at the University of Florida.

Diets and Treatments

Mares were blocked by age, breed, and expected date of foaling and randomly assigned to dietary treatments in a 2 x 2 factorial arrangement. Mares were supplemented with

isoelemental amounts of trace minerals in one of two forms: 1) inorganic zinc, manganese, copper and cobalt (SULF; n=18); or 2) amino acid complexes of zinc, manganese, and copper and cobalt glucoheptonate (4PLEX; n=18). Within each form of trace mineral supplementation, mares originated from one of two populations: 1) mares that had been maintained on a similar program of inorganic or amino acid complex trace mineral supplementation for 6 mo as part of a previous experiment (LONG; n=18); or 2) mares that had no prior exposure to amino acid complex trace minerals (SHORT; n=18). Collectively, this design resulted in the following four treatments: 1) SULF-LONG (n=9); 2) SULF-SHORT (n=9); 3) 4PLEX-LONG (n=9); and 4) 4PLEX-SHORT (n=9). Mares began receiving their respective treatments 84 d prior to estimated foaling date and continued through 112 d postpartum. Foaling season lasted from January through May 2007; thus, the experiment spanned the months of October 2006 to December 2007.

Supplemental trace mineral sources were incorporated into separate protein/vitamin/mineral supplement pellets (Table 3-1). Sources of trace minerals in SULF treatments were zinc sulfate, manganese sulfate, copper sulfate, and cobalt sulfate. Trace mineral sources in 4PLEX treatments included zinc methionine, manganese methionine, copper lysine, and cobalt glucoheptonate provided via 4Plex® (Zinpro Corporation, Eden Prairie, MN), along with additional manganese in the form of manganese sulfate to provide isoelemental amounts of manganese compared to SULF treatments. The nutrient composition of the pellets was similar; the only difference was the source of added zinc, manganese, copper and cobalt (Table 3-2). The amounts of sulfate and amino acid complex trace minerals added to each supplement pellet were formulated to provide 1X the daily zinc and manganese requirements, 1.5X the daily copper requirement, and 44X the daily cobalt requirement for mares in late gestation and lactation

(NRC, 2007). The estimated daily intake of trace minerals in mares in late gestation and lactation are presented in table 3-4.

Throughout the experiment, mares were housed in one of two 16.2 ha (40 acre) pastures of similar forage composition, with mares on all treatments equally distributed among pastures. The basal diet for all treatment groups consisted of whole oats, pasture and hay. From November to March (late gestation, early lactation), mares were offered Coastal bermudagrass hay *ad libitum.* From April to October (lactation), mares only had access to bahiagrass pasture. Twice daily at 0700 and 1500 h mares were individually fed whole oats and the protein/vitamin/mineral pellets containing either 4PLEX or SULF trace minerals. The amount of oats was adjusted to maintain body condition and ranged from 4.5 kg/d during late gestation and 8.5 kg/d during lactation (as-fed basis). The supplement pellets were fed at a rate of 0.24% BW/d during late gestation and 0.32% BW/d during lactation (as-fed basis). The diet was designed to meet or slightly exceed the nutrient requirements of mares in late gestation and lactation (NRC, 2007).

All feeds were periodically sampled for subsequent analysis of nutrient composition. Pasture samples were collected from areas on or near where there was evidence of grazing and analyzed at 1-mo intervals from April to October. Four cores from each round hay bale fed from November to March, as well as random samples of oats collected from each weekly delivery, were composited and each analyzed as a single sample. Random samples of the SULF and 4PLEX supplement pellets were collected from each of the four batch mixes and composited and analyzed by batch. Composite feed samples were dried (60°C for 48 h) and stored at -20°C until further analysis. Feed analysis consisted of DM at 100°C (AOAC 1990), crude protein by an automated nitrogen analyzer (Elementar vario Max; Elementar Americas, Inc., Mt. Laurel, NJ), and NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technologies, Fairfield, NY).

Copper, calcium, zinc, manganese, and cobalt in feeds were determined by atomic absorption spectrophotometry (AAnalyst800; Perkin-Elmer, Norwalk, CT). Phosphorus was determined by colorimetry (AOAC, 1990). The nutrient composition of the supplement pellets is presented in Table 3-2. The nutrient composition of the oats, hay, and pasture are presented in Table 3-3.

Blood Sample Collection and Handling

As summarized in Figure 3-1 and described in further detail in the sections below, blood samples were collected from mares and foals at specific intervals for the analysis of immunoglobulin, trace mineral, cobalamin, and hormone concentrations. In all cases, blood samples were collected by jugular venipuncture into evacuated blood collection tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ) containing either sodium heparin or potassium EDTA for the harvesting of whole blood or plasma, or no anticoagulant for harvesting of serum. With the exception of samples obtained from foals at 24 and 36 h after parturition, all blood samples were collected between 0700 and 0900 h. After collection, blood samples were immediately placed on ice and transported approximately 30 min to the Animal Nutrition Laboratory for further processing.

Blood samples containing no anticoagulant were allowed to clot a minimum of 1 h before centrifugation to facilitate separation of serum. Blood samples were centrifuged at 2058 x g for 15 min for separation of plasma or serum. Serum and plasma were harvested with plastic disposable pipettes and aliquoted into polypropylene cryogenic vials (3-4 vials, 1.0-2.0 mL each). Samples were frozen at -80˚C until further analysis, with the exception of whole blood used in the neutrophil function assay, which was analyzed on the day of collection.

Passive Transfer of Immunity

At 30 d prior to expected foaling, all mares received a booster vaccination containing tetanus, Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE), West Nile

Virus (WNV), influenza, Equine Herpes Virus-1 (EHV-1), and Equine Herpes Virus-4 (EHV-4) in order to facilitate maternal transfer of antibodies in colostrum. Passive transfer of immunity was assessed by measuring immunoglobulin (Ig) concentrations in postpartum mare colostrum and foal serum. Approximately 100 mL of pre-suckle colostrum was obtained by hand-milking shortly after foaling and stored at 4˚C until transfer to the Animal Nutrition Laboratory for further processing. Colostrum was processed by gentle mixing and straining through four layers of cheese cloth to remove any dirt and debris. Samples were remixed and subsamples of colostrum were aliquoted into polypropolene cryogenic vials and stored at -80˚C until analysis of Ig concentrations. Approximately 10 mL of blood was obtained from foals by jugular venipuncture at 24 and 36 h after parturition into evacuated tubes containing no anticoagulant. Blood samples were processed for serum and stored until analyses of Ig concentrations were performed, as described above. Concentrations of IgG, IgA, and IgM were determined in mare colostrum and foal serum by single radial immunodiffusion using commercially available kits (VMRD, Inc., Pullman, WA). Serum and colostrum samples were diluted to an appropriate level to ensure values were within measurable limits of the assay. The detection ranges were 200–1600, 31–250, and 25-200 mg/dL for IgG, IgA, and IgM, respectively. Four standards supplied by the manufacturer of known concentration for each Ig were used to create a standard curve, and unknown Ig concentrations in samples were determined using the Metra Fit computer program (Metra Biosystems, Inc., Mountain View, CA).

Tetanus Antibody Titers

Humoral immune function in foals was evaluated in response to tetanus vaccination. The same inactivated, multivalent vaccine containing tetanus, EEE, WEE, WNV, influenza A-2, EHV-1, and EHV-4 antigens (Fort Dodge Laboratories, Fort Dodge, IA) used for the mares was administered to foals. A primary vaccination was administered i.m. at 112 d of age, followed by

a second and third vaccination at 140 and 168 d of age, respectively. Approximately 10 mL of blood was obtained by jugular venipuncture prior to the administration of vaccinations at 112, 140 and 168 d of age and 4 wk following the vaccination at 196 d of age. Blood was collected into evacuated tubes containing no anticoagulant, processed to obtain serum, and stored until later analysis, as described above. Tetanus-specific IgG titers were determined in serum using an ELISA (Scintilla Development Co., Bath, PA). In addition to determining antibody titers in response to vaccination, maternal influence over tetanus-specific IgG was also evaluated in foal serum obtained 1-2 d postpartum and at 56 and 112 d of age.

Neutrophil Function

Polymorphonuclear (PMN) neutrophil function was assessed in mares and foals as a measure of innate immunity. Blood was collected by jugular venipuncture at 56 d prior to and 56 and 112 d after foaling in mares, and at 1-2, 56, and 112 d of age in foals. On each day of sampling, approximately 10 mL of blood were collected into evacuated tubes containing sodium heparin (for neutrophil function) and approximately 10 mL were collected into tubes containing potassium EDTA (for white blood cell differential analysis) as the anticoagulants. Neutrophil function in whole blood was assessed using a simultaneous phagocytosis and oxidative burst dual-color flow cytometric assay as described by Vineyard et al. (2007). Briefly, whole blood in 100 µL aliquots from each horse was loaded with 4 µM dihydrorhodamine (DHR) for 10 min at 37˚C with continuous mixing. Heat-killed St*aphylococcus aureus* bacteria labeled with propidium iodide (PI) were added to achieve a bacteria:PMN ratio of 30:1. White blood cell differential analysis was used to determine the PMN concentration in whole blood in order to achieve an accurate bacteria:PMN ratio. DHR-loaded whole blood without bacteria served as the negative control, while DHR-loadedwhole blood stimulated with phorbol miristate acetate (PMA) (5µg/mL) served as the positive control. After incubation at 37˚C for 30 min with

constant mixing, samples were placed on ice to immediately halt the phagocytosis and oxidative burst processes. Samples were prepared for flow cytometry utilizing a Q-prep automated lysing system (Coulter Corp., Miami, FL). For completion of hemolysis, 500 µL of de-ionized water was added, and 10 µL of 0.4% trypan blue added to quench extracellular fluorescence. Preparation of the blood for flow cytometry was completed within 4 h of collection. A FACSort flow cytometer (Becton Dickinson, San Jose, CA) was utilized to measure the fluorescent intensity in the prepared sample after processing was complete. Data were collected from 10,000 cells/sample and analyzed using CellQuest software (Becton Dickinson, San Jose, CA) to quantify the percentage of neutrophils that phagocytosed bacteria, as well as the percentage of neutrophils with a phagocytosis induced oxidative burst, as shown in Figure 3-2. Due to an apparent decrease in the fluorescence of the bacteria over time, no attempt was made to examine the effect of time on neutrophil function.

Vitamin B12 Status

Cobalamin concentrations were determined in mare and foal serum as a measure of the gastrointestinal microbial synthesis of vitamin B12 from supplemental cobalt. Approximately 10 mL of blood was obtained by jugular venipuncture from mares at 56 d prior to and at 56 and 112 d after foaling and from foals at 1-2, 56, and 112 d of age. Blood was collected into evacuated tubes containing no anticoagulant, processed to obtain serum, and stored until later analysis, using the procedures described above. Serum was analyzed for cobalamin using an IMMULITE 2000 solid phase, competitive chemiluminescent enzyme immunoassay involving an automated alkaline denaturation procedure (Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A & M University, College Station, TX).

Serum Trace Minerals

Maternal transfer of trace minerals to the foal *in utero* was estimated by analyzing the zinc, copper, and manganese concentrations of umbilical cord blood collected at foaling. In addition, serum trace mineral concentrations were determined in mares 56 d prior to and 56 d after foaling and in foals at 1-2 and 112 d of age. Umbilical cord blood was collected during parturition before the umbilical cord had separated from the foal using a sterile syringe and needle. Subsequent blood samples obtained from mares and foals were obtained by jugular venipuncture. Umbilical cord and venous blood were processed to obtain serum and stored until later analysis, as described above. The concentrations of Zn, Cu, and Mn were determined using atomic absorption spectrometry (AOAC 1990). To determine Zn and Cu concentrations, serum was combined with de-ionized water in a 1:1 ratio and read using a flame atomic absorption spectrometer (AAnalyst 800; PerkinElmer, Inc., Shelton, CT) at wavelengths of 213.9 nm (Zn) and 324.8 nm (Cu). To determine Mn concentration, serum was combined with diluent (8 mL Triton® X-100, 5 g of sodium EDTA per liter of de-ionized water) in a 1:1 ratio and analyzed using a graphite furnace atomic absorption spectrometer (AAnalyst 800 with THGA Graphite Furnace and AS-800 Autosampler; PerkinElmer, Inc., Shelton, CT) at a wavelength of 279.5 nm.

Reproductive Performance

To determine the effect of source and duration of trace mineral supplementation on reproductive performance in the first postpartum estrous cycle (commonly referred to as 'foal-heat'), mares were examined daily by the same investigator with trans-rectal ultrasonography beginning 1 d post-foaling and continuing through 1 d post-ovulation. Reproductive exams were discontinued on mares failing to ovulate within 21 d post-foaling in order to analyze the reproductive data more effectively.

The size and number of follicles developing in the first postpartum cohort were recorded daily without maintaining the identity of individual follicles, as described by (Ginther and Bergfelt, 1992). Follicle development data were used to determine the following: 1) total number of follicles that developed from both ovaries; 2) average size of the 6 largest follicles that developed (3 from each ovary); 3) size of the largest follicle that developed; 4) days to foal-heat ovulation; 5) occurrence of ovulation less than or equal to 10 d or greater than 10 d post-foaling; 6) days to follicle deviation, defined as the day in which the growth rate of the dominant follicle deviated significantly from the growth rate of the subordinate follicles (Ginther et al., 2002); 7) growth rate (mm/d) of the largest follicle that developed; and 8) average growth rate (mm/d) of the 6 largest follicles that developed (3 from each ovary).

Assessment of intrauterine luminal fluid was recorded daily using a 4 point scale, where 1 = no fluid and 4 = extensive fluid occupying the uterine lumen through the body and both horns. Similarly, intrauterine endometrial edema was evaluated daily using a 4 point scale, where 1 = homogenous appearance or no edema and 4 = extreme heterogeneous appearance or excess edema. These data were used to determine the daily change in intrauterine luminal fluid and endometrial edema from foaling to foal heat ovulation.

The diameters of the uterine body (midway between horn bifurcation and cervix) and the gravid and non-gravid uterine horns (at mid-point between uterine bifurcation and the ovarian tip of the horn) were recorded and used to determine the rate of postpartum uterine involution.

Daily blood samples (10 mL) were collected by jugular venipuncture from 1 d postpartum through 1 d post-ovulation for the determination of serum luteinizing hormone (LH) and follicle stimulating hormone (FSH). Hormone concentrations were measured using a radio

immuno assay (RIA) assay (Colorado State University Endocrine Laboratory, Fort Collins, CO). Data were used to determine peak hormone levels relative to day of ovulation.

Bodyweights

Bodyweights were obtained from mares 84 d prior to foaling, within 1-2 d of parturition, and at 28-d intervals through 140 d post-foaling. Bodyweights were obtained from foals within 1-2 d of birth and at 28-d intervals through 168 d of age. All bodyweights were obtained using a calibrated digital livestock scale with an accuracy of ± 0.5 kg.

Statistical Analyses

One foal (mare on 4PLEX-LONG) had a serum IgG level indicative of failure of passive transfer at 12 h of age (as determined by SNAP testing) and was given a plasma transfusion. The same foal was euthanized due to angular limb deformities at 7 wk of age. As a result, the only data included in the analysis from this foal were umbilical trace mineral concentration, serum cobalamin and trace mineral concentrations at birth, and neutrophil function at birth. Another foal (mare on SULF-LONG) was euthanized within 4 wk post-foaling due to severe angular limb deformities. Only serum Ig to assess passive transfer, umbilical trace mineral concentrations, serum cobalamin and trace mineral concentrations at birth, and neutrophil function at birth were included in the analysis for this foal. A third foal (mare on SULF-LONG) died due to complications from *Rhodococcus* pneumonia at approximately 4 wk of age. The data included in the analysis from this foal included serum Ig for assessment of passive transfer of immunity, serum cobalamin and trace mineral concentrations at birth, and neutrophil function at birth were included in the analysis for this foal. It is unlikely the angular limb deformities or pneumonia were resultant of dietary treatment. In all cases of foal loss, mares remained on treatment and all data collected from the mare remained in the analysis.

Three mares required a uterine lavage in the early postpartum period due to a retained placenta (mare on 4PLEX-SHORT), placentitis (mare on 4PLEX-LONG), or extreme intrauterine fluid (mare on SULF-SHORT). As a result, no reproductive data from these mares were included in the statistical analyses. Two mares receiving 4PLEX-LONG, two mares receiving SULF-SHORT, and one mare receiving SULF-LONG failed to ovulate within 21 d postpartum. With the exception of ovulation occurring ±10 d post-foaling, data from these mares was omitted from all other reproductive analyses. Three mares receiving 4PLEX-SHORT double-ovulated and were removed from day of deviation analysis. The gravid and non-gravid horn of two mares on SULF-SHORT and one mare on 4PLEX-SHORT could not be distinguished and thus, were not included in the analysis of these measures. Collectively, the number of mares included in the analysis of various reproductive performance measures was as follows: ovulation occurring ±10 d post-foaling (n=33; 4PLEX-LONG (n=9), 4PLEX-SHORT (n=6), SULF-LONG (n=9), and SULF-SHORT (n=9)); day of deviation (n=25; 4PLEX-LONG (n=6), 4PLEX-NEW (n=4), SULF-LONG (n=9), and SULF-SHORT (n=6)); all other reproductive measures (n=28; 4PLEX-LONG (n=6), 4PLEX-SHORT (n=7), SULF-LONG (n=9), and SULF-SHORT (n=6)).

Statistical analyses of colostrum and foal Ig concentrations, neutrophil phagocytosis and oxidative burst, trace mineral concentrations in umbilical cord serum, and early postpartum reproductive performance were performed using the mixed procedure of SAS (Version 9.1; SAS Institute, Inc., Cary, NC). The model for foal serum Ig concentrations included source, duration, time, and the source x duration, source x time, duration x time, and source x duration x time interactions, whereas the model for the other variables included source, duration, and the source x duration interaction. In addition, the freq procedure of SAS was used to analyze whether the

day of ovulation occurred less than or equal to 10 d or greater than 10 d post-foaling. Tetanus antibody titers, mare and foal serum trace mineral concentrations, and mare and foal serum cobalamin were analyzed using the mixed procedure of SAS (Version 9.1; SAS Institute, Cary, NC) with a covariance test suitable for repeated measures (Littell et al. 1998). The covariance structure was first-order autoregressive, with horse within treatment used as the subject effect. The model for each variable included source, duration, time, and the source x time, duration x time, source x duration, and source x duration x time interactions. All data are expressed as the treatment lsmeans ± SE. For all analyses, $P \leq 0.05$ was considered significant and $P \leq 0.10$ was discussed as a trend.

Table 3-1. The expected and actual concentrations of zinc, manganese, copper and cobalt in the SULF and 4PLEX supplement pellets

	Expected Analysis[1]				Actual Analysis[2]			
	SULF		4PLEX		SULF		4PLEX	
Nutrient	As-fed	DM	As-fed	DM	As-fed	DM	As-fed	DM
DM, %	89.9	100	90.5	100	89.7	100	90.5	100
Zn, ppm	325	361	325	359	449	501	466	515
Mn, ppm	325	361	325	359	422	470	472	521
Cu, ppm	117	130	117	129	133	149	139	153
Co, ppm	23	26	23	25	25	28	26	29

[1] The "Expected Analysis" does not include the Zn, Mn, Cu, and Co that naturally-occur in the feeds included within the supplement pellet. These endogenous levels were estimated at 44 ppm Zn, 38 ppm Mn, 27 ppm Cu, and 0.11 ppm Co (100% DM basis).

[2] Mean of four batches of 4PLEX and SULFATE pellets (see Table 2 for individual batch analyses).

Table 3-2. Nutrient composition of each of the four batch mixes of SULF and 4PLEX supplement pellets (100% DM basis)

	SULF[1]					4PLEX[2]				
Nutrient	1	2	3	4	Mean	1	2	3	4	Mean
Moisture, %	10.3	10.1	10.4	10.3	10.3	9.0	9.6	9.7	9.7	9.5
DE, Mcal/kg	3.8	3.8	3.8	3.7	3.8	3.5	3.5	3.5	3.5	3.5
Crude Protein, %	36.0	36.4	37.0	36.0	36.4	35.5	36.4	36.4	35.8	36.0
NDF, %	9.2	9.8	9.4	9.3	9.4	15.8	16.1	15.5	17.6	16.3
ADF, %	5.1	5.6	4.6	6.4	5.4	11.2	9.9	10.0	10.9	10.5
Ca, %	3.3	3.1	3.4	3.6	3.4	3.5	3.5	3.6	3.7	3.6
P, %	1.9	1.8	2.0	2.0	1.9	1.9	1.9	2.0	1.9	1.9
Zn, ppm	500	545	455	502	501	521	493	540	505	515
Mn, ppm	475	529	418	456	470	583	454	574	474	521
Cu, ppm	142	165	147	141	149	184	136	147	146	153
Co, ppm	30	29	27	27	28	28	31	27	30	29

[1]Represents Zn, Mn, Cu, and Co as sulfate forms.
[2]Represents amino acid complexes of Zn, Mn, Cu, and Co.

Table 3-3. Nutrient composition of whole oats, Coastal bermudagrass hay and bahiagrass pasture in the basal diet (100% DM basis)

			Pasture						
Nutrient	Oats	Hay	April	May	June	July	Aug	Sept	Mean
DE, Mcal/kg	3.3	2.0	2.6	2.4	2.6	2.4	2.0	2.1	2.4
Crude Protein, %	11.9	12.1	19.7	16.8	21.4	17.7	13.3	14.5	17.2
NDF, %	28.0	73.1	58.6	62.3	62.0	59.0	69.8	69.8	63.6
ADF, %	13.2	38.6	29.6	32.0	30.2	31.3	39.9	39.7	33.8
Ca, %	0.07	0.33	0.45	0.56	0.47	0.34	0.37	0.40	0.43
P, %	0.34	0.22	0.31	0.26	0.32	0.37	0.30	0.31	0.31
Zn, ppm	29	41	35	25	32	31	29	22	29
Mn, ppm	48	113	123	120	148	89	70	66	103
Cu, ppm	6	8	10	9	10	11	10	8	10
Co, ppm	0.19	0.46	0.49	0.33	0.26	0.29	0.26	0.29	0.32

Table 3-4. Mineral concentrations in the total diet based on actual intake of supplement pellets and oats and estimated intake of pasture and/or hay for mares in late gestation and lactation.

Nutrient[1]	Zn, ppm	Mn, ppm	Cu, ppm	Co, ppm
Late Gestation[2]				
SULF[3]	84	126	22	3.2
4PLEX[4]	85	131	22	3.3
NRC requirement[5]	40	40	12.5	0.05
Percent of requirement met by supplement pellet	130 %	128 %	125 %	5470 %
Lactation[6]				
SULF[3]	80	115	23	3.3
4PLEX[4]	82	120	23	3.4
NRC requirement[5]	40	40	10	0.05
Percent of requirement met by supplement pellet	138 %	134 %	164 %	6185 %

[1]All nutrient concentrations are presented on a DM basis.
[2]Total daily ration includes oats (0.7% BW) and Coastal bermudagrass hay (estimated at 1.1% BW) and either the SULF or 4PLEX supplement pellet (0.20% BW).
[3]Zn, Mn, Cu, and Co added to the supplement pellet are provided in sulfate forms.
[4]Zn, Mn, and Cu added to the supplement pellet are provided as amino acid complexes and Co provided as glucoheptonate complex.
[5]Nutrient requirements based on total daily intake of 2.0% BW in late gestation and 2.5% BW in lactation (NRC, 2007).
[2]Total daily ration includes oats (0.7% BW) and fresh bahiagrass pasture (estimated at 1.1% BW) and either the SULF or 4PLEX supplement pellet (0.28% BW).

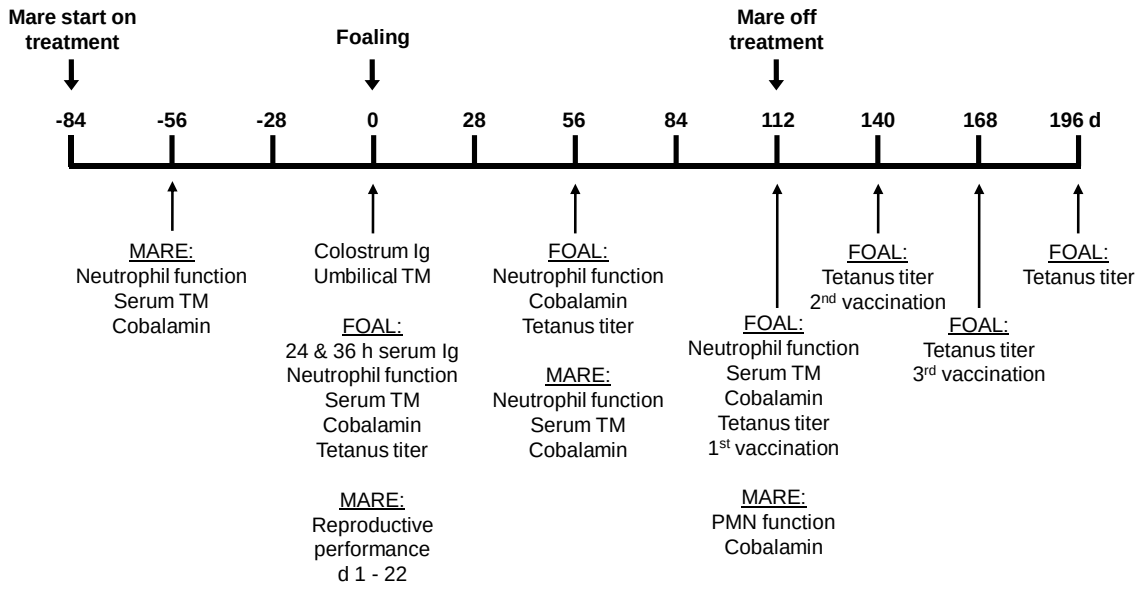

Figure 3-1. Timeline of sample collection

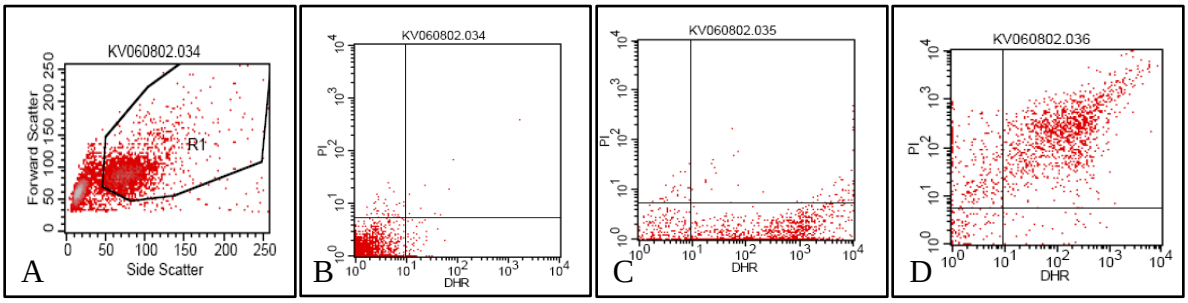

Figure 3-2. Representative scatter plot generated from one mare illustrating neutrophil phagocytosis and oxidative burst determined by flow cytometry.

(A) Neutrophils were gated for analysis on the basis of cell size (forward scatter) and complexity (side scatter). Panels B, C, and D show the propidium iodide (PI)-labeled y-axis which represents the intensity of red fluorescence from bacteria labeled with PI. The dihydrorhodamine (DHR) labeled x-axis represents the intensity of green fluorescence generated by conversion of nonfluorescent DHR to fluorescent rhodamine by the oxidative burst response of neutrophils. (B) Negative control- the lower left quadrant contains DHR loaded neutrophils in the absence of PI-labeled bacteria. (C) Positive control- the lower right and left quadrants contain DHR loaded PMN in the absence of bacteria stimulated with phorbol miristate acetate (PMA). The lower right quadrant contains cells that have undergone an artificial oxidative burst. (D) The lower left quadrant contains PI-labeled bacteria that have not yet undergone phagocytosis or subsequent oxidative burst. The upper left quadrant contains DHR loaded neutrophils that have phagocytosed PI-labeled bacteria. The upper right quadrant contains DHR loaded neutrophils that have phagocytosed PI labeled-bacteria and have undergone a subsequent oxidative burst.

CHAPTER 4
RESULTS

General Observations

The bodyweights of mares and foals are presented in Table 4-1. Dietary trace mineral source and duration of supplementation had no effect on mare or foal body weights. Mares lost an average of 67 kg at foaling, but subsequently maintained or gained body weight during lactation. Mean bodyweight of foals ± SE at birth was 56.9±1.3 kg. From birth to 24 wk of age, average daily gain of foals was 1.2 kg/d.

Routine observations at feeding time, as well as the lack of feed refusals, indicated the mares readily consumed the protein/vitamin/mineral supplement pellets containing either sulfate or amino-acid complex trace mineral sources. In addition, foals were observed to share their dams' whole oats and supplement pellet during the twice daily individual feedings.

Passive Transfer of Immunity

Immunoglobulin concentrations in mare colostrum are presented in Table 4-2. Dietary trace mineral source had no effect on colostrum IgG or IgM concentrations, but did affect IgA. Colostrum from mares receiving 4PLEX had higher (P=0.03) IgA levels than colostrum from mares receiving SULF. Long-term versus short-term duration of trace mineral supplementation had no effect on colostrum IgG, IgM, or IgA concentrations. Similarly, trace mineral source x duration interactions were not detected for any of the colostrum immunoglobulins. Nonetheless, evaluation of the response among the four treatment combinations revealed that colostrum IgA was greater in mares receiving 4PLEX-LONG (P=0.05) and tended to be higher in mares receiving 4PLEX-SHORT (P=0.09) compared to that observed in mares fed SULF-SHORT (Table 4-2). Similarly, colostrum IgA concentrations were numerically higher in 4PLEX-LONG

(P=0.14) and 4PLEX-SHORT (P=0.22) compared to SULF-LONG, but these differences were not significant. Colostrum IgG and IgM were not different among the four treatments.

The Ig concentrations in foal serum mimicked those observed in colostrum (Table 4-3). Trace mineral source fed to the mare had no effect on foal serum IgG or IgM, but trace mineral source did affect foal serum IgA (P=0.02). Foals nursing 4PLEX mares had higher serum IgA concentrations at 24 h of age (P=0.05) and numerically higher IgA concentrations at 36 h of age (P=0.13) compared to foals nursing SULF mares. Duration of trace mineral supplementation in the mare showed a trend to affect foal serum IgG (P=0.10) and IgA (P=0.09), whereby foals from long-term supplemented mares had higher serum IgG and IgA than foals from short-term supplemented mares (Table 4-3). Foal serum IgM concentrations were not affected by the duration mares had been supplemented with trace minerals. Trace mineral source x duration and source x duration x time interactions were not significant for foal serum immunoglobulins. However, differences in foal serum IgG and IgA were found among the four treatment combinations (Table 4-3). Foals nursing 4PLEX-LONG mares had higher serum IgG across all time points (P=0.03) and specifically at 36 h (P=0.04) compared to foals from SULF-SHORT mares, with foals from 4PLEX-SHORT and SULF-LONG exhibiting intermediate concentrations of serum IgG. Foal serum IgG did not differ among treatments at 24 h. Across time, serum IgA was higher in foals suckling 4PLEX-LONG mares compared to 4PLEX-SHORT (P=0.05), SULF-SHORT (P=0.005), and SULF-LONG (P=0.01). Most of this difference among treatments occurred at 24 h, where serum IgA was higher in foals nursing 4PLEX-LONG mares compared to foals nursing mares fed SULF-SHORT (P=0.02) or SULF-LONG (P=0.04). In addition, serum IgA tended to be higher at 24 h in foals from 4PLEX-LONG mares compared to 4PLEX-SHORT (P=0.09). At 36 h, serum IgA continued to show a trend to

be higher in foals nursing 4PLEX-LONG mares compared to foals nursing SULF-SHORT mares (P=0.10). Foal serum IgM was not affected by trace mineral treatment of the mare at either 24 or 36 h of age.

Tetanus Antibody Titers

Tetanus-specific IgG titers measured in the serum of foals are presented in Table 4-4. Time had the greatest effect on antibody titers (P=0.0001). Titers were highest at 1-2 d of age, reflecting the uptake of maternal antibodies in colostrum. Titer levels had decreased by 56 d of age (P=0.001) and subsequently remained unchanged through 112 d of age. Although foals received their first tetanus vaccination at 112 d of age, antibody titers were not different from 112 to 140 d of age and, in fact, continued to decline (Table 4-4). Thus, a serological response to the first vaccination was not observed. Trace mineral source fed to the mare and duration of supplementation had no overall effect on foal serum tetanus antibody titers. However, a marginal response to the second vaccination administered at 140 d was observed in foals from SULF mares, in which antibody titers tended to increase (P=0.10) from 140 to 168 d of age. A similar response to the second vaccination was not observed in foals from 4PLEX mares (Table 4-4). When these responses were analyzed with respect to the four treatment combinations, only the foals from SULF-SHORT mares showed an increase in antibody titers from d 140 to 168 (P=0.03), resulting in higher titers at d 168 compared to foals from 4PLEX-SHORT mares (P=0.05). All foals, regardless of the mare treatment, failed to show a serologic response to the third vaccination administered at 168 d, as antibody titers were similar between d 168 and d 196.

Neutrophil Function

The phagocytic and oxidative burst activities of neutrophils in mares and foals are presented in Tables 4-5 and 4-6, respectively. To avoid potential differences arising from the use of multiple *Staphylococcus aureus* cultures, the same batch of labeled bacteria were utilized in

the neutrophil function assay throughout this 11-mo study. As a result, some loss of bacteria fluorescence was noted over time. Therefore, no attempt was made to analyze the effect of time on mare or foal neutrophil function. When data were analyzed within each day, source and duration of dietary trace mineral supplementation of the mare had no effect on the percentage of neutrophils that underwent phagocytosis or a subsequent oxidative burst in either mares or foals. Similarly, a trace mineral source x duration interaction was not observed for neutrophil function in mares or foals. However, at 56 d of age, foals nursing SULF-SHORT mares tended to have a greater percent phagocytosis (P=0.06) and oxidative burst (P=0.06) compared to foals nursing SULF-LONG mares.

Trace Mineral Concentrations in Umbilical Cord Serum

Trace mineral concentrations in umbilical cord serum are presented in Table 4-7. Dietary trace mineral source had no effect on Cu or Mn concentrations in umbilical cord serum, but mares receiving SULF tended to have higher cord serum Zn than mares supplemented with 4PLEX (P=0.07). Duration of trace mineral supplementation and the trace mineral source x duration interaction did not affect umbilical cord serum Cu, Zn or Mn.

Trace Mineral Concentrations in Mare and Foal Serum

Serum trace mineral concentrations measured during late gestation and early lactation in the mare are presented in Table 4-8. Dietary trace mineral source and duration of supplementation had no effect on mare serum Cu, Zn, or Mn concentrations. Similarly, an overall interaction between trace mineral source x duration of supplementation was not observed for any of these minerals in mare serum. However, SULF-LONG mares had greater serum zinc levels 56 d prior to foaling than 4PLEX-LONG mares (P=0.04), while values remained similar between treatments at 56 d after foaling. An overall effect of time was detected for mare serum Mn (P=0.0002). Serum Mn declined from 56 d before to 56 d after foaling in mares

supplemented with SULF (P=0.0003) and tended to decline in mares supplemented with 4PLEX (P=0.06). Copper and Zn concentrations in mare serum did not differ between pre- and post-foaling samples.

Serum trace mineral concentrations measured in foals at 1-2 and 112 d of age are presented in Table 4-9. An overall effect of time was noted for all trace minerals, describing an increase in serum Cu (P=0.0001) and Zn (0.0001) and a decrease in serum Mn (P=0.002) in foals from 1-2 d to 112 d of age. Source of trace mineral supplemented to the mare and duration of supplementation had no effect on foal serum Cu, Zn, or Mn concentrations. No interactions between trace mineral source, duration of supplementation, and/or time were significant for foal serum Zn or Mn. In contrast, trends for source x duration (P=0.06), source x time (P=0.10), and source x duration x time (P=0.11) interactions were detected for foal serum Cu. Although serum Cu concentrations were not different among treatments at birth, foals belonging to SULF mares had higher serum Cu levels at 112 d of age than foals from 4PLEX mares (P=0.03). When all four treatment combinations were examined, foals from SULF-SHORT mares had higher serum Cu at 112 d of age compared to SULF-LONG (P=0.01), 4PLEX-SHORT (P=0.002), and 4PLEX-LONG (P=0.02) foals. Foal serum Zn increased with age in foals from 4PLEX-SHORT (P=0.03), 4PLEX-LONG (P=0.0001), and SULF-LONG (P=0.02) mares, but not SULF-SHORT mares (P=0.14). Numeric decreases in serum Mn were observed in foals from SULF-SHORT (P=0.27), 4PLEX-LONG (P=0.15), and SULF-LONG (P=0.13) supplemented mares, but the decrease was only significant in foals from 4PLEX-SHORT mares (P=0.01). The high variability in serum Mn measured at 1-2 d of age, coupled with the numerically higher initial serum Mn concentrations in foals from 4PLEX-SHORT mares likely accounted for these responses.

Vitamin B12 Status

Serum cobalamin concentrations measured during late gestation and early and late lactation in the mare are presented in Table 4-10. An effect of trace mineral source (P=0.03), time (P=0.0001), and source x time (P=0.05) were found for mare serum cobalamin. Serum cobalamin was similar among treatments 56 d before and 56 d after foaling. Mares supplemented with 4PLEX exhibited a 2-fold increase in serum cobalamin (P=0.001) from 56 to 112 d post-foaling, whereas serum cobalamin remained unchanged over time in SULF supplemented mares. As a result, serum cobalamin was greater in mares fed 4PLEX at 112 d post-foaling (P=0.001) compared to mares receiving SULF. Duration of trace mineral supplementation had no effect on mare serum cobalamin; however, there was a trend for an effect of source x duration x time (P=0.10). Serum cobalamin concentrations did not change from 56 d pre- to 56 d post-foaling in any of the four treatment combinations and were similar among treatments. From 56 to 112 d post-foaling, 4PLEX-SHORT (P=0.001), 4PLEX-LONG (P=0.07), and SULF-LONG (P=0.07) supplemented mares had an increase in serum cobalamin, while serum cobalamin remained unchanged in SULF-SHORT mares. At 112 d post-foaling, mares fed 4PLEX-SHORT had higher serum cobalamin concentrations than mares fed SULF-SHORT (P=0.0001) and SULF-LONG (P=0.02), and tended to have higher serum cobalamin than mares fed 4PLEX-LONG (P=0.10). In addition, serum cobalamin concentrations were higher in 4PLEX-LONG mares (P=0.01) and tended to be higher in SULF-LONG mares (P=0.07) at 112 d compared to mares receiving SULF-SHORT.

Table 4-11 lists serum cobalamin concentrations measured in foals. Although foals nursing 4PLEX mares had numerically higher serum cobalamin concentrations than SULF foals at birth, the source of trace mineral supplied to the mare had no overall effect on foal serum cobalamin. An overall effect of time was noted (P=0.06). Serum cobalamin decreased from 1-2 to 56 d of

age in foals nursing 4PLEX mares (P=0.02) and remained unchanged from 56 to 112 d of age. A progressive decline in serum cobalamin from birth through 112 d of age was also observed for foals belonging to SULF mares, but these changes were not significant. Duration of trace mineral supplementation in the mare had no overall effect on foal serum cobalamin; however, foals nursing mares that underwent long-term trace mineral supplementation had higher serum cobalamin concentrations at 1-2 d of age than foals from mares that underwent short-term supplementation (P=0.04). The combination of trace mineral source and duration of supplementation in the mare had no overall effect foal serum cobalamin. Nonetheless, at 1-2 d of age, serum cobalamin concentration was greater in foals from 4PLEX-LONG mares compared to foals from 4PLEX-SHORT (P=0.04) and SULF-SHORT (P=0.01) mares. Serum cobalamin in foals from SULF-LONG mares did not differ from 4PLEX-LONG foals at 1-2 d of age, and while numerically higher, also did not differ from SULF-SHORT or 4PLEX-SHORT foals. No differences in foal serum cobalamin were observed among the four treatment combinations at 56 or 112 d of age.

Postpartum Reproductive Performance

Data obtained to ascertain reproductive performance in the early postpartum period are presented in Tables 4-12 – 4-17. Source of dietary trace mineral had no effect on the average diameter of all follicles, the diameter of the largest follicle, or the average diameter of the 6 largest follicles that developed in the early postpartum period (Table 4-12). Similarly, duration of trace mineral supplementation had no effect on the average diameter of all follicles or the diameter of the largest follicle, but tended have an effect on the average diameter of the 6 largest follicles that developed (P=0.10), whereby short-term supplemented mares tended to have a greater average size of the 6 largest follicles than mares receiving long-term supplementation

(Table 4-12). There was no overall source x duration effect, but 4PLEX-SHORT mares tended to have greater average size of the 6 largest follicles than 4PLEX-LONG mares (Table 4-12).

There was no effect of source or duration of trace mineral supplementation on the growth rate of the largest follicle or the average growth rate of the six largest follicles that developed in the early postpartum period (Table 4-13). Although there was also no overall effect of trace mineral source x duration of supplementation on either of these measures, the growth rate of the largest follicle that developed did differ between the four treatment combinations. The growth rate of the largest follicle was greater in 4PLEX-SHORT mares than 4PLEX-LONG mares (P=0.04), and tended to be greater than SULF-LONG (P=0.08) and SULF-SHORT (P=0.08) mares (Table 4-13).

Across treatments, the number of follicles that developed in the first postpartum cohort averaged 20.5 ± 1.3. The number of follicles that developed were not affected by dietary trace mineral source, but showed a trend to be affected by the duration of supplementation (P=0.10) and the source x duration interaction (P=0.09) (Table 4-14). Short-term supplemented mares had greater number of follicles develop in the first postpartum cohort than did long-term supplemented mares (P=0.10). Comparison of the four treatment combinations revealed that mares fed 4PLEX-LONG had fewer follicles develop than mares supplemented with 4PLEX-SHORT (P=0.02), and tended to have fewer follicles compared to SULF-LONG (P=0.09). The day of follicle deviation, where there is a departure in the growth rate between the dominant and subordinate follicles, averaged 6.1 ± 0.7 d post-foaling. Dietary trace mineral source, duration of supplementation, and the source x duration interaction had no effect on the day of follicle deviation (Table 4-14).

The number of days from foaling to foal-heat ovulation is presented in Table 4-15. Across treatments, ovulation occurred 12.1 ± 0.5 d post-foaling. Dietary trace mineral source and the duration of supplementation had no effect on the day of foal-heat ovulation. Across treatments, ovulation occurred within 10 d postpartum for 24% of the mares, whereas 76% of mares ovulated greater than 10 d postpartum. There was no difference in the number of mares ovulating within or after 10 d post-foaling between 4PLEX or SULF trace mineral sources, or among the source and duration combinations.

Changes in uterine dynamics in the early postpartum period are presented in Table 4-16. In all treatments, the net negative change in fluid score, uterine body diameter, and gravid and non-gravid horn diameters were indicative of the process of uterine involution. The net positive change in edema score in all treatments reflected the increase in edema associated with follicle development and impending ovulation. Dietary trace mineral source and duration of supplementation had no effect on the change in intrauterine luminal fluid score or endometrial edema score from foaling to foal-heat ovulation. Similarly, the source and duration of trace mineral supplementation had no effect on the change of the uterine body or non-gravid horn from foaling to foal-heat ovulation (Table 4-16). However, the change in diameter of the gravid horn was affected by duration of supplementation, where mares that had received short-term supplementation had a greater rate of involution compared to mares that had been supplemented long-term (P=0.05). Although the rate of change in diameter of the gravid horn was not affected by trace mineral source or the source x duration interaction, differences were observed among the four treatment combinations. The gravid horn in mares receiving 4PLEX-LONG had a slower rate of involution compared to 4PLEX-SHORT (P=0.02), with SULF-SHORT and SULF-LONG mares exhibiting intermediate rates of involution (Table 4-16).

Dietary trace mineral source and duration of supplementation had no effect on peak LH or FSH concentrations in mare serum, or the day on which the peak in these hormones occurred relative to foaling (Table 4-17). Across treatments, peak LH concentration averaged 74.2 ± 7.2 ng/mL and peaked on or near the time of ovulation (0 ± 0.33 d). Serum FSH concentrations averaged 172.7 ± 24.8 ng/mL and peaked 7.7 ± 1.0 d prior to ovulation. A trace mineral source x duration of supplementation interaction was detected for the day of FSH peak (P=0.06). Mares receiving 4PLEX-LONG tended to have FSH peak closer to foaling (and further from ovulation) than 4PLEX-SHORT (P=0.06) and SULF-LONG mares (P=0.07).

Table 4-1. Bodyweights of mares and foals

Day post-foaling	Mare bodyweight, kg		Foal bodyweight, kg	
	SULF	4PLEX	SULF	4PLEX
(-)56	644.0 (11.6)	625.0 (11.8)	---	---
1-2	575.7 (10.1)	558.3 (10.3)	56.7 (1.3)	57.1 (1.3)
28	581.3 (10.3)	568.4 (11.8)	96.5 (2.8)	95.6 (2.8)
56	588.8 (12.9)	566.6 (12.9)	133.4 (2.9)	143.8 (13.9)
84	590.8 (10.0)	564.2 (10.1)	169.6 (4.4)	163.4 (4.1)
112	593.9 (11.8)	570.2 (12.5)	196.0 (5.7)	198.4 (5.3)
140	596.0 (11.0)	570.7 (12.7)	224.3 (5.4)	224.2 (6.1)
168	---	---	252.4 (5.6)	252.3 (6.5)

Values presented as mean (SE).

Table 4-2. Effects of dietary trace mineral (TM) source and duration of supplementation on immunoglobulin concentrations in mare colostrum

	Colostrum immunoglobulins, mg/dL		
Variable	IgG	IgA	IgM
TM Source			
SULF	10,268 (741)	464 (56) [z]	161 (17)
4PLEX	10,660 (741)	647 (56) [y]	153 (17)
Duration			
SHORT	11,025 (741)	533 (56)	147 (17)
LONG	9,903 (741)	577 (56)	166 (17)
Source x Duration			
SULF-SHORT	11,011 (1048)	436 (80) [z,†]	151 (23)
SULF-LONG	9,526 (1048)	491 (80) [y,z]	155 (23)
4PLEX-SHORT	11,039 (1048)	632 (80) [y,z,†]	144 (23)
4PLEX-LONG	10,281 (1048)	662 (80) [y]	178 (23)
P-values			
TM Source (S)	0.71	0.03	0.74
Duration (D)	0.29	0.60	0.42
S x D	0.73	0.87	0.54

Values presented as lsmeans (SE). [y,z]Within a column, means with different superscripts differ (P<0.05). †Within a column, means differ (P<0.10).

Table 4-3. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on immunoglobulin concentrations in foal serum at 24 and 36 h of age

	Foal serum immunoglobulins, mg/dL					
	IgG		IgA		IgM	
Variable	24 h	36 h	24 h	36 h	24 h	36 h
TM Source						
SULF	2,404 (177)	2,510 (177)	46 [z] (11)	43 (11)	45 (4)	45 (4)
4PLEX	2,600 (177)	2828 (177)	76 [y] (11)	66 (11)	51 (4)	47 (4)
Duration						
SHORT	2,413 (177)	2,460 [†] (177)	49 (11)	47 (11)	48 (4)	44 (4)
LONG	2,592 (177)	2,879 [†] (177)	72 (11)	61 (11)	49 (4)	49 (4)
Source x Duration						
SULF-SHORT	2,236 (251)	2,350 [z] (251)	41 [z] (16)	40 [†] (16)	47 (6)	44 (6)
SULF-LONG	2,571 (251)	2,671 [y,z] (251)	50 [z] (15)	46 (15)	44 (6)	46 (6)
4PLEX-SHORT	2,589 (251)	2,570 [y,z] (251)	56 [y,z,†] (16)	54 (16)	48 (6)	44 (6)
4PLEX-LONG	2,612 (251)	3,087 [y] (251)	96 [y,†] (16)	78 [†] (16)	54 (6)	51 (6)
P-values						
TM Source (S)	0.15		0.02		0.33	
Duration (D)	0.10		0.09		0.49	
S x D	0.87		0.27		0.42	
Time	0.35		0.57		0.62	
S x Time	0.73		0.73		0.65	
D x Time	0.50		0.68		0.69	
S x D x Time	0.48		0.79		0.75	

Values are presented as lsmeans (SE). [y,z]Within a column, means with different superscripts differ (P<0.05). †Within a column, means differ (P<0.10).

Table 4-4. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on tetanus-specific IgG titers (IU/mL) in foal serum

	Foal age, d[1]					
Variable	1-2	56	112	140	168	196
TM Source						
SULF	2.83 [a] (0.27)	1.98 [b] (0.27)	1.94 [b] (0.27)	1.34 [b,‡] (0.27)	1.76 [b,‡] (0.27)	1.47 [b] (0.27)
4PLEX	3.10 [a] (0.26)	2.18 [b] (0.26)	1.86 [b,c] (0.27)	1.41 [c] (0.26)	1.29 [c] (0.27)	1.28 [c] (0.26)
Duration						
SHORT	2.99 [a] (0.25)	1.97 [b] (0.25)	1.83 [b,c] (0.25)	1.25 [c] (0.25)	1.54 [b,c] (0.25)	1.30 [c] (0.25)
LONG	2.94 [a] (0.28)	2.19 [b] (0.28)	1.97 [b,c] (0.29)	1.51 [b,c] (0.28)	1.50 [c] (0.29)	1.46 [c] (0.28)
Source x Duration						
SULF-SHORT	2.87 [a] (0.36)	1.91 [b,c] (0.36)	1.72 [b,c] (0.36)	1.19 [c] (0.36)	2.03 [a,b,y] (0.36)	1.72 [b,c] (0.36)
SULF-LONG	2.80 [a] (0.41)	2.06 [a,b] (0.41)	2.16 [a,b] (0.41)	1.50 [b] (0.41)	1.49 [b,y,z] (0.41)	1.23 [b] (0.41)
4PLEX-SHORT	3.12 [a] (0.36)	2.03 [b] (0.36)	1.93 [b,c] (0.36)	1.31 [b,c] (0.36)	1.06 [c,z] (0.36)	0.88 [c] (0.36)
4PLEX-LONG	3.09 [a] (0.38)	2.33 [a,b] (0.38)	1.78 [b] (0.40)	1.51 [b] (0.38)	1.52 [b,y,z] (0.40)	1.69 [b] (0.38)
P-values						
TM Source (S)	0.87					
Duration (D)	0.61					
S x D	0.51					
Time	0.0001					
S x Time	0.61					
D x Time	0.94					
S x D x Time	0.43					

Values are presented as lsmeans (SE). [1]Foals received a primary tetanus vaccination at 112 d of age, followed by booster vaccinations at 140 and 168 d of age. [a,b,c]Within a row, means with different superscripts differ (P<0.05). [y,z]Within a column, means with different superscripts differ (P<0.05). ‡Within a row, means differ (P<0.10).

Table 4-5. Effects of dietary trace mineral (TM) source and duration of supplementation on neutrophil phagocytosis and oxidative burst in the mare 56 d before and 56 and 112 d after foaling

	Phagocytosis, %			Oxidative burst, %		
Variable	(-) 56 d	56 d	112 d	(-) 56 d	56 d	112 d
TM Source						
SULF	91.3 (2.5)	80.7 (3.3)	65.8 (2.8)	65.0 (3.1)	62.9 (3.8)	45.5 (2.6)
4PLEX	89.6 (2.7)	80.7 (3.4)	64.7 (2.8)	68.4 (3.3)	62.5 (3.9)	46.3 (2.6)
Duration						
SHORT	91.1 (2.6)	80.4 (3.4)	65.6 (2.9)	65.0 (3.1)	59.8 (3.9)	45.1 (2.6)
LONG	89.7 (2.7)	81.1 (3.3)	64.9 (2.7)	68.5 (3.3)	65.5 (3.8)	46.7 (2.5)
Source x Duration						
SULF-SHORT	92.0 (3.6)	79.5 (5.0)	68.1 (4.0)	63.2 (4.4)	59.7 (5.7)	45.5 (3.7)
SULF-LONG	90.5 (3.6)	82.0 (4.4)	63.6 (3.8)	66.9 (4.4)	66.0 (5.1)	45.5 (3.5)
4PLEX-SHORT	90.2 (3.6)	81.3 (4.7)	63.2 (4.0)	66.7 (4.4)	59.9 (5.4)	44.6 (3.7)
4PLEX-LONG	89.0 (4.1)	80.1 (5.0)	66.3 (3.8)	70.2 (4.9)	65.0 (5.7)	47.9 (3.5)
P-values						
TM Source (S)	0.20	0.99	0.78	0.46	0.94	0.84
Duration (D)	0.72	0.88	0.86	0.44	0.31	0.65
S x D	0.97	0.70	0.34	0.99	0.92	0.66

Values presented as lsmeans (SE).

Table 4-6. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on neutrophil phagocytosis and oxidative burst in the foal at 1-2, 56, and 112 d of age

	Phagocytosis, %			Oxidative burst, %		
Variable	1-2 d	56 d	112 d	1-2 d	56 d	112 d
TM Source						
SULF	83.3 (3.5)	70.7 (3.8)	62.9 (3.8)	63.6 (4.2)	56.6 (4.2)	38.9 (5.2)
4PLEX	79.8 (3.6)	68.2 (3.9)	65.0 (4.1)	57.1 (4.3)	55.0 (4.3)	41.8 (5.7)
Duration						
SHORT	83.4 (3.7)	73.3 (3.9)	64.5 (3.9)	62.5 (4.4)	60.2 (4.3)	38.2 (5.4)
LONG	79.6 (3.4)	65.6 (3.8)	63.3 (3.9)	58.2 (4.0)	51.4 (4.2)	42.4 (5.4)
Source x Duration						
SULF-SHORT	82.6 (5.1)	78.1 † (5.8)	66.5 (5.3)	67.1 (6.1)	64.7 † (6.4)	37.6 (7.3)
SULF-LONG	83.9 (4.8)	63.3 † (4.9)	59.2 (5.3)	60.1 (5.7)	48.4 † (5.4)	40.2 (7.3)
4PLEX-SHORT	84.2 (5.4)	68.5 (5.3)	62.6 (5.8)	57.9 (6.5)	55.6 (5.8)	38.9 (8.0)
4PLEX-LONG	75.3 (4.8)	67.9 (5.8)	67.4 (5.8)	56.3 (5.7)	54.4 (6.4)	44.6 (8.0)
P-values						
TM Source (S)	0.49	0.64	0.71	0.29	0.80	0.71
Duration (D)	0.45	0.17	0.82	0.48	0.16	0.59
S x D	0.31	0.21	0.29	0.65	0.22	0.84

Values presented as lsmeans (SE). †Within a column, means differ (P<0.10).

Table 4-7. Effects of dietary trace mineral (TM) source and duration of supplementation on copper, zinc, and manganese concentrations in umbilical cord serum

	Umbilical cord serum trace minerals		
Variable	Copper, mg/L	Zinc, mg/L	Manganese, µg/L
TM Source			
SULF	0.17 (0.01)	1.30 (0.09) †	62.2 (37.0)
4PLEX	0.18 (0.01)	1.05 (0.09) †	90.3 (37.0)
Duration			
SHORT	0.17 (0.01)	1.16 (0.09)	45.4 (37.0)
LONG	0.18 (0.01)	1.20 (0.09)	107.1 (37.0)
Source x Duration			
SULF-SHORT	0.17 (0.01)	1.33 (0.13)	70.7 (52.4)
SULF-LONG	0.17 (0.01)	1.27 (0.13)	53.7 (52.4)
4PLEX-SHORT	0.18 (0.01)	0.98 (0.13)	20.1 (52.4) †
4PLEX-LONG	0.18 (0.01)	1.13 (0.13)	160.4 (52.4) †
P-values			
TM Source (S)	0.32	0.07	0.59
Duration (D)	0.62	0.76	0.24
S x D	0.87	0.43	0.14

Values presented as lsmeans (SE). †Within a column, means differ (P<0.10).

Table 4-8. Effects of dietary trace mineral (TM) source and duration of supplementation on copper, zinc, and manganese concentration in the mare 56 d before and 56 d after foaling

	Mare serum trace minerals					
	Copper, mg/L		Zinc, mg/L		Manganese, µg/L	
Variable	(-)56 d	56 d	(-)56 d	56 d	(-)56 d	56 d
TM Source						
SULF	1.38 (0.07)	1.34 (0.07)	0.74 (0.02)	0.69 (0.02)	190.9 [a] (31.8)	23.6 [b] (31.8)
4PLEX	1.45 (0.07)	1.43 (0.07)	0.69 (0.02)	0.70 (0.02)	140.3 [‡] (31.8)	56.8 [‡] (31.8)
Duration						
SHORT	1.42 (0.07)	1.41 (0.07)	0.72 (0.02)	0.69 (0.02)	156.5 [a] (31.8)	48.2 [b] (31.8)
LONG	1.41 (0.07)	1.35 (0.07)	0.71 (0.02)	0.69 (0.02)	174.6 [a] (31.8)	32.1 [b] (31.8)
Source x Duration						
SULF-SHORT	1.43 (0.10)	1.31 (0.10)	0.72 [y,z] (0.03)	0.71 (0.03)	167.3 [a] (45.0)	38.1 [b] (45.0)
SULF-LONG	1.32 (0.10)	1.37 (0.10)	0.75 [y] (0.03)	0.67 (0.03)	214.4 [a] (45.0)	9.0 [b] (45.0)
4PLEX-SHORT	1.41 (0.10)	1.51 (0.10)	0.71 [y,z] (0.03)	0.68 (0.03)	145.8 (45.0)	58.3 (45.0)
4PLEX-LONG	1.49 (0.10)	1.34 (0.10)	0.66 [z] (0.03)	0.71 (0.03)	134.8 (45.0)	55.2 (45.0)
P-values						
TM Source (S)	0.24		0.34		0.79	
Duration (D)	0.63		0.78		0.97	
S x D	0.89		0.86		0.80	
Time	0.66		0.43		0.0002	
S x Time	0.95		0.21		0.19	
D x Time	0.73		0.90		0.59	
S x D x Time	0.13		0.08		0.51	

Values presented as lsmeans (SE). [a,b]Within a mineral, means in the same row with different superscripts differ (P<0.05). [y,z]Within a mineral, means in the same column with different superscripts differ (P<0.05). [‡]Within a mineral, means in the same row differ (P<0.10).

Table 4-9. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on copper, zinc, and manganese concentrations in foal serum at 1-2 and 112 d of age

	Foal serum trace minerals					
	Copper, mg/L		Zinc, mg/L		Manganese, µg/L	
Variable	1-2 d	112 d	1-2 d	112 d	1-2 d	112 d
TM Source						
SULF	0.28 [a] (0.06)	1.75 [b,y] (0.06)	0.51 [a] (0.04)	0.67 [b] (0.04)	61.4 [a] (22.3)	1.7 [b] (22.3)
4PLEX	0.30 [a] (0.06)	1.57 [b,z] (0.06)	0.47 [a] (0.04)	0.73 [b] (0.04)	84.7 [a] (20.8)	2.0 [b] (21.5)
Duration						
SHORT	0.27 [a] (0.06)	1.72 [b] (0.06)	0.53 [a] (0.04)	0.67 [b] (0.04)	76.7 [a] (20.8)	1.7 [b] (20.8)
LONG	0.31 [a] (0.06)	1.61 [b] (0.06)	0.46 [a] (0.04)	0.73 [b] (0.04)	69.4 [a] (22.2)	1.9 [b] (22.9)
Source x Duration						
SULF-SHORT	0.27 [a] (0.08)	1.91 [b,y] (0.08)	0.53 (0.06)	0.65 (0.06)	47.9 (29.5)	1.5 (29.5)
SULF-LONG	0.29 [a] (0.08)	1.59 [b,z] (0.09)	0.49 [a] (0.06)	0.69 [b] (0.06)	75.0 (33.4)	1.8 (33.4)
4PLEX-SHORT	0.27 [a] (0.08)	1.53 [b,z] (0.08)	0.52 [a] (0.06)	0.69 [b] (0.06)	105.5 [a] (29.5)	2.0 [b] (29.5)
4PLEX-LONG	0.32 [a] (0.08)	1.62 [b,z] (0.09)	0.43 [a] (0.06)	0.77 [b] (0.06)	63.9 (29.5)	1.9 (31.2)
P-values						
TM Source (S)	0.20		0.88		0.59	
Duration (D)	0.51		0.95		0.87	
S x D	0.06		0.99		0.43	
Time	0.0001		0.0001		0.002	
S x Time	0.10		0.26		0.60	
D x Time	0.22		0.13		0.87	
S x D x Time	0.11		0.61		0.44	

Values presented as lsmeans (SE). [a,b]Within a mineral, means in the same row with different superscripts differ (P<0.05). [y,z] Within a mineral, means in the same column with different superscripts differ (P<0.05).

Table 4-10. Effects of dietary trace mineral (TM) source and duration of supplementation on serum cobalamin concentrations in the mare 56 d before and 56 and 112 d after foaling

	Mare serum cobalamin, ng/L		
Variable	(-)56 d	56 d	112 d
TM Source			
SULF	2,507 (428)	2,471 (428)	3,289 (428) [z]
4PLEX	2,644 (428) [a]	2,772 (428) [a]	5,286 (428) [b,y]
Duration			
SHORT	2,602 (428) [a]	2,406 (428) [a]	4,248 (428) [b]
LONG	2,549 (428) [a]	2,837 (428) [a]	4,328 (428) [b]
Source x Duration			
SULF-SHORT	2,465 (606)	2,349 (606)	2,501 (606) [z]
SULF-LONG	2,548 (606)	2,593 (606) [‡]	4,078 (606) [z,y,‡]
4PLEX-SHORT	2,738 (606) [a]	2,463 (606) [a]	5,996 (606) [b.x]
4PLEX-LONG	2,550 (606) [a]	3,082 (606) [a,b]	4,577 (606) [b,x,y]
P-values			
TM Source (S)		0.03	
Duration (D)		0.68	
S x D		0.20	
Time		0.0001	
S x Time		0.05	
D x Time		0.82	
S x D x Time		0.10	

Values presented as lsmeans (SE). [a,b]Within a row, means with different superscripts differ (P<0.05). [x,y,z]Within a column, means with different superscripts differ (P<0.05).

Table 4-11. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on serum cobalamin concentrations in the foal at 1-2, 56, and 112 d of age

	Foal serum cobalamin, ng/L		
Variable	1-2 d	56 d	112 d
TM Source			
SULF	3,272 (429)	2,879 (429)	2,790 (429)
4PLEX	4,127 (413) [a]	2,7569 (413) [b]	2,865 (413) [b]
Duration			
SHORT	3,073 (401) [z]	2,834 (401)	2,876 (401)
LONG	4,325 (440) [a,y]	2,804 (440) [b]	2,779 (440) [b]
Source x Duration			
SULF-SHORT	2,848 (567) [z]	2,961 (567)	2,805 (567)
SULF-LONG	3,965 (643) [y,z]	2,796 (643)	2,774 (643)
4PLEX-SHORT	3,298 (567) [z]	2,707 (567)	2,946 (567)
4PLEX-LONG	4,955 (601) [a,y]	2,811 (601) [b]	2,784 (601) [b]
P-values			
TM Source (S)		0.44	
Duration (D)		0.28	
S x D		0.65	
Time		0.06	
S x Time		0.48	
D x Time		0.21	
S x D x Time		0.85	

Values presented as lsmeans (SE). [a,b]Within a row, means with different superscripts differ (P<0.05). [y,z]Within a column, means with different superscripts differ (P<0.05).

Table 4-12. Effects of dietary trace mineral (TM) source and duration of supplementation on follicle diameter in the first postpartum cohort

	Follicle diameter, mm		
Variable	All follicles[2]	Largest six follicles[3]	Largest follicle[4]
TM Source			
SULF *(n=14)*	12.4 (0.3)	20.4 (0.7)	49.6 (1.1)
4PLEX *(n=14)*	12.7 (0.3)	20.3 (0.7)	49.1 (1.1)
Duration			
SHORT *(n=14)*	12.7 (0.3)	21.2 (0.7) †	50.0 (1.1)
LONG *(n=14)*	12.4 (0.3)	19.5 (0.7) †	48.9 (0.1)
Source x Duration			
SULF-SHORT *(n=6)*	12.7 (0.5)	20.8 (1.1)	51.2 (1.6)
SULF-LONG *(n=8)*	12.2 (0.4)	19.9 (0.9)	48.1 (1.4)
4PLEX-SHORT *(n=8)*	12.8 (0.4)	21.5 (0.9) †	48.6 (1.4)
4PLEX-LONG *(n=6)*	12.5 (0.5)	19.1 (1.1) †	49.7 (1.6)
P-values			
TM Source (S)	0.65	0.94	0.76
Duration (D)	0.45	0.10	0.51
S x D	0.87	0.48	0.18

Values presented as lsmeans (SE). [2]Average of all follicles from the left and right ovaries. [3]Average of the 3 largest follicles from the left and 3 largest from the right ovary. [4]Largest follicle from either ovary. †Within a column, means with different superscripts differ (P<0.10).

Table 4-13. Effects of dietary trace mineral (TM) source and duration of supplementation on follicle growth rate in the first postpartum cohort

	Follicle growth rate, mm/d	
Variable	Largest six follicles[2]	Largest follicle[3]
TM Source		
SULF *(n=14)*	0.33 (0.09)	2.62 (0.16)
4PLEX *(n=14)*	0.46 (0.09)	2.83 (0.16)
Duration		
SHORT *(n=14)*	0.39 (0.09)	2.89 (0.16)
LONG *(n=14)*	0.40 (0.09)	2.56 (0.16)
Source x Duration		
SULF-SHORT *(n=6)*	0.34 (0.13)	2.60 (0.25) [y,z, ‡]
SULF-LONG *(n=8)*	0.33 (0.11)	2.64 (0.22) [y,z,†]
4PLEX-SHORT *(n=8)*	0.45 (0.11)	3.19 (0.22) [y,†, ‡]
4PLEX-LONG *(n=6)*	0.47 (0.13)	2.47 (0.25) [z]
P-values		
TM Source (S)	0.31	0.37
Duration (D)	0.96	0.16
S x D	0.92	0.11

Values presented as lsmeans (SE). [2]Growth rate of the 3 largest developing follicles from the left and 3 largest from the right ovary. [3]Growth rate of the largest developing follicle, regardless of ovary. [y,z]Within a column, means with different superscripts differ (P<0.05). [†]Within a column, means with different superscripts differ (P<0.10). [‡]Within a column, means with different superscripts differ (P<0.10).

Table 4-14. Effects of dietary trace mineral (TM) source and duration of supplementation on number of follicles developing in the first postpartum cohort and day of follicle deviation

Variable	Number of follicles[2]	Day of deviation[3]
TM Source		
SULF *(n=14)*	20.9 (1.3)	6.0 (0.7)
4PLEX *(n=11)*	19.6 (1.3)	6.3 (0.8)
Duration		
SHORT *(n=12)*	21.8 (1.3) [†]	6.4 (0.8)
LONG *(n=13)*	18.8 (1.3) [†]	5.9 (0.7)
Source x Duration		
SULF-SHORT *(n=6)*	20.8 (1.9) [y,z]	6.8 (1.1)
SULF-LONG *(n=8)*	21.0 (1.7) [y,z,†]	5.3 (0.9)
4PLEX-SHORT *(n=8)*	22.8 (1.7) [y]	6.0 (1.1)
4PLEX-LONG *(n=6)*	16.5 (1.9) [z,†]	6.6 (1.2)
P-values		
TM Source (S)	0.48	0.81
Duration (D)	0.10	0.65
S x D	0.09	0.32

Values presented as lsmeans (SE).[2]Number of follicles developing in the left and right ovaries. [3]The day post-foaling in which there was a departure in growth rate between the dominant and subordinate follicles. [y,z]Within a column, values with different superscripts differ (P<0.05). [†]Within a column, values differ (P<0.10).

Table 4-15. Effects of dietary trace mineral (TM) source and duration of supplementation on day of foal heat ovulation

Variable	Day[2] *(n=28)*	Percentage of mares ovulating ≤10 days[3] *(n=34)*	Percentage of mares ovulating >10 days[4] *(n=34)*
TM Source			
SULF	12.5 (0.7)	22.2	77.8
4PLEX	11.9 (0.7)	31.3	68.8
Duration			
SHORT	11.9 (0.7)	35.3	64.7
LONG	12.5 (0.7)	17.7	82.4
Source x Duration			
SULF-SHORT	13.0 (1.0)	22.2	77.8
SULF-LONG	12.0 (0.9)	22.2	77.8
4PLEX-SHORT	10.9 (0.9)	50.0	50.0
4PLEX-LONG	13.0 (1.0)	12.5	87.5
P-values			
TM Source (S)	0.56	0.55	
Duration (D)	0.56	0.24	
S x D	0.11	0.35	

Values presented as lsmeans (SE). [2]Day of ovulation. [3]Percentage of mares that ovulated within 10 d of foaling. [4]Percentage of mares that ovulated more than 10 d after foaling.

Table 4-16. Effects of dietary trace mineral (TM) source and duration of supplementation on postpartum uterine morphology

	Change[2] in score/d		Change[3] in diameter, mm/d		
Variable	Fluid *(n=28)*	Edema *(n=28)*	Uterine body *(n=28)*	Gravid horn *(n=25)*	Non-gravid horn *(n=25)*
TM Source					
SULF	-0.07 (0.02)	0.02 (0.02)	-0.9 (0.2)	-2.8 (0.4)	-2.7 (0.4)
4PLEX	-0.08 (0.02)	0.03 (0.02)	-1.3 (0.2)	-2.7 (0.3)	-2.9 (0.4)
Duration					
SHORT	-0.08 (0.02)	0.03 (0.02)	-1.1 (0.2)	-3.3 [y] (0.4)	-3.1 (0.5)
LONG	-0.07 (0.02)	0.03 (0.02)	-1.1 (0.2)	-2.3 [z] (0.3)	-2.5 (0.4)
Source x Duration					
SULF-SHORT	-0.07 (0.03)	0.01 (0.03)	-0.8 (0.3)	-3.0 [y,z] (0.5)	-2.8 (0.7)
SULF-LONG	-0.06 (0.02)	0.02 (0.03)	-1.0 (0.3)	-2.6 [y,z] (0.4)	-2.5 (0.5)
4PLEX-SHORT	-0.09 (0.02)	0.04 (0.03)	-1.4 (0.3)	-3.5 [y] (0.4)	-3.4 (0.5)
4PLEX-LONG	-0.07 (0.03)	0.03 (0.03)	-1.1 (0.3)	-2.0 [z] (0.5)	-2.5 (0.6)
P-values					
TM Source (S)	0.63	0.61	0.30	0.90	0.64
Duration (D)	0.60	0.99	0.98	0.05	0.32
S x D	0.79	0.86	0.49	0.25	0.58

Values presented as lsmeans (SE). [2]Daily change in intrauterine luminal fluid score and endometrial edema score from 1 d post-foaling to day of ovulation. [3]Daily change in diameter (mm/d) of the uterine body, previously pregnant uterine horn, and previously non-pregnant uterine horn from 1 d post-foaling to day of ovulation. [y,z]Within a column, means with different superscripts differ (P<0.05).

Table 4-17. Effects of dietary trace mineral (TM) source and duration of supplementation on peak serum concentrations of LH and FSH and day of peak concentration relative to ovulation

	LH		FSH	
Variable	Peak, ng/mL	Day	Peak, ng/mL	Day
TM Source				
SULF *(n=15)*	74.4 (10.6)	0.2 (0.5)	157.2 (34.8)	-7.3 (1.3)
4PLEX *(n=13)*	75.5 (11.0)	0.2 (0.5)	195.1 (36.3)	-8.7 (1.4)
Duration				
SHORT *(n=13)*	74.6 (10.6)	0.6 (0.5)	140.4 (34.8)	-7.1 (1.3)
LONG *(n=15)*	75.2 (11.0)	- 0.2 (0.5)	211.9 (36.3)	-8.8 (1.4)
Source x Duration				
SULF-SHORT *(n=6)*	73.9 (15.4)	0.9 (0.7)	136.0 (50.9)	-8.3 (1.9) [y,z]
SULF-LONG *(n=9)*	74.9 (14.4)	- 0.4 (1.7)	178.4 (47.6)	-6.3 (1.8) [z]
4PLEX-SHORT *(n=7)*	75.4 (14.4)	0.4 (0.7)	144.8 (47.6)	-6.0 (1.8) [z]
4PLEX-LONG *(n=6)*	75.5 (16.6)	0.0 (0.8)	245.3 (55.0)	-11.3 (2.1) [y]
P-values				
TM Source (S)	0.94	0.94	0.45	0.47
Duration (D)	0.97	0.25	0.16	0.39
S x D	0.98	0.54	0.56	0.06

Values presented as lsmeans (SE). [y,z]Within a column, means with different superscripts differ (P<0.10).

CHAPTER 5
DISCUSSION

Passive Transfer of Immunity

The mammary gland of the mare is capable of selectively concentrating a wide range of antibodies into colostrum prior to foaling (Jeffcott, 1974, 1975). Immunoglobulin concentrations found in mare colostrum in the current study were similar to those observed by others (Pearson et al., 1984; Kohn et al., 1989; Spearman, 2004; Stelzleni, 2006). Using an inhibition ELISA with specific monoclonal antibodies against equine IgG and IgA subisotypes, Sheoran et al. (2000) reported that IgGb was the dominant isotype in pre-suckle colostrum of mares, followed by IgGa, IgG(T), IgA, and IgGc in order of descending concentration. Although the SRID assays used to determine Ig concentrations in the current study do not differentiate subclasses, the total Ig of colostrum consisted of 93.5% IgG, 5% IgA, and 1.5% IgM.

While there were no treatment differences observed for colostral IgG or IgM, mares supplemented with trace mineral amino acid complexes, regardless of duration of supplementation, had higher colostral IgA than mares supplemented with sulfate trace minerals. The IgA found in mammary secretions can originate from either humoral sources (i.e., serum) or can be produced locally by plasma cells located adjacent to the secretory epithelium (Larson et al., 1980). Tracer studies in sows have found that approximately 60% of IgA in colostrum is produced within the mammary gland, with the remaining colostrum IgA originating from serum (Bourne and Curtis, 1973). Although the proportion of colostral IgA that originates locally has not been studied in the mare, McGuire and Crawford (1972) found a greater mixture of IgA with differing molecular weights in the colostrum of a pony mare compared to that in milk, indicating a significant portion of IgA in colostrum was serum-derived, whereas IgA in milk was almost solely produced within the mammary gland. Serum-derived IgA appears predominantly as a

dimeric, non-secretory form in the horse (Porter, 1973; McGuire and Crawford, 1972). In contrast, locally-produced IgA contains secretory component, which is acquired during the transport of IgA across glandular and mucosal epithelia. Secretory component is derived from proteolytic cleavage of five Ig-like extracellular domains of the epithelial cell surface polymeric-Ig receptor, which is widely distributed in the epithelia lining of the digestive, respiratory and genital tracts, as well as in most exocrine glands, including the mammary gland (Kraehenbuhl and Neutra, 1992). Although not directly demonstrated in the horse, several cytokines and steroid hormones have been shown to regulate expression of this receptor in other animals and, thus, indirectly control the amount of IgA that is delivered into external secretions (Kraehenbuhl and Neutra, 1992). In the current study, supplementation with trace mineral amino acid complexes may have enabled greater quantities of IgA to be produced or, more likely, allowed greater quantities of IgA to be released into colostrum. Zinc is essential for the highly proliferating cells of the epithelium (Maggini et al., 2007); thus, adequate dietary zinc is needed to help support and maintain the integrity of the secretory epithelium of the mammary gland. Zinc is also required for protein synthesis, and manganese plays a role in cholesterol biogenesis and potentially steroid synthesis (Underwood, 1977). If the trace mineral amino acid complexes found in diet of 4PLEX mares were more bioavailable than sulfate trace minerals, they may have been better able to support the health of mammary tissue, as well as influenced polymeric-Ig receptor functioning, resulting in greater transepithelial transport of local and humoral IgA into colostrum.

The greater IgA levels in colostrum from mares supplemented with trace mineral amino acid complexes was directly reflected in their foals, whose serum IgA concentrations were greater than those from SULF mares. In the pig, there is selectivity against the absorption of secretory IgA in colostrum with preferential absorption of monomeric, non-secretory IgA

(Porter, 1973). In contrast, there appears to be no selectivity between secretory and non-secretory IgA in the calf, as the IgA profile of serum rapidly becomes similar to the colostrum ingested (Porter, 1973). Based on the low ratio between colostrum IgA and serum IgA in 1-d old foals, Sheoran et al. (2000) suggested uptake of secretory IgA in foals is minimal, similar to that observed in pigs. In the current study, the higher serum IgA concentrations observed in foals nursing 4PLEX mares, coupled with the higher IgA concentrations in colostrum suggests that the colostrum of 4PLEX mares may have contained greater quantities of non-secretory IgA that was capable of being absorbed by the foal. Secretory IgA secreted on epithelial surfaces throughout the body serves a role in the first line of defense against pathogens, in large part by preventing adherence of bacteria and viruses to epithelial surfaces (Widmann and Itatani, 1998). In contrast, the function of serum IgA, regardless of form, has been largely un-elucidated. Sheldrake et al. (1984) suggested that selective transepithelial transport of serum IgA occurs at a number of mucosal sites, but is dependent on secretory component availability. While the presence of secretory component in neonatal foals has been well documented, Sheoran et al. (2000) found no evidence of IgA in the nasal secretions of foals before 28 d of age. Alternatively, it has been suggested in many species that the hepatobiliary transport of serum IgA serves to reinforce the secretory IgA produced locally in the gastrointestinal tract (Sheldrake et al., 1984). In the current study, the greater supply of IgA in colostrum of mares fed trace mineral amino acid complexes may directly benefit the foal's first line of defense in the intestine. In addition, higher circulating IgA in the foal may function as a second line of defense or serve to further strengthen intestinal mucosal immunity.

Tetanus Antibody Titers

Several trace minerals are thought to play a role in antibody production; however, evidence for the benefits of organic trace minerals over inorganic sources has been somewhat

equivocal. Ferket and Qureshi (1992) reported greater antibody titers in young turkeys supplemented with amino acid complexes of zinc and manganese compared to supplementation with similar amounts of inorganic trace minerals. Dorton et al. (2003) supplemented feedlot steers with copper lysine or copper sulfate and observed an increased antibody response to vaccination with ovalbumin, but not in response to pig red blood cells. Spears et al. (1991) reported a trend for greater primary humoral immune response to vaccination with bovine herpes virus in newly received feedlot cattle fed zinc methionine compared to zinc oxide or no zinc supplementation; however, no differences in antibody titers were observed among the three zinc treatments in response to parainfluenza vaccination. Other studies in cattle have found no difference in humoral immune response between copper sulfate and copper lysine (Ward et al., 1993) or zinc oxide and zinc proteinate (Spears and Kegley, 2002). Only one study has investigated humoral immune response in horses supplemented with organic trace minerals. The investigators reported that weanlings fed zinc, manganese, and copper amino acid complexes had greater primary humoral immune response to a single injection of pig red blood cells compared to weanlings fed isoelemental amounts of these minerals in inorganic form (Siciliano et al. 2003). The lack of consistency between studies likely results from several factors, including the specific antigen used to elicit the humoral response, the animal's previous exposure to the antigen, the rate and duration of trace mineral supplementation, and the different organic and inorganic trace mineral sources fed.

In the current study, tetanus-specific IgG titers were highest at 1-2 d of age in all foals, indicating the uptake of maternal antibodies from colostrum, and progressively declined through 196 d of age, reflecting the waning of maternal antibodies. All foals, regardless of the mare's dietary treatment, failed to respond serologically to the primary vaccination given at 112 d of

age. Furthermore, this priming of the immune system had no effect on the response to the second vaccination given at 140 d of age, with the exception of foals from mares supplemented short-term with SULF. The ability of these foals, but not the others, to show a marginal seroconversion is not clear, although these foals did have the lowest titers at the time of the second vaccination. Subsequently, foals from all treatment groups failed to respond to the third vaccination. Similar results were found by Wilson et al. (2001), in which IgGa, IgGb and IgG(T) titers remained low in 3-mo old foals given a series of three vaccinations of tetanus toxoid. By comparison, a similar tetanus vaccination strategy initiated in foals at 6 mo of age resulted in the desired antibody response. The authors concluded that maternal antibodies, still present in 3-mo old foals, exerted an inhibitory effect on the response of foals to tetanus vaccination (Wilson et al., 2001). In the current study, foals still had significant tetanus titers at 4 mo of age when the first vaccination was administered; thus, the presence of maternal antibodies is likely the cause of the inability of the foals to seroconvert in response to vaccination. Traditionally, it has been recommended that vaccination of foals begin at 3 or 4 mo of age in order to complete the primary series before weaning (Ardans, 1982). But, as demonstrated by Wilson et al. (2001), as well as the current study, beginning vaccinations at this time may be ineffective in the face of antigen-specific maternal immunity. Unfortunately, the presence of maternal antibodies at the time of vaccination also prevented accurate assessment of the influence of trace mineral source fed to the mare on the humoral immune response in the foal.

Achieving greater seroconversion in response to the foal's primary series of vaccinations would be advantageous for farm herd health management. However, the powerful and relatively long-lasting influence of maternal antibodies, coupled with the fact that most foals are consuming significant quantities of solid food by 6 mo of age, suggests it may be more

appropriate to supplement foals directly with trace mineral amino acid complexes when evaluating their response to routine vaccinations. To assess primary and/or secondary humoral immune response in foals younger than 6 mo of age, a novel antigen, in which there are no preexisting maternal antibodies, would have to be used.

Neutrophil Function

In the current study, foal neutrophil function was similar to that observed in adult mares, demonstrating comparable phagocytic and oxidative burst activity throughout the study. These findings agree with Flaminio et al. (2000), who found no age-dependent maturation of phagocyte function in neutrophils isolated from foals.

Supplementation of trace minerals as amino acid complexes appeared to offer no advantage in support of neutrophil function over trace minerals supplied in sulfate form. In humans, copper deficiency results in neutropenia (Percival, 1995), and in animals has been shown to result in decreased phagocytic and killing function of neutrophils (Boyne and Arthur, 1981). However, Arthington et al. (1995) found no depression of neutrophil bactericidal function during copper depletion or repletion of heifers. In addition to copper, a deficiency in zinc has been shown to impair chemotaxis, phagocytosis, and generation of the oxidative burst by neutrophils in humans and primates (Allen et al., 1983; Keen and Gershwin, 1990). In the current study, supplemental trace mineral sources were supplied to mares in amounts to meet 100% of their zinc and manganese requirements and 150% of their copper requirements in late gestation and lactation. These minerals, coupled with those naturally-occurring in forage and oats, presumably met the requirements for normal neutrophil function in the mare. Further, because neutrophil function in foals was not different from mares or among treatments, it is likely that they too received adequate trace minerals *in utero*, from milk, and from the consumption of forage and their dam's feed to support neutrophil function.

Serum Trace Minerals

In the current study, umbilical cord serum zinc concentrations were greater than zinc concentrations observed in both mare and foal serum. However, mare and foal serum manganese values were similar to those manganese concentrations observed in umbilical cord serum. Umbilical cord serum copper was less than serum copper concentrations observed in the mare, and similar to serum concentrations observed in the foal at birth. Mare serum zinc and copper concentrations were relatively similar during late gestation and early lactation, whereas serum manganese decreased. In the foal, serum zinc and copper increased and serum manganese decreased from birth to 112 d of age. These findings differ from that of Ott and Asquith (1994), in which a decrease in serum copper and zinc was observed in foals from birth to 112 d of age. Okumura et al. (1998) reported an increase in foal serum copper until 1 mo of age, with little variation in serum zinc and manganese. Tawatsin et al. (2002) also observed an increase in serum copper concentrations in foals from birth to 25 wk of age. Bell et al. (1987) reported that during the first year of extrauterine life, concentrations of zinc in foal serum are variable and apparently unrelated to the age of the animal. However, copper and its major transport protein, ceruloplasmin, increased in parallel from birth to 28 d of age. The comparative differences found in postnatal development of serum trace minerals in foals is likely due to several factors, including placental transfer *in utero* and the resulting body stores of trace minerals at birth, as well as trace mineral intake from solid feeds made available to the foal in addition to milk.

The source of trace mineral supplied to the mare appeared to have no effect on serum zinc and manganese concentrations in either mares or their foals. However, while not different at birth, serum copper in foals belonging to mares supplemented with sulfate trace minerals was higher at 112 d age compared to foals nursing mares supplemented with trace mineral amino acid complexes. It is difficult to determine whether this was a result of supplementation of the mare

or the result of external factors that were not controlled for, such as greater forage or concentrate consumption by the foal. Increasing the zinc and copper concentration of the mare's diet above NRC requirements has been reported to have little effect on the content of the mare's milk (Baucus et al., 1987; Kavazis et al., 2002), and Ullrey et al. (1974) concluded that when trace mineral concentration in mare milk is compared to foal requirements, milk values are low. In contrast, there is some evidence that supplementation of the pregnant mare in late gestation may effectively increase fetal trace mineral stores, which may be of use after birth (Pearce et al., 1998). Therefore, placental transfer of trace minerals *in utero* and consumption of the dam's concentrate likely contributed significantly as sources of trace minerals available to the foal in the current study.

Although assessment of trace mineral status is routinely completed using blood measurements, especially in horses, the predictive relationship between tissue and blood levels and the presence of a deficient or non-deficient state is variable. Trace minerals are distributed into a number of different pools, including storage, transport, and biochemical function pools (Suttle, 1986). Thus, the serum levels in the current study most likely represent the transport pool of trace minerals.

Serum Vitamin B12

The only known function of dietary cobalt is as a component of vitamin B12 (cobalamin), which can be synthesized by microorganisms in the hindgut of the horse. Cobalt dependent vitamin B12, along with iron and copper, are involved in hematopoiesis (Ammerman, 1970). Vitamin B12 has a long biological half-life and is stored primarily in the liver of most animals (McDowell, 2000).

Mean serum cobalamin concentrations measured in mares and foals in the current study were similar to those observed by Roberts (1983). Roberts (1983) observed a decrease in serum

cobalamin in mares during gestation with a subsequent rise during lactation, which suggests possible transfer of vitamin B12 from mare to fetus *in utero*. Because only one sample was taken during gestation in the current study, it is not known if gestation affected vitamin B12 status similarly. Nonetheless, serum cobalamin concentration in the foal was highest at 1-2 d of age compared to 56 and 112 d of age, particularly in foals born to mares that had been supplemented with trace mineral for a longer duration. Therefore, it is reasonable to presume some placental transfer of vitamin B12 occurred in the current study. Although cobalamin concentrations were not measured in colostrum or milk, higher serum cobalamin in 1-2 d old foals could also represent uptake from early postpartum nursing. Kincaid et al. (2004) found that serum cobalamin reserves are depleted in high producing dairy cows in early to mid-lactation, despite the provision of cobalt well above the requirement, reflecting losses of vitamin B12 in milk. A decline in mare serum cobalamin from late gestation to early lactation was not observed in the current study, but cobalamin concentrations were significantly lower at these time points compared to late lactation. Milk production in the mare progressively declines after approximately 2 mo (NRC, 2007), which may help to conserve vitamin B12 in the mare.

In addition to changes that occurred over time, serum cobalamin in mares and foals was influenced by dietary trace mineral treatment. Foals born to mares that underwent long-term cobalt supplementation had higher serum cobalamin concentrations than foals from mares that underwent short-term supplementation, suggesting that long-term supplementation of the broodmare with cobalt may enhance the vitamin B12 status of the foal at birth. In addition, mares fed trace mineral amino acid complexes had greater serum cobalamin in late lactation than mares fed sulfate trace minerals. The combination of trace mineral source and duration of supplementation also appeared to affect serum cobalamin in the mare in late lactation. Serum

cobalamin was greater in mares receiving short-term 4PLEX supplementation compared to either short- or long-term SULF supplementation. Mares receiving long-term 4PLEX had greater serum cobalamin concentrations that short-term SULF, but intermediate between short-term 4PLEX and long-term SULF. Finally, mares receiving long-term SULF had considerably higher serum cobalamin than short-term SULF, although this difference was not found to be significant. Collectively, these findings suggest greater availability of cobalt glucoheptonate during repletion of serum vitamin B12 after the losses that occurred during gestation and lactation. Furthermore, these findings suggest that mares may benefit from long-term cobalt supplementation to better support placental transfer during pregnancy, as well as the supply of vitamin B12 in colostrum and milk. Although others have shown that horses respond to cobalt supplementation (above the NRC requirement) with an increase in serum cobalamin (Alexander and Davies, 1969), this is the first study describing a greater response with cobalt glucoheptonate compared to cobalt sulfate. Griffiths et al. (2007) reported higher serum cobalamin in lactating dairy cows supplemented with trace mineral amino acid complexes, including cobalt glucoheptonate, from 35 d prior to calving through 270 d of lactation. However, the control diet in their study contained no supplemental trace minerals; therefore, it is unclear whether the increase resulted from greater availability of cobalt glucoheptonate or a greater supply of cobalt in the diet.

Early Postpartum Reproductive Performance

The exact biological roles of trace minerals in reproduction are largely unknown. However, reports of compromised reproduction during dietary trace mineral deficiencies suggest their necessity for optimal reproductive performance. In copper deficient animals, estrous cycles and conception rates appear to remain unaffected, however, reproductive failure due to fetal death and resorption has been known to occur (Underwood, 1977). Copper is also necessary for the formation of connective tissue (McDowell, 2003), and as such would be necessary for proper

fetal development. Zinc is known to be centrally involved in cell division, suggesting its importance during fetal growth and during the physiologic events such as uterine involution that occur in the postpartum female. In addition, zinc is intimately associated with several hormones; for example, the steroid hormones androgen and estrogen function by binding to transcription factors that contain zinc fingers (McDowell, 2003). Zinc deficiency in the female has been shown to impair the synthesis and or secretion of FSH and LH, as well as cause abnormal ovarian development and disruption of the estrous cycle (Bedwal and Bahuguna, 1994). Doisey (1974) proposed that insufficient manganese interrupts the synthesis of cholesterol and its precursors, which would ultimately inhibit the synthesis of sex hormones, adversely affecting reproduction. Hostetler et al. (2003) hypothesized that manganese may play a role in the secretion of progesterone based on the findings by Hidirolgou and Shearer (1976) that the manganese concentration in the corpus luteum of ewes increased during early pregnancy, and that inadequate progesterone concentrations are known to cause early embryonic loss.

Reports addressing the effects of organic trace mineral supplementation on reproductive performance of mares are limited. Ott and Asquith (1994) observed a reduction in the number of cycles bred and services per mare when mares were provided proteinated vs. inorganic trace minerals, although the differences were not statistically significant. Similarly, Ley et al.(1990) found that barren mares supplemented with inorganic trace minerals experienced no first cycle pregnancies, two early embryonic losses, and higher number of services per 17-d conception compared to mares receiving chelated trace mineral supplementation; but again, these differences were not statistically different. Studies evaluating reproduction in response to organic trace mineral supplementation in other species of livestock are more numerous but not necessarily consistent. Boland et al. (1996) reported that dairy cattle receiving proteinated trace minerals had

a non-significant reduction in days to follicle deviation, and 5 fewer days to first ovulation. Manspeaker et al. (1987) found that dairy cattle supplemented with chelated trace minerals had increased ovarian activity, greater postpartum uterine involution of the pregnant horn, and less embryonic mortality, although results were not significantly different from cattle receiving no trace mineral supplementation. More recently, Siciliano-Jones et al. (2008) supplemented dairy cattle 3 wk prepartum through 35 wk postpartum with isoelemental amounts of trace minerals as sulfates or amino acid complexes and found no differences in days to first service, services per conception, or number of days open.

In the current study, mares that underwent short-term supplementation with trace mineral amino acid complexes exhibited a faster rate of uterine involution in the gravid horn, an earlier peak in FSH, developed a greater number of follicles, had a faster growth rate of the largest follicle, and fewer days to foal heat ovulation compared to mares supplemented long-term with amino acid complexes and mares supplemented short- or long-term with trace mineral sulfates. When bred on foal heat, a higher pregnancy rate has been observed in mares that ovulate 10 or more days after foaling compared to mares that ovulate on or before 10 days after foaling (Loy, 1980). Thus, enhancing follicular development and subsequent ovulation may not always be beneficial. The return of the endometrium to its pre-gravid morphological state is critical for support of the next pregnancy. On average, the equine embryo enters the uterus 5 days after ovulation. Therefore, ovulation 10 or more days after foaling ensures that the endometrium has returned to normal prior to arrival of the embryo (Arrott et al., 1994). However, if a more rapid rate of uterine involution occurs concomitantly with faster follicular development and ovulation, as occurred in short-term supplemented 4PLEX mares, the potential exists for the pregnancy to be successful.

Mares in the present study were managed as a commercial herd, which included breeding mares by artificial insemination (Quarter Horses and Standardbreds) or live cover (Thoroughbreds) to several different stallions, as well as strategic timing of breedings to yield a subsequent foal crop from mid-January through March in order to be economically competitive in an equine market. Because of these extraneous variables, conception rates, number of cycles per conception, and services per conception were not evaluated. Although follicle dynamics and uterine involution were marginally altered in mares supplemented with trace mineral amino acid complexes, conception should be considered the ultimate end-point. A synergy of many physiologic factors and events must take place in order for conception to occur. Many of the physiologic events that occur in preparation for early postpartum conception were enhanced by substituting trace mineral amino acid complexes in place of sulfate trace minerals in the diet. Therefore, further investigation is warranted to evaluate the impact trace mineral amino acid complexes have on mare conception rates, as well as early embryonic loss.

CHAPTER 6
IMPLICATIONS

The present study demonstrated that supplementing mares in late gestation with trace mineral amino acid complexes can influence immunoglobulin concentrations in mare colostrum and subsequently influence passive transfer of immunity in the nursing foal. Immunoglobulin A concentrations were greater in mares supplemented with trace mineral amino acid complexes compared to mares supplemented with trace mineral sulfates. This was further reflected in the foal, in which foals nursing mares supplemented with trace mineral amino acid complexes had greater serum IgA concentrations than foals nursing mares supplemented with trace mineral sulfates. Increased IgA levels may render the foal more immunologically competent by making greater amounts of the immunoglobulin available to act as both a second, and more importantly, a first line of defense in the neonatal foal. The presence of maternal antibodies precluded the evaluation of the effect of trace mineral supplementation of the mare on humoral immune response to routine tetanus vaccination in the foal. Further research is warranted to determine if supplementing the foal directly with organic trace minerals will elicit a greater response to routine vaccinations administered after 6 mo of age. Neutrophil function in mares and foals was not affected by dietary trace mineral treatment of the mare, suggesting that innate immunity was not limited by trace mineral supply. The current study suggests greater availability of cobalt glucoheptonate during repletion of serum vitamin B12 after the losses that occurred during gestation and lactation. Furthermore, these findings suggest that mares may benefit from long-term cobalt supplementation to better support placental transfer during pregnancy, as well as the supply of vitamin B12 in colostrum and milk. Finally, variable effects of trace mineral source and duration of supplementation were observed with respect to early postpartum reproductive performance. The ability to detect such effects may have been limited by the small sample size

resulting from the elimination of mares from statistical analysis for various reasons. Nonetheless, follicular development and uterine involution appeared to be marginally enhanced in mares receiving short-term supplementation with trace mineral amino acid complexes. Such physiological events may better prepare the mare for conception when breeding at foal heat. Additional research is needed to reevaluate the role that duration of supplementation plays in reproductive performance, as well as to determine if supplementation with trace mineral amino acid complexes influences conception rates in the mare.

APPENDIX A
PROCEDURE FOR ASSESSMENT OF EQUINE NEUTROPHIL FUNCTION

Neutrophil function was assessed in mare and foal whole blood as described by Vineyard et al. (2007). The neutrophil function assay procedure was as follows:

1. Label 3 tubes for each horse: negative control (DHR only), positive control (DHR + PMA), and SA (DHR + *Staphylcoccous aureus)*, or 6 tubes for duplicates.

2. Prepare 50 µM working solution of DHR from 500 µM stock solution (100 µL DHR stock + 900 µL PBS = 1000 µL of 50 µM DHR).

3. Add 100 µL of heparanized whole blood to each tube.

4. Add 10 µL of 50 µM DHR into all the tubes (final concentration/tube = 4 µM).

5. Incubate tubes at 37˚C for 10 min with constant rotation to load the DHR into the neutrophils.

6. Prepare 5 µg/L working solution of PMA from 1 mg/mL stock solution (5 µL PMA stock + 995 µL PBS = 1000 µL 5 µg/mL PMA solution). Store working solution on ice pending use.

7. Add 10 µL of the PMA working solution to the positive control. The final concentration of PMA per tube = 50 ng.

8. Add the appropriate amount of bacterial suspension (10^6 cells/µL) to the SA tubes for a bacterial:neutrophil ration of 30:1.

9. Incubate all tubes at 37˚C for 30 min with constant rotation.

10. Immediately place tubes on ice to stop phagocytosis and oxidative burst activity.

11. Process the tubes for flow cytometry using the automated Q-Prep Epics immunology workstation set on the 35-second cycle (600 µL reagent A, 265 µL reagent B, 100 µL reagent C).

12. Add 500 µL of cold distilled water to each tube for completion of hemolysis.

13. Add 10 µL of 0.4% trypan blue to each tube to quench extracellular fluorescence.

DHR working stock: To make 500 µM stock solution, add 11.5 mL DMSO to 2 mg DHR. To make 500 µM working solution, add 100 µL DHR stock to 900 µL PBS.

Q-Prep Reagents:

Reagent A

Formic Acid:	1.2 mL/1000 mL de-ionized water

Reagent B

Sodium carbonate:	6.0 g/1000 mL de-ionized water
Sodium chloride:	14.5 g/1000 mL de-ionized water
Sodium sulfate:	31.3 g/1000 mL de-ionized water

Reagent C

1% Paraformaldehyde:	10.0 g/1000 mL PBS

Preparation of Bacterial Targets:

1. Obtain a preparation of *Staphylcoccus aureus* bacteria grown in tryptic soy broth at final volume of 10 mL and final concentration of $5x10^9$ cells/mL.

2. Kill the bacteria by heating the broth culture at 56˚C for 30-60 min. Harvest the heat-killed bacteria by centrifugation at 2000 rpm for 15 min.

3. Decant the supernatant and re-suspend the bacterial pellet in 10 mL sterile PBS. Vortex. Centrifuge at 2000 rpm for 15 min.

4. Decant the supernatant and re-suspend the bacterial pellet in 10 mL of 300 µg/mL PI solution (7mL PBS + 3 mL PI stock solution (1 mg/mL)).

5. Cover tube in aluminum foil to protect against light and mix by continuous rotation at 23˚C for at least 12 h.

6. Harvest the PI-labeled bacteria by centrifugation at 2000 rpm for 15 min. Decant the supernatant and re-suspend the bacteria in 10 mL sterile PBS.

Flow Cytometry Settings:

Cytometer Type: FACSort

Detectors/Amps:

Param	Detector	Voltage	AmpGain	Mode
P1	FSC	E00	1.20	Lin
P2	SSC	370	1.00	Lin
P3	FL1	400	1.00	Log
P4	FL2	485	1.00	Log
P5	FL3	412	1.00	Log
P6	FL2-A		1.00	Lin
P7	FL2-W		1.00	Lin

Threshold:
Parameter: FSC
Value: 120

Compensation:
FL1 – 3.60 % FL2
FL2 – 99.9 % FL1
FL2 – 0.00 % FL3
FL3 – 0.00 % FL2

APPENDIX B
PROCEDURE FOR SERUM MANGANESE ANALYSIS

Equipment: AAnalyst 800 Atomic Absorption Spectrometer with THGA Graphite Furnace and AS-800 Autosampler (PerkinElmer, Inc., Shelton, CT).

The THGA is a transversely-heated graphite furnace for electrothermal atomization in atomic absorption spectrometry. The furnace incorporates the electromagnet required for the application of the Zeeman effect background correction.

Standard Preparation: Stock manganese solution of 1000 ppm (1g/L) and deionized water was used to prepare two intermediate solutions of 10 µg/mL and 100 µg/L. The two intermediate solutions were used to prepare the 20 µg/L working standard in a diluent of 8 mL Triton® X-100 and 5 g of sodium EDTA per liter of deionized water. From this working standard another standard of 5 µg/L was also prepared in diluent.

Sample Preparation: 0.5 mL serum diluted with 0.5 mL of diluent. A 20 µL injection volume was used for AA analysis. Automatic recovery of spiked samples was programmed for every 10 samples. The furnace program was modified to include a special gas type (**air)** for steps preceding atomization step.

Method:

Spectrometer

Element:	Mn
Wavelength (nm):	279.5
Slit width (nm):	0.2L

Signal

Type:	AA-BG (Zeeman)

Furnace Program

Step	˚C	Ramp Time	Hold Time	Internal Flow	Gas Type
1	110	2	30	250	Air
2	150	5	20	250	Air
3	300	15	15	250	Air
4	950	10	30	250	Argon
5	1900	0	3	0	Argon
6	2600	2	2	250	Argon

APPENDIX C
PROCEDURE FOR SERUM COPPER AND ZINC ANALYSIS

Equipment: AAnalyst 800 Flame Atomic Absorption Spectrometer and AS-800 Autosampler (PerkinElmer, Inc., Shelton, CT).

Standard Preparation:

Copper:

Automatic Zero (AZ)
Standard contained 50 mL serum matrix, 20 mL 50% glycerol, and 0 mL 100 ppm Cu stock.

Standard (S1)
Standard contained 50 mL serum matrix, 20 mL 50% glycerol, and 5 mL 100 ppm Cu stock.

Zinc:

AZ
Standard contained 50 mL serum matrix, 20 mL 50% glycerol, and 0 mL 100 ppm Zn stock.

S1
Standard contained 50 mL serum matrix, 20 mL 50% glycerol, and 1 mL 100 ppm Zn stock.

All standards brought to final 100 mL total volume with deionized water. Standards consisted of 50 % glycerol so that their viscosity matched that of the serum.

Sample Preparation: Serum samples were prepared using a 1:1dilution with deionized water for the determination of Cu and Zn.

Method:

Spectrometer

Element:	Cu	Zn
Wavelength (nm):	324.8	213.9

APPENDIX D
PROCEDURE FOR IMMUNOGLOBULIN ANALYSIS

Immunoglobulin (Ig) G, A, and M were analyzed using a single radial immunodiffusion (SRID) kit (VMRD, Inc., Pullman, WA). Detection ranges were 200–1600, 31–250, and 25-200 mg/dL for IgG, IgA, and IgM, respectively. The procedure used for IgG, A, and M was as follows:

1. Samples were allowed to come to room temperature and vortexed thoroughly.

2. Samples with high expected Ig values were diluted with deionized water to ensure that readings were within measurable levels. Dilution ratios (sample:deionized water) were as follows

   IgG
   Colostrum: 1:25
   Foal serum (24 h): 1:5
   Foal serum (36 h): 1:5

   IgA
   Colostrum: 1:8
   Foal serum (24 h): No dilution
   Foal serum (36 h): No dilution

   IgM
   Colostrum: 1:4
   Foal serum (24 h): No dilution
   Foal serum (36 h): No dilution

3. Three µL of Standards A through D for each Ig were pipetted into wells 1 through 4 of plate 1. The pipette was lifted off the bottom of the well simultaneously as the plunger of the pipette was depressed to ensure excess sample did not overflow the well.

4. Three µL of each sample to be tested was pipetted into the remaining wells of all plates using the method discussed above. Sample identification specifying well number and dilution rates of each sample were recorded.

5. Plate covers were reattached and plates were left undisturbed, and incubated rightside up at room temperature for 18 – 24 h outside of their mylar pouches.

6. After the incubation period, ring diameter was recorded, with measurements taken in mm using a monocular comparator (VMRD, Inc., Pullman, WA). Used plates were inverted and placed back into their mylar pouches and stored at 4-8˚C for possible further reference.

7. Immunoglobulin concentrations were determined by entering standard and sample diameters along with the appropriate sample dilutions into the computer program MetraFIT (Metra Biosystems, Inc., Mountain View, CA). Concentrations were determined using a standard model equation provided by the program.

Standards:

IgG	**IgA**	**IgM**
Standard A: 200 mg/dL	Standard A: 31 mg/dL	Standard A: 25 mg/dL
Standard B: 400 mg/dL	Standard B: 62 mg/dL	Standard B: 50 mg/dL
Standard C: 800 mg/dL	Standard C: 125 mg/dL	Standard C: 100 mg/dL
Standard D: 1600 mg/dL	Standard D: 250 mg/dL	Standard D: 200 mg/dL

APPENDIX E
MARE AND FOAL SERUM FOLATE

Table E-1. Effects of dietary trace mineral (TM) source and duration of supplementation on serum folate concentrations in the mare 56 d before and 56 and 112 d after foaling

	Mare serum folate, ng/L		
Variable	(-) 56 d	56 d	112 d
TM Source			
SULF	8.05	7.80^{x}	7.42
	(0.40)	(0.40)	(0.40)
4PLEX	7.97^{a}	$9.24^{b,y}$	$7.03^{a,b}$
	(0.40)	(0.40)	(0.40)
Duration			
SHORT	8.09^{a}	8.93^{b}	7.11^{c}
	(0.40)	(0.40)	(0.40)
LONG	$7.93^{a,b}$	8.11^{a}	7.35^{b}
	(0.40)	(0.40)	(0.40)
Source x Duration			
SULF-SHORT	7.91	7.74^{z}	7.17
	(0.57)	(0.57)	(0.57)
SULF-LONG	8.18	7.86^{z}	7.67
	(0.57)	(0.57)	(0.57)
4PLEX-SHORT	$8.29^{a,\dagger}$	$10.12^{b,y}$	$7.04^{a,c,\dagger}$
	(0.57)	(0.57)	(0.57)
4PLEX-LONG	7.67^{a}	$8.37^{b,x,z}$	$7.02^{a,c}$
	(0.57)	(0.57)	(0.57)
P-values			
TM Source (S)		0.47	
Duration (D)		0.58	
S x D		0.23	
Time		0.0001	
S x Time		0.001	
D x Time		0.13	
S x D x Time		0.41	

Values presented as lsmeans (SE). ^{a,b,c}Within a row, means with different superscripts differ (P<0.05). †Within a row, means differ (P<0.10). ^{x,y,z}Within a column, means with different superscripts differ (P<0.05).

Table E-2. Effects of dietary trace mineral (TM) source and duration of supplementation in the mare on serum folate concentrations in the foal at 1-2, 56, and 112 d of age

	Foal serum folate, ng/L		
Variable	1-2 d	56 d	112 d
TM Source			
SULF	6.75[a]	5.02[x,b]	5.63[a,b]
	(0.50)	(0.52)	(0.52)
4PLEX	5.79	6.74[y]	6.32
	(0.50)	(0.50)	(0.55)
Duration			
SHORT	6.70	5.80	6.08
	(0.49)	(0.49)	(0.49)
LONG	5.84	5.97	5.87
	(0.54)	(0.54)	(0.58)
Source x Duration			
SULF-SHORT	6.05[x,y,z,‡]	4.46[y, ‡]	5.23[‡]
	(0.69)	(0.69)	(0.69)
SULF-LONG	7.45[x,z,†]	5.59[x,y,†]	6.03
	(0.78)	(0.78)	(0.78)
4PLEX-SHORT	7.34[x,z]	7.13[x]	6.93[‡]
	(0.69)	(0.69)	(0.69)
4PLEX-LONG	4.24[y, ‡]	6.35[x,y, ‡]	5.74
	(0.73)	(0.73)	(0.73)
P-values			
TM Source (S)		0.28	
Duration (D)		0.51	
S x D		0.003	
Time		0.72	
S x Time		0.03	
D x Time		0.58	
S x D x Time		0.34	

Values presented as lsmeans (SE). [a,b]Within a row, means with different superscripts differ (P<0.05).[†]Within a row, means differ (P<0.10). [x,y,z]Within a column, means with different superscripts differ (P<0.05). [‡]Within a column, means differ (P<0.10).

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BIOGRAPHICAL SKETCH

Jerome "J.G." George Vickers IV was born in Houston, Texas along with his twin brother. In a childhood most aptly described as nomadic, horses seemed to be the one constant, and his passion for the equine industry ensued.

J.G. graduated third in his class from Liberty County High School in 2001, and attended Chipola College in Marianna, FL shortly thereafter. He then moved to Williston, FL to work at his parents' farm, and earned his Associate of Arts degree from Santa Fe Community College in Gainesville, FL. He enrolled in the University of Florida, declaring animal sciences as a major. While in school, he discovered in himself a great passion for science in general, and for equine nutrition in particular. The chance to complete a Master of Science in equine nutrition with Dr. Lori K. Warren presented itself just prior to the completion of his B.S.

During his tenure as a graduate student, J.G. worked as a teaching assistant for multiple equine science courses. He also served as Vice President to the Animal Sciences Graduate Student Association during the 2007 and 2008 terms. During this time, he was also a representative for the College of Agricultural and Life Sciences Ag Council, giving insight and instruction on issues facing the agricultural industry. Upon graduation, J.G. will continue with the pursuit of his passion, and enter into the equine feed industry, with the possibility of furthering his education one day in the future.