TRACE MINERALS ON THE IMMUNE RESPONSE FOLLOWING VACCINATION AGAINST BOVINE RESPIRATORY DISEASE

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ABSTRACT

Bovine respiratory disease (BRD) has a major impact on the profitability of the cattle industries in North America, resulting in substantial economic losses. Effective transfer of maternal antibodies in conjunction with appropriate biosecurity measures and vaccination programs are essential to prevent and control BRD. Trace minerals (TM) are crucial for the development of an adequate immune response in cattle, especially in stressed animals. Field and experimental studies focused on improving the TM status of stressed feeder calves have shown positive effects of administration of TM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits. Our studies revealed that administration of injectable TM (Se, Zn, Cu and Mn) concurrently with modified-live virus vaccination in dairy calves resulted in earlier and increased antibody titer and leukocyte proliferation upon stimulation with BRD viruses compared to the control group. Additionally, TM provided additional beneficial effects when administered concurrently with respiratory MLV vaccine: 1. Injectable TM increased average daily gain during the 14 day post- BVDV challenge period. 2. Clinical scores were lower for TM compared to saline-injected group on days 5 and 8 post challenge. 3. Administration of TM prevented BVDV-induced thrombocytopenia compared to saline-injected calves and positive control group, and 4. TM enhanced protection from BVDV-2 infection by MLV vaccine, helping to maintain adequate health and performance of growing calves. Addition of injectable TM supplements to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

THE IMMUNE SYSTEM IN CATTLE

Innate Immunity

The innate immune system represents the first line of defense against a pathogen before the adaptive system can develop the appropriate response. The innate immunity is not antigen specific and does not include anamnestic or memory response. Components of nonspecific immunity are present both prior to and following antigen exposure and they do not discriminate against most foreign substances. These components include natural barriers (skin, epithelial lining, anti-microbial substances, enzymes, etc.), complement proteins, and white blood cells (granulocytes, macrophages) that engulf and eliminate pathogens (phagocytosis and killing).

Further, the innate immunity also includes type I interferon (IFN) antiviral response and natural killer cells (NKC) which are lymphocytes able to destroy infected cells. Granulocytes are also called polymorphonuclear leukocytes (PMN), mainly neutrophils. The main function of neutrophils is to monitor for infection to perform phagocytosis and killing of pathogens. Neutrophils identify pathogens by the recognition of pathogen-associated molecular patterns (PAMPs) using specific receptors, called Toll-like receptors. Microbes have molecules not typically found in mammalian cells (e.g. double-stranded RNA, CpG DNA sequences, and unusual carbohydrate residues), so that neutrophils are able to detect these molecules to eliminate the infectious agents. After binding of PAMPs to Toll-like receptors, neutrophils activate and initiate the phagocytosis and killing. Microbial destruction by phagocytosis involves the production of reactive oxygen species (ROS) which are bactericidal.

Adaptive Immunity

The adaptive or acquired immunity has the capacity to recognize specific antigens and has memory. The primary components of the adaptive immune system involve humoral (antibody production by B lymphocytes) and cell mediated immunity (developed mainly by CD4$^+$ helper T cells, CD8$^+$ cytotoxic T cells and WC1$^+$ γδ T cells).

B-cells mature in bone marrow and are released into blood, where they circulate and populate lymphoid tissues. These cells also act as antigen presenting cells (APC) so that they can recognize antigens and present them to helper T cells, which enhance further antibody production. B lymphocytes activate and undergo proliferation and differentiation, a process termed "clonal expansion". Each clone of B lymphocytes can recognize a specific target antigen. Production of antibodies by B lymphocytes following interaction with the antigen requires input of interleukins-2 (IL-2), IL-4 and IFN-γ. These cytokines also cause formation of B memory cells from the activated B-cell population. Antibodies are Y-shaped proteins, containing a constant (C) region and a variable (V) region. The V region contains the antigen binding site. The constant region is assembled from distinct genes that yield different antibody isotopes, such as IgG, IgM, IgD, IgA and IgE. Each of these isotopes has different physical properties and specific roles in the animal's immune system. The roles of antibodies in adaptive immune response include: neutralization or microbe and toxins, opsonization and phagocytosis, antibody-dependent cellular cytotoxicity, and complement activation.

T-cells are produced in the bone marrow and mature in the Thymus (hence the name "T"-cell). T-cells are responsible for "cell-mediated immunity". Similar to B cells, clonal expansion and differentiation of T-cells results in the development of effector and memory T-cells. During cell-mediated immunity, T lymphocytes recruit and stimulate phagocytic (nonspecific) activity as well as participate in direct lysis of infected cells (e.g. viral infected cells). Helper T-lymphocytes (CD4$^+$) orchestrate the adaptive immune response through the production of cytokines and costimulatory molecules, while cytotoxic T-cells (CD8$^+$) have the capacity to destroy infected cells (e.g. during virus infection).

VACCINATION TO PREVENT BOVINE RESPIRATORY DISEASE IN DAIRY CALVES

Bovine respiratory disease (BRD) has a major impact on the profitability of the dairy and beef industries in North America, resulting in substantial economic losses that exceed $1 billion annually (Griffin, 1997; McVey, 2009). The infectious agents most consistently implicated in BRD include *Bovine viral diarrhea virus* (BVDV), *Bovine herpes virus 1* (BHV1), *Bovine respiratory syncytial virus* (BRSV), *Parainfluenza 3 virus* (PI3V), *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma bovis*. Appropriate biosecurity measures and vaccination program are crucial to prevent and control BRD.

Vaccination against BVDV1, BVDV2, BoHV-1, BRSV, and PI3V is considered a key management strategy to minimize mortality and economic losses associated with BRDC in weaned calves. However, vaccines are not 100% effective. Several factors affect the immune response and protection elicited by vaccination in dairy calves, including nutrition, stress, climate, presence of maternal antibodies, vaccination route, vaccine handling.

There are two types of commercially available respiratory vaccines in the USA: modified-live virus (MLV) and inactivated. These two types of vaccines have advantages and disadvantages. The major advantage of MLV vaccines, in general, is activation of all phases of the immune system yielding a balanced systemic and local immune response, and a balanced humoral and cell-mediated response. Modified-live virus vaccines contain limited antigen mass, requiring viral replication in the host to develop optimal immunity. During the replication cycle, a live virus could recombine or mutate and occasionally revert to virulence and be shed to other susceptible individuals, resulting in clinical consequences. A further disadvantage of MLV vaccines is the immunosuppressive effect on leukocyte function by BVDV, resulting in increased susceptibility to other infections. Furthermore, MLV vaccines have the potential risk for contamination with adventitious virulent strains becoming a source of spread of BVDV infections. On the other hand, the major advantages of inactivated vaccines are that they are not either immunosuppressive nor fetopathogenic. Disadvantages of inactivated BVDV vaccines include a weaker neutralizing antibody response and shorter duration of protection (which indicates a need for increased frequency of administration). Also, inactivated vaccines may have a disadvantage in relation to modified-live vaccine with respect to limited cell mediated immune response, which plays an important role in recovery from and resistance to disease.

Before developing a vaccination program, it is important to develop a risk assessment in order to know which vaccines will be needed. This risk determination will be based on the presence of certain pathogens (and their infection pressure) and the level of passive immunity in the group of calves.

The vaccination program of the dairy calf will depend mostly on the management practices as well as the quantity and quality of colostrum consumed during the first hours of life. Neonatal dairy calves, are separated from the dam at birth or shortly later. Thus, titers of maternally derived antibody varies widely depending on the quality and

quantity of the colostrum consumed, as well as the efficiency of immunoglobulins' intestinal absorption. Chamorro et al., 2014 reported high variability in the level of neutralizing antibodies in young dairy calves; with a high proportion of calves having low titers to BVDV, BoHV1, PI3 and BRSV, which represents a high risk for developing respiratory disease. This suggests that a high percentage of neonatal dairy calves have failure of immunoglobulin transfer. Therefore dairy calves should be vaccinated during the first 3 months of age. Moreover, the newborn calf has a functional immune system that is able to respond to antigens, provided maternal antibody is not present. Therefore, if calves are at high risk of digestive and respiratory disease due failure of passive transfer, priming vaccination during the first weeks of life to develop humoral and cellular protection is required.

Some calves might have moderate to high level of maternal antibodies, which may interfere with the immune response after SQ or IM vaccination. One strategy to overcome the presence of maternal antibody in these animals is to use intranasal vaccines (IN), which will result in the development of antibodies on the respiratory mucosa (i.e. immunoglobulin A; IgA). This local immune response has shown to elicit protection against BRD, since most respiratory pathogens gain entry though this route. Mucosal antibody response neutralize infectious agents at the respiratory mucosa; thus preventing infection. Furthermore, IN vaccines also induce interferon production at the mucosal surface, which will provide a nonspecific antiviral environment and may stimulate maturation of the immune system.

A common protocol used in young dairy calves include a priming dose of IN vaccination containing BoHV1, PI3 and BRSV accompanied by a booster of SQ MLV vaccine containing BVDV, BoHV1, PI3, and BRSV 60-90 days after priming vaccination. In these programs, calves may be vaccinated several times during the first 6 months of life.

One of the goals of vaccinating adult dairy cows is to achieve an adequate level of protection and induce increased level of antibodies in colostrum. However, cows in a dairy herd are normally at various reproductive stages. This limits selection of vaccines to those that are safe to use in pregnant animals. Healthy replacement heifers should be vaccinated at least twice with MLV vaccine before breeding. These animals need to be isolated from pregnant cows during and after vaccination. Vaccination should be scheduled so that maximal protection is achieved during the critical first 4 months of gestation to maximize the potential for adequate duration of immunity and enhance protection against fetal infection. Replacement dairy heifers are commonly vaccinated with MLV vaccines at 3 months of age (when there is a significant decay in antibody titers), at 6 months of age and 30-60 days before breeding. If inactivated vaccines are administered, vaccination of heifers before breeding should be timed so that maximal responses are afforded by first 4 months of gestation to maximize protection against fetal infection. Thus, the second booster should be given 2-4 weeks before breeding. Cows should be revaccinated annually, 4 weeks before breeding.

ROLE OF TRACE MINERALS IN THE IMMUNE RESPONSE

Nutritional status, and particularly mineral levels, have been demonstrated to impact cattle health and performance (Enjalbert et al., 2006; Galyean et al., 1999; Underwood and Suttle, 1999). Trace minerals such as Zinc (Zn), Manganese (Mn), Copper (Cu), and Selenium (Se) are important for optimal immune function (Chirase et al., 1991; Percival, 1998; Underwood and Suttle, 1999) and growth (Spears and Kegley, 2002) in cattle, particularly in highly stressed, and newly received feeder calves (Duff and Galyean, 2007). Zinc contributes with the structure and function of more than 2,500 enzyme systems involved in metabolism (Andreini et al., 2009; Cousins and King, 2004). Zinc activates the enzyme superoxide dismutase, which plays a crucial role in stabilizing cell membranes against reactive oxygen species (ROS) (Bonaventura et al., 2015; Haase and Rink, 2014). Zinc is involved in DNA replication through the actions of ribonucleotide reductase, and is necessary for lymphocytes proliferation and differentiation. Zinc's major roles in the immune response involve signaling and adhesion of neutrophils and macrophages (Bonaventura et al., 2015), production of pro-inflammatory cytokines by monocytes (Rink and Kirchner, 2000), regulation of IL-2 secretion, signal transduction for T cell activation, clonal expansion, differentiation and T_H cells polarization (Haase and Rink, 2014), B-cell function, and antibody production (Pinna et al., 2002; Tomlinson et al., 2008).

Copper is important in the mitochondrial metabolic cascades for energy production to supply different organs, including those of the immune system (Failla, 2003). Copper also plays a role in superoxide dismutase activity and neutralization of ROS (Maggini et al., 2007), and contributes to the process of phagocyte killing (Linder, 1991). Ceruloplasmin is a copper-containing enzyme whose production increases dramatically during inflammation in response to the necessity of scavenging oxygen radicals released by immune cells (Percival, 1998). In rodents, copper deficiency is associated with decreased IL-2 production, lymphocyte proliferation and T cells counts (Bala and Failla, 1993; Bonham et al., 2002; Klotz et al., 2003; Linder and Hazegh-Azam, 1996; Minatel and Carfagnini, 2000; O'Dell, 1993; Pan and Loo, 2000; Percival, 1998). Similarly, studies in cattle fed a copper-deficient diet showed a significant reduction in B-lymphocytes and impaired neutrophil activity (Cerone et al., 1998).

Selenium appears to be very important to the migration of neutrophils into tissues and subsequent inflammation (Maddox et al., 1999). Selenium is a component of the enzyme glutathione peroxidase that inactivates ROS production and prevents released ROS from causing cellular damage (Maddox et al., 1999; Neve, 1991). Selenium deficiencies have been associated with depressed neutrophil migration and killing ability, and reduced B-cell response and antibody production. Moreover, Se supplementation enhanced both humoral and cell-mediated and immune responses (Maggini et al., 2007). The level of Se in tissues and blood affected the total IgM levels and BHV1-specific antibody titers after challenge (Reffett et al., 1988). Evidence that Mn plays a role in the immune system is limited. However, Mn has an essential function in removing ROS produced by active phagocytic cells (Tomlinson et al., 2008).

EFFECT OF INJECTABLE TRACE MINERALS (ITM) ON THE PROTECTION ELICITED BY VACCINATION AGAINST BOVINE RESPIRATORY DISEASE (BRD).

Arthington and Havenga (2012) assessed the effect of administration of ITM on the humoral immune response after BRD specific MLV vaccination in cattle. That study demonstrated that ITM given concurrently with viral vaccination enhanced the production of neutralizing antibodies to BHV1 in beef calves. Additionally, recent studies have shown that treatment with ITM concomitantly with MLV vaccination induced a faster BVDV-specific antibody response in newly received, highly stressed calves (Roberts et al., 2015).

A growing body of evidence suggests that both humoral and cell mediated immune (CMI) responses are critical in protection against viral agents involved in BRD (Collen and Morrison, 2000; Howard 1990; Nobiron et al., 2003). A more complete evaluation of the immune responses induced by MLV vaccination requires the use of methods to assess both humoral (antibody response) and cellular effector mechanisms (recall antigen induced proliferation and induction of IFN-γ as the core Th1 cytokine).

A study was performed at University of Georgia to evaluate the effect of an injectable trace mineral (ITM) supplement containing zinc, manganese, selenium, and copper on the humoral and CMI responses to vaccine antigens in dairy calves receiving a modified-live viral (MLV) vaccine containing BVDV, BHV1, PI3V and BRSV (Palomares et al., 2016). A total of 30 dairy calves (3.5 months of age) were administered a priming dose of the MLV vaccine containing BHV1, BVDV1 & 2, BRSV, PI3V, and an attenuated-live *Mannheimia-Pasteurella* bacterin subcutaneously (SQ). Calves were randomly assigned to 1 of 2 groups: (1) administration of ITM SQ (ITM, Multimin 90® Multimin USA®, n = 15) or (2) injection of sterile saline SQ (Control; n = 15). Three weeks later, calves received a booster of the same vaccine combination SQ, and a second administration of ITM, or sterile saline, according to the treatment group.

Throughout the study, the calves grazed Bermuda grass (*Cynodon dactylon*) and Fescue grass (*Festuca arundinacea*) with no access to mineral supplementation. Animals had access to hay (Bermuda grass and Fescue grass) and water *ad libitum*. Additionally, calves received 2.7 kg/head/day of concentrate supplement (Bulk Cattleman's Special; Godfrey's Warehouse; Madison-GA; Palomares et al., 2016) divided into two meals.

In this study, calves had adequate liver tissue concentration of all the study trace minerals assessed at all sampling dates according to standard reference values (Herdt and Hoff, 2011). Administration of ITM resulted in increased concentrations of liver Se (on days 21 and 56), Cu (on day 56) and Mn (on day 56) compared to saline injected calves (Palomares et al., 2016). Administration of ITM concurrently with MLV vaccination resulted in higher antibody titers to BVDV1 on day 28 after priming vaccination compared to the control group ($P = 0.03$). This higher BVDV1 specific humoral immune response was associated with both higher production of antibody (Fig.

1A) and a numerical tendency of greater proportion of sero-conversion (≥ 4-fold increase compared to day 0) to BVDV1 on day 28 in the ITM group (12/15; 80.0%) compared to the control group (8/15; 53.3%; *P* = 0.13; Fig. 1B).
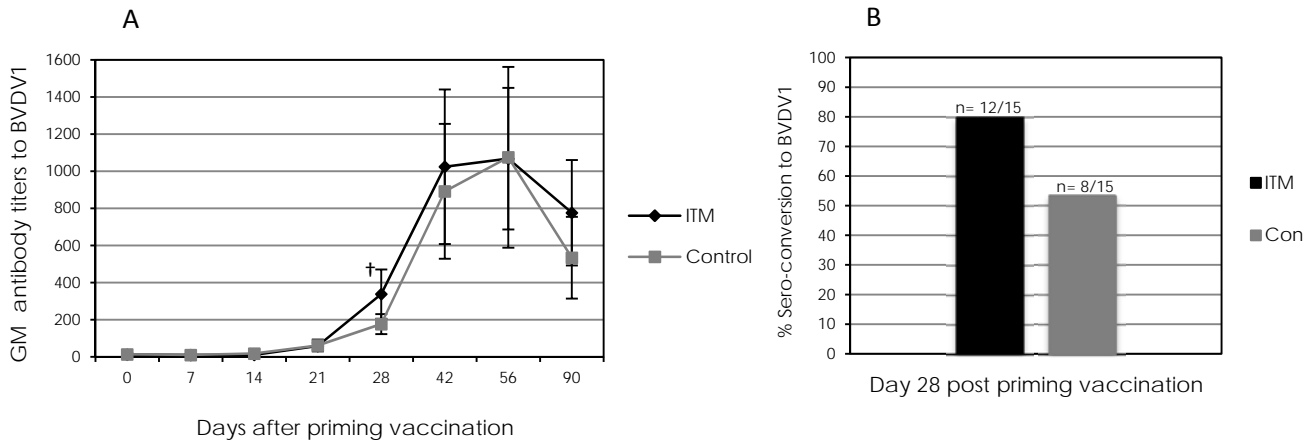

Figure 1. Geometric means serum neutralizing (SN) antibody titers to BVDV1 (A), and proportion of sero-conversion or ≥4-fold increase in SN antibody titer to BVDV1 on day 28 relative to the day of priming vaccination (B) in dairy calves that received injectable trace minerals (ITM) or saline (Control) concurrently with a MLV vaccine. [†]Significant differences between groups for each day (P = 0.03).

This quicker BVDV-specific antibody response elicited by an MLV vaccine delivered concurrently with ITM might be of significant value by conferring earlier protection following vaccination. This could be particularly important in newly received, highly stressed cattle that are at high risk of respiratory viral infections. A rapid increase in serum neutralizing antibodies against BVDV after vaccination may be beneficial to prevent infection and disease development.

The antibodies may neutralize extracellular virus particles, inhibiting attachment of the virus to host cells, and contribute to antibody dependent cell mediated cytotoxicity (Forthal, 2014). Similarly, previous reports have shown that ITM enhanced humoral immune response to pathogens of clinical significance in cattle production, including BVDV (Roberts et al., 2015), BHV-1 (Arthington and Havenga 2012), *E. coli* (Panousis et al., 2001) and *Pasteurella haemolytica* (Droke and Loerch, 1989). In the study performed by Arthington and Havenga (2012), administration of trace minerals concurrently with an MLV vaccine to steers induced a significant increase in BHV-1 serum neutralizing antibody titers on days 14, 30, and 60 post-vaccination compared to the base line titers on day 0 and to the titers in the saline injected steers.

Calves treated with ITM showed an earlier enhancement in mononuclear leukocyte proliferation to BVDV1 following vaccination compared to the control group

(peak of leukocyte proliferation occurred 14 days later). Proliferation of mononuclear leukocytes after BVDV stimulation tended to be higher on day 14 after priming vaccination in calves treated with ITM than in the control group (P = 0.08; Fig. 2A). Calves that received ITM showed higher mononuclear leukocyte proliferation to BRSV stimulation on day 7 after priming vaccination compared to the control group (P = 0.01; Fig. 2B). Moreover, calves in the ITM group also had an enhanced production IFN-γ by PBMC after stimulation with BRSV on day 21 after priming vaccination compared to day 0 (P < 0.01).
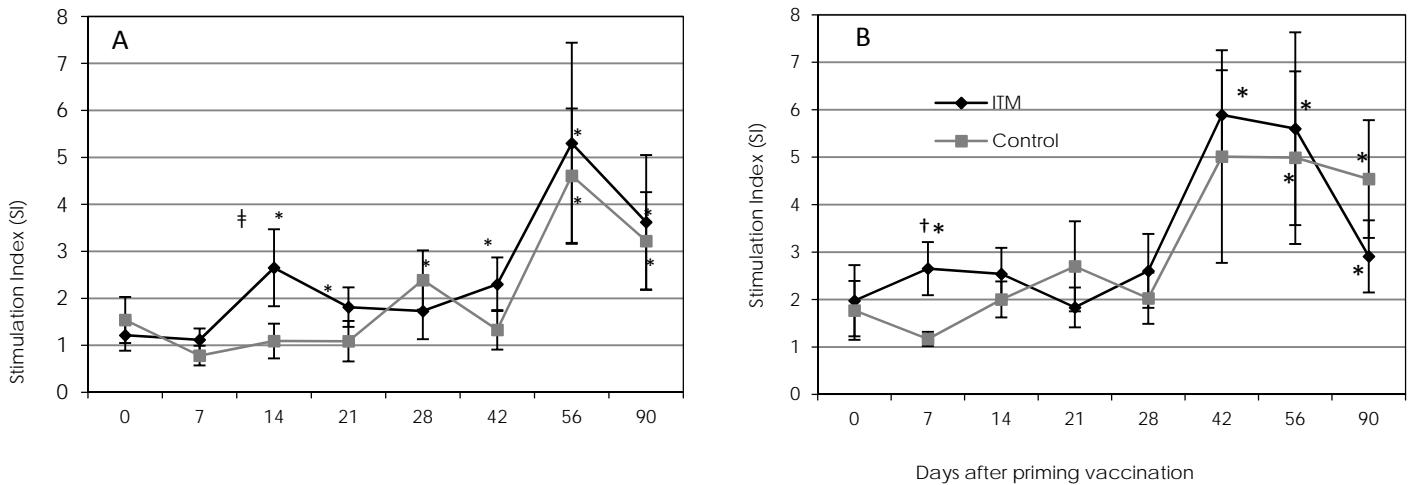

Figure 2. Peripheral blood mononuclear leukocyte proliferation (expressed as stimulation index; SI) in response to BVDV1 (A), and BRSV (B) in dairy calves injected with a trace mineral supplement (ITM) or saline (Control) concurrently with administration of a MLV vaccine. * Value differs significantly from the value on day 0 for each group (3A: P < 0.05; 3B: P < 0.01). ‡Suggests a tendency of higher leukocyte proliferation compared to the control group (3A, P = 0.08). †Significant difference between groups (3B, P= 0.01).

In a subsequent study, 20 dairy calves belonging the previous trial (ITM, n=10; Control, n=10) were intranasally challenged with ncp BVDV-2 (5 mL containing 5 x 10^5 $CCID_{50}$) five months after vaccination and ITM treatment to evaluate the effect of ITM administered concurrently with a MLV vaccine on protection against acute BVDV-2 infection. Additionally, five BVDV-naïve dairy calves were also inoculated and served as positive control group.   In that study, respiratory MLV vaccine (in both ITM and Control groups) conferred protection against acute BVDV infection, preventing moderate clinical disease, leukopenia, lymphopenia, and viremia. Further, ITM provided additional beneficial effects when administered concurrently with respiratory MLV vaccine: 1. ITM increased average daily gain during the 14 day post-challenge period. 2. Clinical scores were lower for ITM compared to saline-injected group on days 5 and 8 post challenge. 3. Administration of ITM prevented BVDV-induced thrombocytopenia compared to saline-injected calves and positive control group (unpublished data). A more recent study using 45 beef calves demonstrated that ITM administered with a MLV vaccine appeared to mitigate the decrease in $CD4^+$ and $CD8^+$ T cells associated with BVDV-2

acute infection in calves that were challenged five days after immunization (unpublished data), which supports our previous findings on increased mononuclear leukocyte proliferation (Palomares et al., 2016). It was concluded that ITM enhanced protection from BVDV-2 infection by MLV vaccine, helping to maintain adequate health and performance of growing calves.

Adequate supply of Zn, Cu, Se and Mn has been documented to be essential for cell signaling and cytokine production during lymphocyte activation (Puertollano et al., 2011; Spears, 2000). These trace minerals are fundamental elements in the structure and function of several metalloproteins that participate in general housekeeping processes involved in cellular clonal expansion including metabolic cascades for energy production, DNA replication and transcription, as well as protection against ROS (Failla, 2003). The enhancement of these general cellular functions might be contributing to the higher leukocyte proliferation response observed in the ITM group.

Trace minerals are crucial for the development of an adequate immune response in cattle, especially in stressed animals. Field and experimental studies focused on improving the trace mineral status of stressed feeder calves have shown positive effects of ITM improving feed efficiency, decreasing morbidity and treatment costs and improving performance and production traits (Arthington et al., 2014; Berry et al., 2000; Clark et al., 2006; Genther, and Hansen, 2014; Richeson et al., 2011). The results of the current study support our hypothesis, and recapitulate the findings of previous studies demonstrating the benefits of trace mineral supplementation on the immune response to MLV vaccines in cattle. This suggests that addition of ITM to calf management protocols might represent a promising tool to improve livestock health on commercial farms.

REFERENCES

Andreini, C., Bertini, I., Rosato, A., 2009. Metalloproteomes: a bioinformatic approach. Acc. Chem. Res. 42, 1471-1479.

Arthington, J.D., Moriel, P., Martins, P.G.M.A., Lamb, G.C., Havenga, L.J., 2014. Effects of trace mineral injections on measures of performance and trace mineral status of pre- and post-weaned beef calves. J. Anim. Sci. 92, 2630-2640.

Arthington, J.D., Havenga, L.J., 2012. Effect of injectable trace minerals on the humoral immune response to multivalent vaccine administration in beef calves. J. Anim. Sci. 90, 1966-1971.

Bala, S., Failla, M.L., 1993. Copper repletion restores the number and function of CD4 cells in copper-deficient rats. J. Nutr. 123, 991-996.

Berry, B.A., Choat, W.T., Gill, D.R., Krehbiel, C.R., Ball, R., 2000. Efficacy of Multimin™ in improving performance and health in receiving cattle. Anim. Sci. Rep. Oklahoma Agric. Exp. Stn. Accessed Nov. 19, 2008. http://www.ansi.okstate.edu/research/2000rr/12.htm.

Bonaventura, P., Benedetti, G., Albarède, F., Miossec, P., 2015. Zinc and its role in immunity and inflammation. Autoimmun. Rev. 14, 277-285.

Bonham, M., O'Connor, J.M., Hannigan, B.M., Strain, J.J., 2002. The immune system as a physiological indicator of marginal copper status? Br. J. Nutr. 87, 393-403.

Brar, J.S., Johnson, D.W., Muscoplat, C.C., Shope Jr, R.E., Meiske, J.C., 1978. Maternal immunity to infectious bovine rhinotracheitis and bovine viral diarrhea viruses: duration and effect on vaccination in young calves. Am. J. Vet. Res. 39, 241-4.

Cerone, S.I., Sansinanea, A.S., Streitenberger, S.A., Garcia, M.C., Auza, N.J., 1998. The effect of copper deficiency on the peripheral blood cells of cattle. Vet. Res. Commun. 22, 47-57.

Chirase, N.K., Hutcheson, D.P., Thompson, G.B., Spears, J.W., 1994. Recovery rate and plasma zinc and copper concentrations of steer calves fed organic and inorganic zinc and manganese sources with or without injectable copper and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 72, 212-219.

Clark, J.H., Olson, K.C., Schmidt T.B., Larson, R.L., Ellersieck, M.R., Alkire, D.O., Meyer, D.L., Rentfrow, G.K., Carr, C.C., 2006. Effects of respiratory disease risk and a bolus injection of trace minerals at receiving on growing and finishing performance by beef steers. The Profess. Anim. Sci. 22, 245-251.

Collen, T., Morrison W.I., 2000. CD4 (+) T-cell responses to bovine viral diarrhoea virus in cattle. Virus Res. 67, 67-80.

Cousins, R. J., King, J.C., 2004. Zinc. in Modern Nutrition in Health and Disease. M. E. Shils, A. C. Ross and R. J. Cousins, eds. Philadelphia: Leas and Febiger.

Droke, E.A., Loerch, S.C., 1989. Effects of parenteral selenium and vitamin E on performance, health and humoral immune response of steers new to the feedlot environment. J. Anim. Sci. 67, 1350-1359.

Duff, G.C., Galyean, M.L., 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 2007 85:823-840.

Ellermann-Eriksen, S., 2005. Macrophages and cytokines in the early defense against herpes simplex virus. Virol. J. 2: 59, 1-30.

Ellis, J., West, K., Cortese, V., 2001. Effect of maternal antibodies on induction and persistence of vaccine-induced immune responses against bovine viral diarrhea virus type II in young calves. J. Am. Vet. Med. Assoc. 219, 351-356.

Endsley, J.J., Roth, J.A., Ridpath, J., Neill, J., 2003. Maternal antibody blocks humoral but not T cell responses to BVDV. Biologicals. 31, 123-125.

Enjalbert, F., Lebreton, P., Salat, O., 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: Retrospective study. J. Anim. Physiol. Anim. Nutr. 90, 459-466.

Failla, M.L., 2003. Trace elements and host defense: Recent advances and continuing challenges. J. Nutr. 133, 1443-1447.

Forthal, D.N., 2014. Functions of antibodies. Microbiol. Spectrum. 2(4) AID-0019-2014.

Galyean, M.L., Perino, L.J., Duff, G.C., 1999. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. 77, 1120-1134.

Genther, O.N., Hansen, S.L., 2014. Effect of dietary trace mineral supplementation and a multi-element trace mineral injection on shipping response and growth performance of beef cattle. J. Anim. Sci. 92, 2522-30.

Griffin, D., 1997. Economic impact associated with respiratory disease in beef cattle. Vet. Clin. North Am. Food Anim. Pract. 13, 367-377.

Haase H, Rink L. 2014. Multiple impacts of zinc on immune function. Metallomics. 6, 1175-1180.

Harrison, J.H., Hancock, D.D., Conrad, H.R., 1984. Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci. 67, 123-132.

Herdt, T.H., Hoff, B., 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am.: Food Anim. Pract. 27, 255-283.

Howard, C.J., 1990 Immunological responses to BVDV infection. Rev. Sci. Tech. Off. Int. Epizoot. 9, 95-104.

Kirkpatrick, J.G., Fulton, R.W., Burge, L.J., Dubois, W.R, Payton, M. 2001. Passively transferred immunity in newborn calves, rate of antibody decay, and effect on subsequent vaccination with modified live virus vaccine. Bovine Pract. 35, 47-55.

Klotz, L.O., Kroencke, K.D., Buchczyk, D.P., Sies, H., 2003. Role of copper, zinc, selenium, and telluriumin the cellular defense against oxidative and nitrosative stress. J. Nutr. 133, 1448S-1451S.

Lemaire, M., Weynants, V., Godfroid J., Schynts, F., Meyer, G., Letesson, J.J., Thiry, E., 2000. Effects of bovine herpesvirus type 1 infection in calves with maternal antibodies on immune response and virus latency. J. Clin. Microbiol. 38, 1885-1894.

Linder, M.C., 1991. Biochemestry of Copper. Earl Frieden, Ed. Plenum Press, New York, NY.

Linder, M.C., Hazegh-Azam, M., 1996. Copper biochemistry and molecular biology. Am. J. Clin. Nutr. 63, 797S-811S.

Machado, V.S., Bicalho, M.L.S., Pereira, R.V., Caixeta, L.S., Knauer, W.A., Oikonomou G., Gilbert, R.O., Bicalho, R.C., 2013. Effect of an injectable trace mineral supplement containing selenium copper, zinc, and manganese on the health and production of lactating Holstein cows. The Vet. Journal. 197, 451-456.

Maddox, J.F., Aherne, K.M., Channa Reddy, C., Sordillo, LM., 1999. Increased neutrophil adherence and adhesion molecule mRNA expression in endothelial cells during selenium deficiency. J. Leuk. Biol. 65, 658-664.

Maggini, S., Wintergerst, E.S., Beveridge, S., Hornig, D.H., 2007. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. Br. J. Nutr. 98, S29-35.

Malmgaard, L., Paludan, S.R., Mogensen, S.C., Ellermann-Eriksen, S., 2000. Herpes simplex virus type 2 induces secretion of IL-12 by macrophages through a mechanism involving NF-kappa B. J. Gen. Virol. 81, 3011-3020.

McVey, D.S., 2009. BRD research needs in the next 10-20 years. Anim. Health Res. Rev. 10, 165-167.

Minatel, L., Carfagnini, J.C., 2000. Copper deficiency and immune response in ruminants. Nutr. Res. 2010, 1519-1529.

Neve, J. (1991) Physiological and nutritional importance of selenium. Experientia. 47, 187–193.

Nobiron, I., Thompson, I., Brownlie, J., Collins, M.E., 2003. DNA vaccination against bovine viral diarrhoea virus induces humoral and cellular responses in cattle with evidence for protection against viral challenge. Vaccine. 21, 2082-2092.

NRC. 2001. Nutrient requirements of dairy cattle. 7th Edition National Academy Press. Washington, D.C

O'Dell, B.L., 1993. Interleukin-2 production is altered by copper deficiency. Nutr. Rev. 51, 307-309.

Pan, Y.J., Loo, G., 2000. Effect of copper deficiency on oxidative DNA damage in Jurkat T-lymphocytes. Free Rad. Biol. Med. 28, 824-830.

Palomares RA, Hurley DJ, Bittar JH, et al. 2016. Effects of injectable trace minerals on humoral and cell-mediated immune responses to Bovine viral diarrhea virus, Bovine herpes virus 1 and Bovine respiratory syncytial virus following administration of a modified-live virus vaccine in dairy calves. Vet Immunol Immunopathol. 2016 Oct 1;178:88-98.

Panousis, N., Roubies, N., Karatzias, H., Frydas, S., Papasteriadis, A. 2001. Effect of selenium and vitamin E on antibody production by dairy cows vaccinated against Escherichia coli. Vet. Rec. 149, 643-646.

Percival, S.S., 1998. Copper and immunity. Am. J. Clin. Nutr. 67, 1064S-1085S.

Pinna, K., D.S. Kelley, P.C. Taylor, and J. C. King. 2002. Immune functions are maintained in healthy men with low zinc intake. J. Nutr. 132: 2033-2036.

Platt, R., Widel, P.W., Kesl, L.D., Roth, J.A., 2009. Comparison of humoral and cellular immune responses to a pentavalent modified live virus vaccine in three age groups of calves with maternal antibodies, before and after BVDV type 2 challenge. Vaccine. 27, 4508-4519.

Pogge, D.J., Richter, E.L., Drewnoski, M.E., Hansen, S.L., 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90, 2692-2698.

Puertollano, M.A., Puertollano, E., Álvarez de Cienfuegos, G., de Pablo, M.A., 2011. Dietary antioxidants: Immunity and host defense. Curr. Top. Med. Chem. 11, 1752-1766.

Puschner, B., Choi, Y., Tegzes, J.H., Thurmond, M.C., 2004. Influence of age, sex, and production class on liver zinc concentration in calves. J. Vet. Diagn. Invest. 16, 278-282.

Puschner, B., Thurmond, M.C., Choi, Y., 2004. Influence of age and production type on liver copper concentrations in calves. J. Vet. Diagn. Invest. 16, 382-387.

Reber, A.J., Tanner, M., Okinaga, T., Woolums, A.R., Williams, S., Ensley, D.T., Hurley, D.J., 2006. Evaluation of multiple immune parameters after vaccination with modified live or killed bovine viral diarrhea virus vaccines. Comp. Immunol. Microbiol. Infect. Dis. 29, 61-77.

Reffett, J.K., Spears, J.W., Brown, T.T.J. 1988. Effect of dietary selenium on the primary and secondary immune response in calves challenged with infectious bovine rhinotracheitis virus. J. Nutr. 118, 229-235.

Rhodes, S.G., Cocksedge, J.M., Collins, R.A., Morrison, W.I., 1999. Differential cytokine responses of CD4+ and CD8+ T cells in response to bovine viral diarrhea virus in cattle. J. Gen. Virol. 80, 1673-1679.

Richeson, J.T., Kegley, E.B., 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. The Profess. Anim. Sci. 27, 461-466.

Rink, L., Kirchner, H., 2000. Zinc-altered immune function and cytokine production. J. Nutr. 130, 1407S-1411S.

Ridpath, J.E., Neill, J.D., Endsley, J., Roth, J.A., 2003. Effect of passive immunity on the development of a protective immune response against bovine viral diarrhea virus in calves. Am. J. Vet. Res. 64, 65-69.

Roberts, S.L., May, N.D., Brauer, C.L., Gentry, W.W., Weiss, C.P., Jennings, J.S., Richeson, J.T., 2015. Effect of injectable trace mineral administration on health, performance and vaccine response of newly received beef cattle. J. Anim. Sci. 93, suppl. S3/J. Dairy Sci. 98, Suppl. 2.

Sandbulte, M.R., Roth, J.A., 2003. Priming of multiple T cell subsets by modified-live and inactivated bovine respiratory syncytial virus vaccines. Vet. Immunol. Immunopathol. 95, 123-133.

Spears, J.W., 2000. Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59, 587-594.

Spears, J.W., and Kegley, E.B., 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80, 2747-2752.

Tomlinson, D.J., Socha, M.T., DeFrain, J.M., 2008. Role of trace minerals in the immune system. 2008 Penn State Dairy Cattle Nutrition Workshop. November 12-13. Grantville, PA. 39-52.

Underwood, E.J., Suttle, N.F., 1999. The mineral nutrition of livestock. 3rd rev. Ed. CABI publishing, New York, NY.

Van Donkersgoed, J., van den Hurk, J.V., McCartney, D., Harland, R.J., 1991. Comparative serological response in calves to eight commercial vaccines against infectious bovine rhinotracheitis, parainfluenza-3, bovine respiratory syncytial, and bovine viral diarrhea viruses. Can. Vet. J. 32, 727-733.

Vollstedt, S., Arnold, S., Schwerdel, C., Franchini, M., Alber, G., Di Santo, J.P., Ackermann, M., Suter, M., 2004. Interplay between alpha/beta and gamma interferons with B, T, and natural killer cells in the defense against herpes simplex virus type 1. J. Virol. 78, 3846-3850.

Windeyer, M.C., Leslie, K.E., Godden, S.M., Hodgins, D.C., Lissemore, K.D., LeBlanc, S.J., 2015. Association of bovine respiratory disease or vaccination with serologic response in dairy heifer calves up to three months of age. Am. J. Vet. Res. 76, 239-245.