The role of trace minerals (TM) in animal production is an area of strong interest for producers, nutritionists, feed manufacturers, vets and scientists. It is also one of the most complex and possibly most poorly understood areas of animal nutrition. Adequate TM intake, absorption and metabolism is required for numerous metabolic functions including immune response, reproduction and growth (Baker et al., 2003). Building, maintaining or enhancing mineral status through feeding and supplementation is complex because of TM requirements by the dairy cow as necessary to obtain and maintain optimum production. This is especially true given current economic and management systems where optimal efficiency is critical (Larsen, 2005). Clinical deficiencies are observed infrequently. Subclinical deficiencies may be a significantly larger problem than acute mineral deficiency. Substantial problems can be created when there is a lack of apparent signs which allow the producer, and often his nutritionist or vet, to recognize the deficiency. In many cases animals continue to grow, reproduce or produce but at a reduced rate, greatly undermining productivity and profits. As trace mineral status declines immunity and enzyme functions are compromised first, followed by a reduction in maximum growth and fertility. Finally, normal growth and fertility decrease prior to evidence of clinical deficiencies (Wikse 1992). In all cattle, and especially the high producing dairy cow, in today’s economy, even small deviations from optimal performance and health can have serious economic implications, especially when expanded over the economies of scale of many dairy operations. It is critical that all nutrients be delivered at levels that provide for optimal health and production efficiency and TM are not exception. In order to maintain animals in adequate trace mineral status, balanced delivery of these traces are essential.

EFFECTS OF TRACE MINERAL STATUS ON PERFORMANCE

In the dairy cow, performance is often defined solely on her ability to produce milk. But numerous other factors are involved including:

- The cow’s ability to efficiently convert feed, forages and water into milk.
- The ability to rebreed in a timely fashion.
- The ability to maintain individual heath.
- The ability to produce quality milk.
- The ability to remain in the herd for as long as possible.

All of these factors can be tied together one way or another. A reduction in a specific level of performance in each of these results in reduced revenues to the producer either because of reduced production of milk and corresponding components, increased cull rates or because of overall added costs. It should be noted that in
addition to these factors, which can be directly related to the cow’s intake of an appropriate diet, her genetics and overall management play a very large role in the end result. For the purpose of this paper however, the focus will be on nutritional levels as reflected in the body, the role of TM status and methods for maintaining an appropriate status.

TRACE MINERAL STATUS IN DAIRY CATTLE – WHAT SHOULD IT BE?

Accurately quantifying the requirements of dairy cattle for TM is very challenging. Trace minerals are needed in very small amounts. At the same time variation in ingredient and diet composition as well as dry matter intake can be high. These circumstances make obtaining even reasonably accurate measurements of intake of TM difficult. Many TM can be stored in various body tissues (especially the liver). With typical turnover, including elevated excretion during times of stress (Orr et al., 1990), a given diet may have to be fed for several months before body stores equilibrate with dietary intake. Overall, two of the largest stumbling blocks to an accurate determinate of TM requirements are measuring endogenous fecal losses (Weiss, 2008) and assessing the effects of the various antagonists to TM absorption in the diet (Harrison and Conrad, 1984; Suttle, 1991). While several methods exist for performing these measurements, at the end of the day estimates of TM requirements are usually much less accurate than estimates of requirements for macro-minerals (Weiss, 2008).

Extensive research has determined reasonably accurate estimates of TM status in dairy cattle (Puls, 1988; Wikse, 1992; Gerloff, 1992; Knowles et al., 1999, Kincaid, 2000). Tables 1 and 2 present various levels of trace elements used to define the status of the animal in both blood components and liver tissue. These values can vary depending on breed, production status, level of stress and so on.

<table>
<thead>
<tr>
<th>Status</th>
<th>Zn (μg/mL)</th>
<th>Cu (μg/mL)</th>
<th>Se (μg/mL)</th>
<th>Mn (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plasma</td>
<td>plasma</td>
<td>serum</td>
<td>serum</td>
</tr>
<tr>
<td>Deficient</td>
<td>0.2 to 0.4</td>
<td>&lt;0.2 to 0.5</td>
<td>&lt;0.025</td>
<td>&lt;5.0</td>
</tr>
<tr>
<td>Marginal</td>
<td>0.5 to 0.8</td>
<td>0.5 to 0.7</td>
<td>0.03 to 0.06</td>
<td>5 to 6</td>
</tr>
<tr>
<td>Adequate</td>
<td>0.8 to 1.4</td>
<td>0.7 to 0.9</td>
<td>0.08 to 0.3</td>
<td>6 to 70</td>
</tr>
<tr>
<td>High</td>
<td>2 to 5</td>
<td>0.9 to 1.1</td>
<td>&gt;2.5</td>
<td>--</td>
</tr>
<tr>
<td>Toxic</td>
<td>3 to 15</td>
<td>&gt;1.2</td>
<td>&gt;3.5</td>
<td>--</td>
</tr>
</tbody>
</table>

Adapted from Kincaid, 2000.
Table 2. Liver concentrations for assessing trace mineral status in cattle

<table>
<thead>
<tr>
<th>Status</th>
<th>Zn μg/g DM</th>
<th>Cu μg/g DM</th>
<th>Se μg/g DM</th>
<th>Mn μg/g DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>&lt;20 to 40</td>
<td>&lt;33</td>
<td>0.1 to 0.5</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Marginal</td>
<td>25 to 40</td>
<td>33 to 125</td>
<td>0.6 to 1.25</td>
<td>7 to 15</td>
</tr>
<tr>
<td>Adequate</td>
<td>25 to 200</td>
<td>125 to 600</td>
<td>1.25 to 2.5</td>
<td>&gt;13</td>
</tr>
<tr>
<td>High</td>
<td>300 to 600</td>
<td>600 to 1250</td>
<td>&gt;2.5</td>
<td>-.</td>
</tr>
<tr>
<td>Toxic</td>
<td>&gt;1000</td>
<td>&gt;1250</td>
<td>-.</td>
<td>-.</td>
</tr>
</tbody>
</table>

Adapted from Kincaid, 2000.

1/ concentrations in adult cattle
2/ classified as severely deficient
3/ classified as marginally deficient
4/ classified as high adequate

As research and practice has shown, Cu deficiency is a commonly encountered nutritional problem in ruminants. Clinical signs of deficiency can result in an array of adverse effects which can include reduced growth rates, decreased feed conversion, abomasal ulcers, lameness, poor immune function, sudden death and impaired reproductive function (Puls, 1994). Due to rapid changes in Cu in blood components, the best method for diagnosing Cu status is by analysis of liver tissue. Deficiency within a herd will result in some animals that have low serum Cu concentrations, but serum content does not fall until liver Cu is fairly depleted. In herds that have tested livers and found a high incidence of deficiency, it is not uncommon for a high percentage of the animals to show “normal” serum concentrations (Hall, 2006). Even with a perceived herd deficiency, low serum Cu concentrations may only be seen in 20% of the individuals. Herds that may be classified as marginally deficient based on liver testing may have predominantly “normal” serum Cu concentrations. This said serum Cu analysis should be viewed as a screening method only. Another factor that can influence diagnosis of Cu deficiency in serum is the presence of high serum molybdenum. As the copper-sulfur-molybdenum complex that forms is not physiologically available for tissue use (Suttle, 1991), “normal” serum Cu content in the presence of high serum molybdenum should always be considered suspect. In addition, the form of selenium supplementation can alter the normal range for interpretation of serum Cu status, with selenite supplemented cows having a lowered normal range for serum Cu.

Copper deficiency can be diagnosed via analysis of Cu-containing enzymes. The two most common enzymes that are utilized are ceruloplasmin and superoxide dismutase. Low concentrations of these enzymes in serum and whole blood, respectively, can be used effectively to diagnose for Cu deficiency. However, ceruloplasmin concentrations can increase with inflammatory disease so these levels must be evaluated carefully. Finally, higher costs for analysis of these enzymes than that of liver copper analysis often limits their utilization.
Because of the interest over the last few years, excessive supplementation of copper in cattle is a relatively common finding, especially in dairy cattle. Liver Cu concentrations greater than 200 ppm are routinely identified.

Manganese deficiency in ruminants is associated with impaired reproductive function, skeletal abnormalities in calves, and less than optimal productivity. Cystic ovaries, silent heat, reduced conception rates, and abortions are the typical reproductive effects. Calves that are Mn deficient can be weak, small, and develop enlarged joints or limb deformities. In many cases this appears to be linked to reduced activity of Mn dependent enzymes required for cartilage development (Leach, 1971). Manganese deficiency, although not reported often, is identified routinely in dairy cattle when tested. It is interesting that most testing of beef cattle finds normal Mn concentrations in liver, blood, and serum. Of the samples available, liver is the most indicative of whole body status, followed by whole blood and then serum. Overall, response to supplementation has frequently been used as a means of verifying manganese deficiency, but it is critical that a bioavailable form be utilized since availability of Mn is typically very low (NRC, 2001).

As an essential mineral, Se is commonly found to be deficient in ruminants. Selenium deficiency in ruminants has been related to a variety of with adverse effects on growth, reproduction, immune response, fetal development and muscle tissue (Hogan, et al., 1993). “White muscle disease”, a necrosis and scarring of cardiac and/or skeletal muscle, is linked to severe Se deficiency, although it can be caused by Vitamin E deficiency as well (Hostetler, et al., 2003). Reduced growth rates, poor immune function, and impaired reproductive performance can be observed with less severe Se deficiency.

Diagnosis of a deficiency can be made by analysis of liver, whole blood, or serum for Se content or by analysis of whole blood for activity of glutathione peroxidase, a Se-dependent enzyme (Kincaid, 2000). The most specific analysis is that of whole blood glutathione peroxidase, as it verifies true functional Se status. Liver is the optimal tissue to analyze for selenium content, as it is a primary storage tissue. With serum and whole blood, the former better reflects recent intake, while the latter better reflects long term status. Whole blood has a Se concentration that is approximately three times higher (Scholz and Hutchinson, 1979) and is often better for Se determination because any hemolysis of the erythrocytes will cause serum to show a false high value for Se (Maas et al., 1992). Since seleno-proteins are incorporated into the red blood cells when they are made and the cells have a long half-life, Se content can be a reflection of intake over the previous months. In order to adequately diagnose Se deficiency, the dietary form of the selenium intake by the animals is important to determine. Natural Se, predominantly in the form of selenomethionine, is metabolized and incorporated into Se dependent proteins, but can also be incorporated into non-specific proteins in place of methionine. This means that Se from these sources can be deposited in muscle and other tissues. Inorganic Se is metabolized and only incorporated into Se dependent proteins but this does include the very important availability to the synthesis of glutathione peroxidase. Thus, “normal” concentrations in serum and whole blood differ
depending on whether the dietary Se is a natural organic form or an inorganic supplement.

Zinc is an essential mineral that is required by all cells in animals. Zinc plays a role in numerous enzymatic reactions. Deficiencies of Zn are associated with reduced growth, poor immune function, diminished reproductive performance, and poor offspring viability, as well as skin lesions in severe cases. For instance, Zn has been identified as having a role in or as a co-factor of over 300 enzymes within all 6 classes of enzyme systems (Dibley, 2001). In this capacity, Zn is obviously tied to a wide assortment of processes within the body of the dairy cow related to basic metabolism, health, reproduction and milk quality and production. It is a component of many metalloenzymes including copper-zinc superoxide dismutase, carbonic anhydrase and RNA Polymerase. These among others affect the metabolism of carbohydrates, proteins, lipids and nucleic acids. Additionally, Zn status may directly alter prostaglandin synthesis which directly affects luteal function (NRC 2001). Zinc is also required for maintenance of skin integrity and stabilization of various membranes (Spain, 1993). Additionally it is required in the activated of the cell-mediated immune system (Kellogg et al., 2004; Miller and Madsen, 1992). As Zn status is reduced, along with stress (which can lead to a further depression in Zn status as noted by Orr et al., 1990), a reduction in immune response and compromise of skin integrity the mammary glands natural defense system is weakened. This results in higher somatic cell counts and increased incidence of mastitis. Additionally, in trials where Zn status is increased either by level or source fed, an improvement in overall milk production was observed (Kellogg, 2004; Aguilar et al., 1988). Tissue Zn concentrations do not reflect body status well. Of the common samples tested, liver and serum are the best indicators of zinc status. But, serum and liver Zn can be altered by age, infectious diseases, trauma, fever, and stress. It has been suggested that pancreas Zn content is the best means of truly identifying Zn deficiency.

TRACE MINERAL FEEDING, SUPPLEMENTATION AND DELIVERY

The absolute need for TM in the diet has been identified and is considered common knowledge. Nutritionists and veterinarians alike have labored to identify what the appropriate status of the TM for dairy cattle should be through the testing of a variety of tissues and organic compounds in the body (Hall, 2006, Kincaid, 2000, Levander, 1986). Finally, numerous studies have reported the effects of the different sources of TM on respective status in the animal as well as health and performance parameters (Weiss, 2008; Nocek et al., 2006; Larsen, 2005; Mitchell and Smith, 2003; Daugherty et al., 2000; Orr et al., 1990; Harrison et al., 1984).

Ongoing work in academia and corporately has assessed the usefulness of the variety of TM sources available for oral supplementation based upon respective bioavailabilities. This work has resulted in a variety of results as noted by Greene et al (1998) and is commonly reported as the comparison of a given source to the sulfate version of the TM in question (Table 3).
Table 3. Relative bioavailability of trace minerals from different sources.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Sulfate</th>
<th>Oxide</th>
<th>Carbonate</th>
<th>Chloride</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>100</td>
<td>0</td>
<td>105</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>100</td>
<td>58</td>
<td>28</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>100</td>
<td>0</td>
<td>60</td>
<td>40</td>
<td>159, 206</td>
</tr>
</tbody>
</table>

Adapted from Greene et al., 1998.

Subsequent studies have shown variable results in relative bioavailabilities (Spears 2003). Actual absorption of TM, provided orally and regardless of source is fairly low and is related to a variety of issues (NRC, 2001). In many cases this is related purely to basic TM absorption dynamics (Ammerman et al., 1995). In other cases, breed effects can come into play (Engle, et al., 1999, Du et al., 1996, Ward et al., 1995). In many other situations and of significant importance, poor intestinal absorption is related to the presence of compounds (antagonists) that would bind or interfere with the absorption of the TM in question (Peres, 2001; Ward and Spears, 1997; McDowell, 1992; Suttle, 1991; Harrison and Conrad, 1984; Suttle and Field, 1968). Generally these antagonisms are related to the presence of other minerals in the diet. However, it has been shown that antagonistic effects can be related to type of diet and fiber source (Spears, 2003; Wanger et al., 1972).

Actual absorption of several TM was estimated in the 2001 Dairy NRC (summarized in Table 4). As noted, a great deal of work has been conducted to show that the organic complexes of these TM increase bioavailability. In general it is commonly assumed that organics will show improved relative bioavailabilities respective to their inorganic counterparts of 100 to 200% (NRC 2001). But when the overall, actual absorption of the base TM source is taken into account, even with utilization of an organic complex, actual absorption is still low.

Table 4. Trace mineral absorption in mature cattle

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Estimated Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>1 to 5%</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.5 to 1%</td>
</tr>
<tr>
<td>Selenium</td>
<td>up to 35%(^1)</td>
</tr>
<tr>
<td>Zinc</td>
<td>5 to 15%</td>
</tr>
</tbody>
</table>

\(^1\) Se from Selenomethionine has shown greater absorptive characteristics.

With such low levels of actual absorption of these TM and because of inherent variability of the TM content in feedstuffs and rations, it is a common practice to overformulate these nutrients in an effort to overcome these issues. Unfortunately this overformulation can further antagonize absorption of certain minerals. Additionally it can result in excretion of significant amounts of the various TM sources that can lead to environmental concerns. Ongoing work by Castillio (2008) indicate that elevated levels in feces and urine on the pen surface may increase the microbial production of certain bio gasses. As this research emerges and as greater regulatory influence becomes common, more efficient means of providing for the TM requirements of the dairy cow.
must be identified. Especially for those minerals such as Mn which are shown to have such an exceedingly low net absorption.

As the feeding industry and academia search for optimal methods to maintain and enhance TM status in the dairy cow, building on existing feeding and supplementation techniques and considering the challenges discussed above certain alternatives have become of greater interest. Over the years, injectable TM sources have been evaluated with varying degrees of success. Recently the use of injectable forms of TM has gained increasing acceptance, especially when used in combination with properly designed oral mineral supplementation programs.

Injectable forms of copper containing compounds have been shown to be effective in preventing Cu deficiency in ruminants. Studies as early as 1955 (Sutherland et al., 1955; Allcroft and Uvarov, 1959) both reported that Cu glycinate injected subcutaneously was an effective and safe source of Cu for sheep and cattle (Baker and Ammerman, 1995). These early studies also suggested that multiple injections yearly would be effective in maintaining normal Cu status for animals grazing Cu-deficient forages. Other, later studies have shown positive results with other injectable forms as well. Hemingway, et al (1970) illustrated that injectable forms of Cu (Cu glycinate, Cu-Ca-EDTA, Cu methionate) were effective in maintaining normal blood and liver copper levels in pregnant ewes. Multiple later studies have shown the effectiveness of injectable Cu (Fisher et al 2007, Michael et al 2004, Mitchell and Smith, 2003; Daugherty et al., 2000; Rogers and Poole, 1988). Not all injectable forms were as effective considering the potential for injection-site reactions. Copper glycinate and methionate were both shown to produce severe localized reactions at the point of injection. Copper EDTA showed a much lower degree of reactivity.

Similarly, use of injectable forms of Se have also been investigated. Of all the TM Se is possibly the most commonly delivered via injection with several commercial products available. As with oral forms, use of injectable Se has been shown to increase Se status and specifically the activity of glutathione peroxidase (GSH-Px) in the animal (Maas et al 1992). Common perspectives are that Se injections are short-lived and are transitory at best. In many cases use of injectable Se is indicated therapeutically (Ishler, et al., 2008) although research shows that ongoing effects can be recognized as illustrated in Table 5. More recent work has also shown the increase in enzymatic activity (GSH-Px) to be prolonged (Fisher et al., 2007; Mass et al., 1992).
Table 5. Effect of selenium supplementation in brahman cattlea.

<table>
<thead>
<tr>
<th>Fluid Tested</th>
<th>No.</th>
<th>Control</th>
<th>Dietary Seb</th>
<th>Dietary Seb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow Serum</td>
<td>66</td>
<td>.024d</td>
<td>.055e</td>
<td>.045e</td>
</tr>
<tr>
<td>Colostrum</td>
<td>24</td>
<td>.015d</td>
<td>.049f</td>
<td>.031e</td>
</tr>
<tr>
<td>Milk</td>
<td>44</td>
<td>.0046d</td>
<td>.0097e</td>
<td>.0062e</td>
</tr>
<tr>
<td>Calf Serum</td>
<td>58</td>
<td>.03d</td>
<td>.053e</td>
<td>.049e</td>
</tr>
</tbody>
</table>

a McDowell et al., 1990
b Control + 0.25 ppm Se + IM Inj of 5 mg Se + 1500 mg Vit E
c Control + 0.25 ppm Se
d-f Means in the same row with different subscripts differ (<.05).

Injectable forms of Zn and Mn have not been researched as extensively as Cu and Se. However, ongoing work shows positive responses when these TM are delivered in this fashion. Daugherty and co-workers showed increased (P<.05) liver concentrations of Zn and Mn in beef cows injected with Na EDTA complexes of these TM.

More extensive research into the use of injectable forms of TM combinations have shown promise. Mitchell and Smith (2003) evaluated the use of an injectable TM product (MultiMin®, 20 mg/ml Zn, 20 mg/ml Mn, 10 mg/ml Cu and 5 mg/ml Se) in dairy cattle at various stages of production. A cooperating dairy consisting of 1250 head, 24,000 lbs RHA with average heat detection and pregnancy rates was selected for the trial. Beginning August 2000 cattle were divided by number with even numbered cattle (n=615) receiving 5 ml subcutaneous injections in the neck and control cattle (n=635) receiving no injection. This injection protocol continued through August 2001. Treated cattle were injected as they moved into the close-up group (~30 days prior to freshening) and then again at 6 weeks fresh. All cattle received their standard close-up rations initially and subsequent lactation rations after freshening. Reproductive data (conception rates, pregnancies) and health events (mastitis, somatic cell count, retained placentas, hoof heath/lameness) were recorded from September 1, 2000 to June 2002.

Numerical differences were noted (P>.05) in cases of mastitis between treated and control cows. At 30 days in milk (DIM), 22% fewer cattle showed cases of clinical mastitis in the treatment group. At 60 DIM, the treated group had 13% fewer cases of mastitis. This is in agreement with work by Weiss et al (1990) who showed a high correlation between increased blood Se and reduced incidences of mastitis. According to Hutjens & Tomlinson (2001), University of Missouri research showed that organic zinc supplementation increased production of keratin in the teat. Keratin acts as a physical barrier to bacteria and as a bactericidal plug to the teat, decreasing the incidence of mastitis and somatic cell count.

The conception rate (cows pregnant as a percentage of cows bred within a specific time frame) of all Multimin® treated cows was significantly (P<.05) higher than the conception rate of control cows at days 70, 80 and 90 in milk. (Fig. 1).
Figure 1. The effect of MultiMin® on conception rate in all cows on days 70, 80 and 90 in milk. All differences were statistically significant (P<.05).

Similar results were seen in early lactation cows with conception rates in treated cows increasing at DIM 70, 80 and 90 by 49.7% (P<.05), 45.7% (P<.05) and 50.5% (P<.01) respectively.

A similar study was conducted by Michael et al., 2004 to evaluate similar parameters using a similar injectable TM product (MultiMin Cattle 70®, 40 mg/ml Zn, 10 mg/ml Mn, 15 mg/ml Cu and 5 mg/ml Se). The trial was set up similarly using a herd in WI of 589 head with a rolling herd average of 27,800 and milking 3X daily. The dairy was characterized by an aggressive TM supplementation program (Table 6) which included a portion (undetermined) of TM content from organic sources (Zn methionine, Mn methionine, Cu lysine, Co glucoheptonate).

Table 6. Ration trace mineral concentrations

<table>
<thead>
<tr>
<th>Trace mineral</th>
<th>Concentration a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobalt</td>
<td>1.2</td>
</tr>
<tr>
<td>Copper</td>
<td>27.0</td>
</tr>
<tr>
<td>Iodine</td>
<td>3.4</td>
</tr>
<tr>
<td>Iron</td>
<td>183.0</td>
</tr>
<tr>
<td>Manganese</td>
<td>89.0</td>
</tr>
<tr>
<td>Zinc</td>
<td>95.0</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.5</td>
</tr>
</tbody>
</table>

a parts per million in ration dry matter.
Cows with odd ID’s (n=278) were injected with 5 ml Multimin® (MM – treatment group) 3 to 4 weeks prior to freshening and 3 to 4 before the end of the voluntary waiting period (VWP = 45 DIM). Cows with even ID’s (n=311) were injected with 8 ml of MuSe™ (MS - control group) at 3 to 4 weeks before expected calving date. Control cows received MS as part of the dairy’s standard management procedures. Reproductive and health events were recorded.

A numerical (24%) reduction was seen in mastitis incidences between the cows receiving MM and those receiving MS. Cows receiving MM tended to have lower incidences of retained placentas (RP) n=28 versus control cows n=38 (P=0.29). Although not statistically significant, the 21% reduction in RP events may indicate not only selenium, but also zinc, copper and/or manganese are involved in the prevention of retained placentas. Alternatively, selenium in MM may have been utilized more efficiently within the balanced multi-mineral formulation compared to only selenium in MS. Treated cows tended to have lower (46%) incidences of lameness reported n=8 versus control cows n=16 (P=0.13). Although it not significant, it represents the potential of MM to improve nutritional balance or TM status and therefore hoof health.

Percent cows pregnant (within a specific time frame) of all MM treated cows trended higher than the percent cows pregnant of control cows at days 80, 90, 100, 110, 120, 130, 140 and 150 in milk with varying levels of statistical significance.

The percentage increase in pregnancy production resulting from the two MM injections was very similar to that found by Mitchell (2003) indicating injectable TM supplementation helps increase optimal TM status and therefore improved reproductive performance. The effect of MM on percent pregnant (within a specific time frame) was enhanced in second lactation cows, also seen by Mitchell (2003). This difference was highly significant (P<0.01) early in lactation (Figure 2). Increased conception and pregnancy resulted in a decrease in median days open (MDOPN) from 119 days in the MS group to 99 days in the MM group (P<0.05) (Figure 3). This is equal to a 20 day or 16.8% reduction in MDOPN.
Figure 2: The Effect of MULTIMIN® vs MuSe on Percent Cows Pregnant in Second Lactation Cows on Days 80,90,100,110,120,130,140 & 150 in Milk

<table>
<thead>
<tr>
<th>Days In Milk</th>
<th>MuSe</th>
<th>MULTIMIN</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>21.7</td>
<td>40.8</td>
<td>0.01</td>
</tr>
<tr>
<td>90</td>
<td>25</td>
<td>44.8</td>
<td>0.01</td>
</tr>
<tr>
<td>100</td>
<td>29.4</td>
<td>47.4</td>
<td>0.02</td>
</tr>
<tr>
<td>110</td>
<td>35.9</td>
<td>48.47</td>
<td>0.09</td>
</tr>
<tr>
<td>120</td>
<td>40.2</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>130</td>
<td>43.5</td>
<td>54</td>
<td>0.18</td>
</tr>
<tr>
<td>140</td>
<td>44.6</td>
<td>56.6</td>
<td>0.12</td>
</tr>
<tr>
<td>150</td>
<td>48.9</td>
<td>59.2</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Figure 3. The effect of MULTIMIN® on days open in All Cows between 80 and 200 days in milk of which the regression analysis were used to calculate the 95% confidence limits in decreased Median days Open.

These trial data would suggest that use of an injectable TM solution could have positive effects when incorporated as part of a complete mineral supplementation program. Delivery of critical TM subcutaneous or intramuscular injection by-passes the gastrointestinal tract and the inherent absorption problems created by various antagonists. The Wisconsin data illustrates the effectiveness of an injected TM component into the overall program resulting in an enhanced TM status that can improve reproductive and health performance in the animal.
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Study (Case 1). Trace Minerals and Dairy Cattle. Bovine Veterinarian, Sept 2003, p 32-34.


