Preface:
This proceeding will review the role of manganese (Mn) in bovine fetal development and summarize the necropsy findings and liver mineral analysis of 62 bovine cases from January 1 through June 30, 2010. During this period, bovine fetuses with skeletal deformities from both dairy and beef herds were submitted to the Minnesota Veterinary Diagnostic Laboratory (MVDL) for routine diagnostic investigations. All deformed fetuses were found to have low liver Mn levels.

Introduction:
The fetus is completely dependent on the dam for its supply of nutrients and minerals (Hidiroglou and Knipfel, 1981). Mineral deficiencies of the fetus can result in impaired fetal growth and abnormal development of the skeletal and central nervous systems (Widdowson et al., 1974). In addition to Mn, copper (Cu), iodine (I), iron (Fe) and zinc (Zn) are known to influence embryonic and fetal survival (Hostetler et al., 2003). Manganese is required to activate glycosyltransferase, a necessary enzyme for the proper synthesis of cartilage and bone (Howes and Dyer, 1971). Manganese is also an essential trace mineral involved in enzyme and cholesterol synthesis which is required for progesterone production (Davis et al., 1990). Consequently, Mn may impact estrus and early fetal development.

Published manganese requirements for dairy cattle have changed in the last ten years. Prior to 2001, the National Research Council (NRC) dairy requirements model for gestating dairy cows was 40 mg of Mn/kg of DM. As of 2001, the requirement was lowered to 17.8 mg of Mn/kg of DM. This lower requirement (~17 mg of Mn/kg of DM) is similar to levels of Mn that produced deficiency signs in beef cows (Rojas et al., 1965). More recent research indicates that lactating and dry dairy cows require 1.6 and 2.7 times higher concentrations than those calculated using the 2001 NRC model (Weiss and Socha, 2005).

Gross exams, necropsy and diagnostic results:
Deformed fetuses and stillborn calves from multiple dairy and beef farms were submitted to the MVDL during the first half of 2010. All fetuses were tested as outlined in a protocol that includes bacteriological culture of liver, lung, stomach contents and placenta (when available), PCR tests for Bovine Viral Diarrhea virus (BVDv), Infectious Bovine Rhinotracheitis virus (IBRv) and Leptospira spp., and histopathological exam of brain, lung, liver, heart, diaphragm, placenta and skeletal muscle. Trace mineral analysis of fetal liver is not routinely request on aborted fetuses because reference values for fetuses are lacking. One publication showed that lower liver Cu, Fe, Mn, and Zn concentrations in aborted fetuses when compared to their dam is a nonspecific change of trace element status and that low fetal trace minerals is an effect of abortion, not the cause of abortion (Graham et al., 1994). Nevertheless, trace mineral analysis of 70 fetal livers from normal and deformed specimens were performed and those results are reported and compared.

Skeletal deformities of the fetuses included, but were not limited to, twisted and short limbs, swollen joints, disproportionate dwarfism, superior brachygnathism and shortened nasomaxillary bones (short upper jaw exposing the lower teeth). A few producers also reported that live calves were unsteady, weak and some shook and trembled when walking. The
producers also reported that this problem did not affect more than 10% of all pregnancies. All deformed fetuses had Mn levels below an adjusted reference range for bovine fetuses (0.6X adult). The mean liver manganese level in eight deformed fetuses was 0.88 ppm wet weight (fetal reference 1.75 – 2.5 ppm wet weight). Three additional fetal livers from deformed fetuses were analyzed on a dry matter basis and had low mean Mn levels of 3.1 ug/g (fetal reference range 3.5 – 6.3 ug/g dry matter). The mean copper and zinc levels of the deformed fetuses were within the reference range for adults. One fetus had a slightly low copper level and three had an elevated liver copper. None of the deformed fetuses had low zinc and four reported values above the reference range. Table 1 summarizes the mineral levels of the deformed fetuses.

Table 1: Summary of liver Mn, Cu and Zn in deformed fetuses. (Jan 1 – June 30 2010)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean Liver Ref range</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>0.88 ppm (wet, 0.6 X of adult)</td>
<td>10 0 0</td>
</tr>
<tr>
<td>Copper</td>
<td>62 ppm (wet, adult)</td>
<td>1 6 3</td>
</tr>
<tr>
<td>Zinc</td>
<td>200 ppm (wet, adult)</td>
<td>0 6 4</td>
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The fetal mineral analysis results appeared to mirror those of other bovine livers (calves, adults, etc.) during the same time period. Sixty nine percent (26/41) of liver from non-deformed fetuses had liver Mn levels below the reference range whereas a majority of the liver copper (84%) and zinc (98%) levels were within the reference range for adult cattle. Many of the livers were analyzed with two different analytical methods (toxic element ICP and trace mineral ICP, Michigan State University) and both methods produced similar results.

Table 2: Summary of mineral analyses of bovine livers excluding deformed fetuses (Jan 1 – June 30 2010)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean Liver Ref range</th>
<th>Distribution</th>
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</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>1.2 ppm (wet wt., adult)</td>
<td>26 12 3</td>
</tr>
<tr>
<td>Copper</td>
<td>62 ppm (wet, adult)</td>
<td>1 31 5</td>
</tr>
<tr>
<td>Zinc</td>
<td>95 ppm (wet wt., adult)</td>
<td>0 40 1</td>
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</table>

None of the deformed fetuses were positive for BVDv, IBRv, Leptospira spp. by polymerase chain reaction (PCR) or had microscopic lesions consistent with neosporosis. None of the farms reported diseases of the dams and there was no obvious genetic or breed predisposition. There was minimal evidence of an infectious disease because all but one of the fetuses failed to produce IgG/IgM antibodies as detected in fetal thoracic fluid. None of the fetuses had gross or microscopic evidence of goiter (iodine deficiency).

Nutrition of the Dam:
Total mineral consumption of the dams is variable and difficult to measure. In the cases examined, liver zinc, copper and iron (not reported) were normal suggesting that a total trace mineral deficiency was unlikely and that low liver fetal mineral concentration was not an effect of abortion or perinatal mortality, but the cause of Mn-associated skeletal deformities and fetal loss. These findings are similar to a 2006 study in beef heifers where calves born to heifers receiving a diet containing 15.8 mg of Mn/kg of DM weighed less at birth and suffered from a variety of Mn deficiency signs including disproportionate dwarfism, swollen joints and superior brachygnathism (Hansen et al., 2006). In that study, diagnostic blood tests did not reveal significant Mn deficiencies. Whole blood Mn concentrations were not affected by treatment, but
a more reliable liver Mn analysis was not performed. The diagnosis of Mn deficiency was
determined only by characteristic skeletal deformities.

**Manganese in feed and the relationship with Glyphosate (Roundup®):**
Supplemental manganese is often fed as a mineral premix and the source of Mn include
Mn-sulfate, Mn-oxide, Mn-chloride and Mn-carbonate. The relative availability varies
significantly among different sources. Approximately 1.0 to 1.2 % of Mn from Mn sulfate and
Mn chloride is absorbed. Only 0.25% of Mn from Mn oxide is absorbed and 0.15% of Mn from
Mn carbonate is absorbed.

Manganese levels in cattle feedstuffs are variable. Generally, legume and grass hays
have more Mn than corn or corn silage and Mn is reported to be more available in hay than
silage (Puls,1994). Feed Mn can be highly variable as illustrated in Table 3. The data in the
table are results from selected feeds tested at Dairyland Labs (St. Cloud) from June through
December 2010. The bio-availability of Mn in these feeds is not known.

**Table 3: Feed analysis of selected feed at Dairyland Labs.**

<table>
<thead>
<tr>
<th>Feed</th>
<th>Mean Mn Range</th>
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<tbody>
<tr>
<td>Shelled corn</td>
<td>16 15 ppm 0.01 – 57.65 ppm</td>
</tr>
<tr>
<td>Corn Silage</td>
<td>176 37 ppm 0.01 - 89.43 ppm</td>
</tr>
<tr>
<td>Grass Hay</td>
<td>182 50 ppm 0.01 – 125.20 ppm</td>
</tr>
<tr>
<td>Mixed Haylage</td>
<td>96 57 ppm 0.55 – 113.45 ppm</td>
</tr>
</tbody>
</table>

Recently, Mn and its relationship with glyphosate has gained attention among
nutritionists, agronomists and livestock producers. Plants’ disease defense mechanisms rely on
adequate amounts of Mn (Thompson and Huber, 2007). Some have proposed that glyphosate
interferes with plant absorption and utilization of Mn, thus increasing plant susceptibility to
disease and decreasing amounts of Mn in feedstocks. Research has failed to document a
reduction in Mn concentrations in plants following glyphosate application (Bott et al., 2008;
Rosolem et al., 2009; Nelson 2009). Manganese deficiency in corn and soybeans is not a new
phenomenon and has been described for decades prior to the use of glyphosate. High soil pH
and low soil moisture promote Mn deficiency in plants. Higher organic matter has also been
associated with Mn deficiency in plants (Camberato et al., 2010).

**Corrective actions:**
Herd that produced deformed fetuses had one or more of the following:
1. The mineral contained Mn, but in quantities below 50 mg of Mn/kg of DM (all herds);
2. The source of Mn was the poorly available Mn-oxide (6 herds);
3. One herd did not feed any supplemental mineral;
4. Gestating cow diets lacked hays and/or legumes and the diet was comprised mostly of
corn stalks, distillers grains, corn silage, or a combination thereof (one beef herd).

Dairy herds that switched to the more bio-available manganese sulfate continued to
produce deformed fetuses for 9 months after the feed change. This finding supports previous
research showing that Mn has an important role throughout gestation and likely impacts fertility
and skeletal development throughout gestation (Hostetler et al, 2003). One dairy herd
complained of poor conception, but the role of manganese and poor conception could not be
confirmed. Beef herds that corrected the problem in 2010 are reporting no deformed calves in
2011 and calves born to cows fed 50 mg of Mn/Kg of DM of Mn-sulfate have more vigor. Of
the herds that switched from Mn-oxide to 50 mg/kg of Mn-sulfate, three physically normal
stillbirth fetuses collected in 2011 had liver manganese levels 2 to 3 times higher than 2010
fetuses and levels.

**Summary:**

Bovine fetuses with skeletal deformities and features of incomplete cartilage and bone formation had low levels of liver Mn when compared to fetuses with normal skeletal features. Examination of the gestating cow diets revealed a lack of Mn or Mn from poorly available sources. These findings support supplemental feeding of at least 50 mg of Mn/Kg of DM to gestating cattle and feeding Mn from an available source such as Mn-sulfate and/or Mn-amino acid complex. This recommendation is higher than the 2001 NRC model’s recommendation. Analyzing fetal livers for trace minerals and establishing reference ranges appears justified as this data could provide useful information when assessing the mineral status of gestating cattle.

**References:**