Correction of Subnormal Fertility with Copper and Magnesium Supplementation

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ABSTRACT

Two hundred and four Holstein cows and heifers were randomly assigned to mineral supplement groups 30 d prior to expected calving. Supplement treatment groups were Cu, Mg, Cu plus Mg, and no mineral supplement. The total diet of supplemented groups averaged 15 mg/kg of Cu and .30% of Mg. Pastures consisted of bermudagrass, bahiagrass, and millet during the summer and oats and ryegrass mixture during the winter. Corn and sorghum silage were also fed. Blood samples were taken just prior to initiation of mineral supplementation and at wk 1, 2, 4, 6, and 8 postpartum. Hemoglobin and packed cell volume were determined and plasma was assayed for Cu and Mg. First service conception rates were 57% for the Cu plus Mg treatment and 27, 38, and 33% for treatments 1, 2, and 4, respectively. Ninety-two percent of the cows in the Cu plus Mg-group conceived by 210 d postpartum as opposed to an average of 75% for the other groups. Plasma Mg was different among cows grouped on a fertility basis and hemoglobin was correlated with days to conception. Plasma Mg was correlated with hemoglobin. Both were inversely related to postcalving infection and uterine involution. In summary, cows supplemented with both Cu and Mg showed improved fertility, whereas those supplemented with Cu or Mg alone did not.

INTRODUCTION

Recent reviews concerning the biological significance of Cu (16) and Mg (1, 13) suggest that deficiencies of either may be related to infertility, anemia, or to suppressed immune response. Plant and animal relationships concerning these minerals are complex. Forage concentration of either mineral may be altered by season (11) and cultural practices (2, 12) while other dietary components may alter their availability to the animal (3, 15). In addition, there are problems in determining the Mg (14, 20) and Cu (16) status of the animal. Although plasma concentrations are used, they are not particularly sensitive indicators.

Other studies have related anemia to depressed ovarian function and a consequent delay in fertility following calving (9, 26). A delay in fertility and consequent extension of the open period has important economic consequences, especially for cows calving in spring in the southeastern United States. If cows are not pregnant by June (the beginning of the hot season in Louisiana), it is unlikely that they will conceive before October (the beginning of the cool season) (4, 5).

This experiment was conducted in a dairy herd with a history of relatively low fertility. An intensive investigation was initiated in the herd to identify possible causes of the low fertility. It included blood profiling and the monitoring of postcalving uterine involution and ovarian function (5, 6). Results, which led to the present investigation, indicated a relationship between the number of days to conception and plasma Cu during the 1st and 2nd wk postpartum (9) and plasma Mg at the 2nd, 3rd, and 4th wk postpartum (7). The present experiment was undertaken to determine whether the relationships between plasma Cu and Mg and fertility could be verified.

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MATERIALS AND METHODS

The experiment was conducted at the Southeast Research Station, Franklinton, LA, and utilized a total of 204 Holstein cows and heifers. Cows in replicates 1 (n = 62), 2 (n = 70), and 3 (n = 72) calved between October 17, 1981 and February 17, 1982; July 23 and October 28, 1982; and October 29, 1982 and February 19, 1983, respectively. This gave two groups of fall and winter calving cows and one group of summer calving cows. This calving arrangement allowed the fall and winter calving cows 160 ± 60 d to be inseminated prior to the onset of heat stressing weather in June.

Equal numbers of animals were assigned to each of four supplement groups 30 d before expected calving, at which time supplementation was started. Some cows were dropped from the experiment because they had twins or were culled for mastitis or other ailments early in the postcalving period. Mineral supplements consisted of Cu as copper sulfate, Mg as magnesium oxide, Cu plus Mg, and a control group. The mineral supplements were formulated in a corn meal base and 245 g topdressed once daily on the concentrate ration. The control group received corn meal only. Test animals were individually fed the concentrate portion of the diet twice daily. The supplements were formulated to give a total diet that contained 15 mg/kg Cu and .30% Mg on a dry matter basis or 150% of NRC recommendations (18). Forage and concentrate analyses for the previous 2 yr (10, 11) were used to estimate the amount of Cu and Mg needed in the supplements. Forage was estimated to be 40% of the total rations (11). Concentrations of Cu, Mg, and Mo in feeds were determined by atomic absorption spectroscopy.

The concentrate ration had a crude protein content of 20% and consisted of 63.5% ground corn, 33.5% soybean meal, and 3% mineral mix. The mineral mix contained 5% P, 24 to 30% Ca, and 20% NaCl. All animals received 2.4 kg/d of the concentrate ration and the designated supplement beginning 30 d prior to expected calving date. After calving, the concentrate ration for each cow was increased to 12.7 kg/d fed in two equal portions twice daily for the first 90 d of lactation. Subsequently, individual cows received .4 kg of concentrate/kg of milk for cows producing over 20 kg of milk/d and .33 kg for cows producing less than 20 kg. Pastures consisted of an oats and ryegrass mixture during winter and bahiagrass, bermudagrass, or millet during summer. Corn or sorghum silage plus 5 kg of alfalfa hay per cow per day were fed prior to grazing winter pasture. After winter grazing became available, alfalfa hay was limited to 2 kg/d per cow and fed with silage.

Heparinized blood samples were obtained from the tail vein before initiating mineral supplementation and during wk 1, 2, 4, 6, and 8 postpartum. Hemoglobin (Hb) and packed cell volume (PCV) determinations were made before separating the cells. Mean corpuscular hemoglobin concentration (MCHC) was calculated from the following formula (27):

\[
MCHC (g/dl) = \frac{Hb (g/dl)}{PCV (%)}
\]

Plasma was stored at -20°C until Cu and Mg were determined by atomic absorption spectroscopy.

Ambient temperature was monitored by hygrothermograph and maximum-minimum thermometer. Mean daily temperature was estimated by averaging readings taken at 2-h intervals. The mean temperature used as a covariate in the analysis of variance was an average of the temperatures for the day on which the sample was taken and the 3 previous d.

Uterine involution and cervical discharges were monitored by rectal palpation and vaginal speculum. Animals with cervical discharges containing pus or uteri not involuted normally (as determined by the veterinarian at rectal palpation) were infused with either 60 ml of 2% Lugols iodine solution or 2 g of oxytetracycline diluted to 60 ml in water. Infusions were repeated at weekly intervals if the condition was not resolved. If two or more infusions appeared necessary, the kinds of infusions given were usually alternated. Cows having twins were not included in the statistical evaluation. Estrous detection patches and penectomized bulls will chin ball markers were kept in pastures with the cows to aid in estrous detection. Cows were observed for standing estrus in the early morning and at evening. All breeding was by artificial insemination performed on a once per day breeding schedule.
Forages, concentrates, and mineral supplements were sampled weekly. Statistical computations were performed using the general linear models procedure and the correlation procedure of the Statistical Analysis System (25). The model used for ANOVA included the following main effects.

**Replicate**
Three replicates, as defined earlier, were used.

**Mineral Supplement**
Four groups, as defined previously, were used.

**Fertility Groups**
Cows were allotted to three groups to determine the relationship of the various blood constituents to days to conception. Group 1 conceived within 80 d after calving; group 2 between 80 and 120 d; and group 3 required more than 120 d to conceive.

**Milk Production Groups**
Cows were ranked into three milk production groups on the basis of monthly DHIA records averaged over the first 6 mo of lactation. Group 1 cows averaged less than 22.7 kg/d milk; group 2 averaged more than 22.7 but less than 27.2 kg/d; and group 3 animals produced in excess of 27.2 kg/d.

**Lactation Number Groups**
Cows were allotted to three groups according to lactation number; groups 1, 2, and 3 consisted of first lactation heifers, second lactation cows, and cows in their third or later lactation, respectively.

**Infusion Groups**
Persistence of postcalving uterine infection was categorized by the number of uterine infusions given to the cows and heifers before first service. Cows were allotted to two groups; group 1 was not treated or treated only once; group 2 was treated two or more times. Animals receiving only one infusion often were the cows for which the need for treatment was debated. Because of this and since others (17) have proposed that cows receiving only one infusion were not significantly different in days to conception from cows receiving no infusions, animals in these two categories were placed in one group.

**Average Ambient Temperature**
Temperature was defined earlier.

**Sample**
Sample represented to six blood sampling times.

The model also included all first-order interactions, except for milk production × lactation number and interactions involving sampling days. Previous experience had indicated that too few first lactation heifers were in the high production group, causing some of the least square means to be unestimable. It also was believed that the small sample size would bias results of the analysis. Therefore, the milk production × lactation number interaction source of variation was allowed to fall into the residual error term. All higher order interactions were omitted from the model due to numerous missing cells. The F values, calculated as ratios of mean squares in the analysis of variance, were used to determine significant sources of variation. Where significant differences were detected in the main effects or first order interactions, t tests, as calculated by the general linear models procedure, were conducted between selected pairs of least square means. All higher order interactions were omitted from the model due to numerous missing cells. In addition to the model shown in Table 1, a split-plot design containing replicate, mineral supplement group, and their interaction was also used to test for the significance of mineral supplements across the six samples. Cows within replicates × mineral groups interaction were used as the error term for the main effects and their interaction. Separate analyses were also conducted on the data obtained at each sampling day to determine the times in the postcalving period when significant differences existed. Average daily temperature was used as a covariate in the latter. Correlations were obtained between the mean milk production of each cow, measures of fertility, plasma Cu and Mg, PCV, and Hb.
TABLE 1. Analyses of variance for hemoglobin (Hb), packed cell volume (PCV), plasma magnesium and plasma copper computed across the 6 sampling wk.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Hb</th>
<th>PCV</th>
<th>Mg</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication (R)</td>
<td>2</td>
<td>9.58*</td>
<td>1.79</td>
<td>2.60*</td>
<td>.079*</td>
</tr>
<tr>
<td>Mineral treatment (M)</td>
<td>3</td>
<td>.94</td>
<td>1.12</td>
<td>.30*</td>
<td>.076*</td>
</tr>
<tr>
<td>Open group (O)</td>
<td>2</td>
<td>.98</td>
<td>2.82</td>
<td>.37*</td>
<td>.02</td>
</tr>
<tr>
<td>Production group (P)</td>
<td>2</td>
<td>.07</td>
<td>1.05</td>
<td>.01</td>
<td>.003</td>
</tr>
<tr>
<td>Lactation group (L)</td>
<td>2</td>
<td>17.11*</td>
<td>50.08*</td>
<td>.48*</td>
<td>.021</td>
</tr>
<tr>
<td>Infusion group (I)</td>
<td>1</td>
<td>13.84*</td>
<td>20.83*</td>
<td>.24</td>
<td>.351*</td>
</tr>
<tr>
<td>Sample no. (S)</td>
<td>5</td>
<td>31.08*</td>
<td>354.81*</td>
<td>3.90*</td>
<td>.005</td>
</tr>
<tr>
<td>R X M</td>
<td>6</td>
<td>2.52*</td>
<td>17.18*</td>
<td>.16</td>
<td>.880*</td>
</tr>
<tr>
<td>R X O</td>
<td>4</td>
<td>3.54*</td>
<td>13.63*</td>
<td>.07</td>
<td>.058*</td>
</tr>
<tr>
<td>R X P</td>
<td>4</td>
<td>8.35*</td>
<td>28.60*</td>
<td>.15</td>
<td>.026</td>
</tr>
<tr>
<td>R X L</td>
<td>4</td>
<td>9.53*</td>
<td>21.71*</td>
<td>.13</td>
<td>.021</td>
</tr>
<tr>
<td>R X I</td>
<td>2</td>
<td>9.63*</td>
<td>39.06*</td>
<td>.01</td>
<td>.081*</td>
</tr>
<tr>
<td>O X P</td>
<td>4</td>
<td>1.08</td>
<td>3.15</td>
<td>.05</td>
<td>.085*</td>
</tr>
<tr>
<td>O X L</td>
<td>4</td>
<td>.60</td>
<td>3.21</td>
<td>.08</td>
<td>.022</td>
</tr>
<tr>
<td>M X P</td>
<td>6</td>
<td>1.00</td>
<td>15.94*</td>
<td>.12</td>
<td>.064*</td>
</tr>
<tr>
<td>M X O</td>
<td>6</td>
<td>4.48*</td>
<td>16.61*</td>
<td>.10</td>
<td>.096*</td>
</tr>
<tr>
<td>M X L</td>
<td>6</td>
<td>2.95*</td>
<td>26.36*</td>
<td>.12</td>
<td>.053</td>
</tr>
<tr>
<td>M X I</td>
<td>3</td>
<td>2.63*</td>
<td>27.84*</td>
<td>.11</td>
<td>.140*</td>
</tr>
<tr>
<td>O X I</td>
<td>2</td>
<td>.44</td>
<td>4.31</td>
<td>.10</td>
<td>.035</td>
</tr>
<tr>
<td>P X I</td>
<td>2</td>
<td>4.30*</td>
<td>22.22*</td>
<td>.15</td>
<td>.026</td>
</tr>
<tr>
<td>L X I</td>
<td>2</td>
<td>3.13*</td>
<td>2.47</td>
<td>.36*</td>
<td>.036</td>
</tr>
<tr>
<td>Error df</td>
<td></td>
<td>1076</td>
<td>1047</td>
<td>1092</td>
<td>1094</td>
</tr>
<tr>
<td>Error MS</td>
<td></td>
<td>.765</td>
<td>4.76</td>
<td>.076</td>
<td>.026</td>
</tr>
</tbody>
</table>

*(P<.05).

RESULTS

Mineral Content of Feeds

Concentrations of Cu, Mg, and Mo in the feeds are in Table 2. As shown in a previous study (10, 11), forages grown in the area tended to be marginal in Mg and deficient in Cu. Molybdenum concentrations were well below those considered to interfere with Cu utilization in ruminants (19).

Milk Production

Milk production was not significantly different among mineral supplement groups or

TABLE 2. Mean (+ SD) content of magnesium, copper, and molybdenum in forage and concentrate feeds consumed by animals on study.

<table>
<thead>
<tr>
<th></th>
<th>Magnesium (%)</th>
<th>Copper (µg/g)</th>
<th>Molybdenum (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
<td>X</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>.20</td>
<td>.04</td>
<td>7.8</td>
</tr>
<tr>
<td>Bahiagrass</td>
<td>.29</td>
<td>.02</td>
<td>6.0</td>
</tr>
<tr>
<td>Corn silage</td>
<td>.24</td>
<td>.07</td>
<td>5.2</td>
</tr>
<tr>
<td>Concentrate (20% CP)</td>
<td>.26</td>
<td>.23</td>
<td>10.5</td>
</tr>
<tr>
<td>Ryegrass pasture</td>
<td>.28</td>
<td>.05</td>
<td>4.8</td>
</tr>
<tr>
<td>Sorghum silage</td>
<td>.26</td>
<td>.08</td>
<td>4.2</td>
</tr>
<tr>
<td>Sorghum greenchop</td>
<td>.41</td>
<td>.09</td>
<td>3.3</td>
</tr>
</tbody>
</table>

fertility groups. Daily milk production per cow by mineral supplement group is in Table 3.

**Reproduction**

Several measures of reproductive efficiency are reported by mineral supplement in Table 3. Supplementation with Cu plus Mg affected the percentage of cows that had conceived by 75, 100, 125, and 150 d postpartum ($P<.01$, Table 4). Reproductive efficiency of cows with Cu or Mg alone was not different from that of controls. Number of days open was correlated with days to first service ($P<.0001; r = .438$) and services per conception ($P<.001; r = .786$).

**Blood Constituents**

Because the split-plot model described in Methods did not give significantly different results for replication and mineral supplement effects than the model in Table 1, only the latter is reported. Differences among replications were particularly significant for Mg and Hb and to a lesser extent for Cu. Because replicates 1 and 3 started in the winter and replicate 2 started in July, some of these differences could be regarded as seasonal effects. Plasma concentrations of all constituents varied across lactation groups and sampling weeks but none varied significantly among milk production groups.

**Magnesium**

Plasma Mg declined between the first and second samples and increased at the 2nd wk postpartum (Figure 1C). Significant effects were indicated for replication, mineral supplement, fertility group, and lactation group on plasma Mg concentration (Table 1).

Plasma Mg increased ($P<.0001$) with each replicate; successive replicates started at 2.2, 2.4, and 2.5 mg/dl, respectively. The general profile (Figure 1C) did not change with replicate. Although the analysis across samples indicated a significant effect of mineral supplementation on plasma Mg, it was only significant ($P<.02$) at the 4th wk postpartum (Figure 1C). Second lactation cows had the highest Mg after the 2nd wk postpartum (Figure 2C). The trend for the third or more lactation cows to have lower Mg at the 1st wk postpartum was consistent across mineral

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**TABLE 3. Mean (± SD) of milk production and measures of reproductive efficiency by mineral supplement groups.**

<table>
<thead>
<tr>
<th>Supplements</th>
<th>n</th>
<th>Days to first service</th>
<th>Days to first estrus</th>
<th>Number of services per conception</th>
<th>Services per conception</th>
<th>Milk production (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49</td>
<td>24.9 ±1.6</td>
<td>52.2 ±1.6</td>
<td>81.3 ±1.6</td>
<td>1.5 ±0.2</td>
<td>2.1 ±0.2</td>
</tr>
<tr>
<td>Cu</td>
<td>54</td>
<td>24.3 ±1.6</td>
<td>52.2 ±1.6</td>
<td>81.3 ±1.6</td>
<td>1.9 ±0.2</td>
<td>1.8 ±0.2</td>
</tr>
<tr>
<td>Mg</td>
<td>53</td>
<td>24.8 ±1.6</td>
<td>52.2 ±1.6</td>
<td>81.3 ±1.6</td>
<td>1.7 ±0.2</td>
<td>2.1 ±0.2</td>
</tr>
<tr>
<td>Mg + Cu</td>
<td>49</td>
<td>25.0 ±1.6</td>
<td>51 ±1.6</td>
<td>74.1 ±1.6</td>
<td>1.2 ±0.2</td>
<td>1.4 ±0.2</td>
</tr>
</tbody>
</table>

1 Number of cows in group.
2 Cows that conceived by 210 d postpartum.
3 Number contributing to the mean in parentheses.
TABLE 4. Cows conceiving by 75, 100, 125, and 150 d postcalving by mineral supplement group. Statistical differences were determined by chi-square.

<table>
<thead>
<tr>
<th>Supplement</th>
<th>75 (%)</th>
<th>100 (%)</th>
<th>125 (%)</th>
<th>150 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49</td>
<td>22</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>Cu</td>
<td>51</td>
<td>16</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>Mg</td>
<td>55</td>
<td>16</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>Cu + Mg</td>
<td>49</td>
<td>39</td>
<td>63</td>
<td>73</td>
</tr>
</tbody>
</table>

* Different from other numbers in column (P<.01).
*b Different from other numbers in column (P<.005).

Supplement groups. Plasma Mg varied among fertility groups (P<.008, Table 1). However, the analysis at each sampling time indicated that only the precalving sample was significant (P<.05, Figure 3C).

There was an interaction between lactation number and uterine infusion group for plasma Mg (Table 1). First lactation heifers did not fit the pattern of succeeding lactations (Figure 4A). The first postcalving sample for older cows that received two or more infusions had (P<.01) lower plasma Mg (Figures 4B and 4C). The group receiving more infusions also had lower (P<.01) Mg in the third or more lactation group at the 4th wk postcalving (Figure 4C). Correlation analysis indicated that plasma Mg and the number of postpartum uterine infusions were negatively correlated at the first postcalving sample (P<.004; r = −.211). Plasma Mg was positively correlated with PCV and Hb in postcalving sample wk 2 through 8 (Table 5).

**Hemoglobin**

Hemoglobin concentration declined following calving and tended to stabilize after the 4th wk postcalving (Figure 1B). The response of Hb to mineral supplementation was not significant (Table 1). Lactation groups added significantly to the variance in Hb at the precalving sample (P<.001) and the 4th (P<.0002) and 6th wk (P<.04) postcalving due mostly to the higher Hb in the heifers.

The ANOVA did not show a significant relationship between Hb and fertility groups. Because Hb varied with lactation number, with first-calf heifers having higher Hb (Figure 2B), the distribution of animals by lactation in each of the fertility groups was evaluated. The most fertile group consisted of 36% first lactation heifers, 15% second lactation, and 49% of the animals in the third lactation group. Actually, a higher percentage of the older cows conceived early compared with first-calf heifers (33 vs. 25%).

Hemoglobin at wk 4 postcalving was negatively correlated (P<.0007; r = −.218) with number of services per conception, suggesting the possibility that the effects of low Hb were not simply a matter of delay in the time to first service. Plasma Mg was correlated with Hb from the 2nd through the 7th wk postcalving (Table 5).

Hemoglobin was significantly different among infusion groups (Table 1). This relationship is presented by production group in Figure 5 since the production group × infusion group interaction was also significant. The relationship was significant at the first postcalving sample (P<.02) and the 4th wk postcalving (P<.04). The picture is similar to that seen between plasma Mg and uterine infusion groups (Figure 1). Most individuals in the low production group were first lactation heifers. Higher production animals were more dramatically affected.

The temperature covariate was significant for Hb at the precalving sample (P<.01) and wk 6 (P<.004) and wk 8 (P<.0001) postcalving.

**Packed Cell Volume**

Positive correlations between PCV and Hb were highly significant at all sampling times in Table 5. Plasma Mg was correlated with PCV beginning with the 2nd wk postcalving.

The average MCHC value for dry cows and heifers at the first sample was 35.3 g/dl. It was not significantly different in the three lactation groups or mineral supplement groups. Both PCV and Hb had declined significantly by sample 4; however, MCHC averaged 35.9 and was still not significantly different across these same groups.

The ANOVA model indicates that Hb and PCV showed similar responses except that PCV was not significantly affected by replicate. Figures 1A, 2A, and 3A confirmed this view.

COPPER AND MAGNESIUM IN DIET AND INFERTILITY

Figure 1. Mean packed cell volume (PCV), hemoglobin, and plasma magnesium and copper concentrations by sample week and mineral supplement group. Numbers in parentheses indicate the number of animals per group.

**Copper**

Plasma Cu increased between the presupplement sample 30 d before expected calving and the sample during the 1st wk postcalving. However, the increase was most evident in the first lactation heifers (Figure 2D), which had lower (P<.0001) Cu at the first sample. The group of first-calf heifers that received Cu supplementation alone had (P<.02) higher plasma Cu at postcalving sample wk 6 and 8 than the other three supplement groups. However, this group of heifers had the lowest average PCV at these same sample weeks. The response of plasma Cu to the mineral supplements is given in Figure 1D.

Ambient temperature was related to plasma Cu during the 2nd (P<.03), 4th (P<.04), 6th
Figure 2. Mean packed cell volume (PCV), hemoglobin, and plasma magnesium and copper concentrations by sample week and lactation group. Numbers in parentheses are average milk (kg/d) for each group. There were 78, 45, and 81 cows in the first, second, and third or more lactation groups, respectively.

(P<.02), and 8th wk (P<.003) of postcalving sampling.

Plasma Cu was not correlated with either Hb or PCV. It was negatively correlated with plasma Mg in the precalving sample (P<.002; r = -.224) and at the 4th wk postcalving (P<.007; r = -.193).

Ambient Temperature

The averages ± SD of the ambient tem-
DISCUSSION

This investigation revealed two relationships of particular interest in addition to the synergistic effect of Cu and Mg supplementation on fertility. One involves the relationship between postcalving infection and Hb, Mg, and PCV values. Low PCV was related to infection in a previous investigation into causes of infertility in this herd (9). The second concerns a possible relationship between reduced plasma Mg and the transient anemia that follows calving.

Figure 3. Mean packed cell volume (PCV), hemoglobin, and plasma magnesium and copper concentrations by sample week and fertility (days open) groups. Numbers in parentheses indicate the number of animals per group.
Delayed uterine involution (13) and an impaired immune response (1, 13, 22) have been attributed to Mg deficiency in rats. Although severe deficiencies resulted in fetal malformation with resorptions and abortions, moderate deficiencies during gestation resulted in delayed uterine involution attributed to a deficiency of a Mg dependent collagenase (21). Suppression of the immune system has been attributed to a hypogammaglobulinemia resulting from impaired protein synthesis and reduced numbers of antibody producing cells (13). Tempting as it may be to hypothesize that the postcalving uterine infections noted resulted from a simple Mg deficiency, such a hypothesis is not fully supported by data from this experiment. The Mg-supplemented group received about as many infusions as the control group (Table 3). The Cu-supplemented group on the average received fewer infusions and had fewest average days to first estrus. The Cu plus Mg group averaged one infusion per cow (Table 3) but contained only 8 nonpregnant cows at 150 d postcalving. There was a trend for the

Figure 4. Mean plasma magnesium concentrations by sample week, uterine infusion groups, and lactation number. Weeks at which there are significant \( P<.01 \) differences are marked by an asterisk. Numbers in parentheses indicate the numbers of animals per group.
TABLE 5. Correlation coefficients by sample week for relationships that were relatively consistent across samples.

<table>
<thead>
<tr>
<th>Hemoglobin vs.</th>
<th>Weeks relative to calving</th>
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</thead>
<tbody>
<tr>
<td>PCV</td>
<td>1</td>
</tr>
<tr>
<td>Mg</td>
<td>.796&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Days open</td>
<td>NS</td>
</tr>
<tr>
<td>Average milk</td>
<td>-.345&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk fat</td>
<td>NS</td>
</tr>
<tr>
<td>Copper vs. Mg</td>
<td>-.224&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCV vs. Mg</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>p<.05.  
<sup>b</sup>p<.01.  
<sup>c</sup>p<.001.  
<sup>d</sup>p<.0001.  
<sup>1</sup>PCV = Packed cell volume.  
<sup>2</sup>NS = Not significant.

animals that received two or more infusions and conceived by 210 d postcalving to have a longer open period (114 ± 45 vs. 102 ± 39 d). This was largely due to a longer period to first service (85 ± 22 vs. 76 ± 18 d). Only 69% of the cows that received two or more infusions conceived by 210 d postcalving, as opposed to 82% of the animals receiving none or one infusion. After 210 d postcalving, 22 of the nonpregnant cows were sent to slaughter. Their reproductive tracts were retained for culture and examined for gross and microscopic pathology (8). Pathologic conditions that would have interfered with conception were found in only one tract. This cow had been identified early by rectal palpation. No *Campylobacter fetus* or other specific pathogens were found, indicating that the long-term infertility in these animals was not related to pathology or bacterial infection. For the winter calving cows, long-term infertility may be related to heat stress during the summer.

A postcalving decline in PCV and Hb has been reported (5, 9, 15, 23). Its negative relationship with the postcalving rise in milk production suggests that the decline results from the nutritional stress of early lactation. Manston et al. (15) related Hb and PCV in lactating cows to dietary protein. Cows receiving low protein diets did not recover from postcalving PCV depression. High dietary protein also depressed plasma Cu concentrations. Magnesium and serum albumin increase with milk production in the early postcalving period (24). As mentioned previously, Mg deficiency has been related to depressed protein synthesis in rats (22). Also, Mg deficiency is reported to result in a shortened life of red blood cells in calves (13). Because Hb and PCV were positively correlated with plasma Mg in the current experiment, it is possible that a relative deficiency in Mg in the early postpartum period may, in part, be responsible for the postcalving depression in PCV and Hb. A relationship between serum Mg and PCV has been reported in swine on a Mg-deficient diet (20). These investigators speculated that intracellular Mg from the erythrocytes was transferred to the serum in the deficient animal. They reported that serum Mg values decreased in concert with PCV values as the number of days on the deficient diet increased. The correlations are of particular interest because of the relationship of PCV and Hb to depressed fertility (9, 23). Magnesium supplementation may also have application for treating prob-
lems encountered in the delayed return of ovarian activity in unsupplemented beef heifers. When this project was initiated, it was anticipated that if the hypothesis were correct, there would be some improvement in fertility in the groups receiving Mg or Cu alone. It was not expected that only supplementation with both minerals together would generate a response. Thus far, efforts to explain the synergistic effect of Mg and Cu on reproduction have not been fruitful. It is possible that combination of the minerals minimized the postcalving depression in Hb (Figure 1B). However, Hb and PCV were not significantly different among fertility groups. Age-related differences in the response of Hb to Cu and Mg supplementation may be important. First-calf heifers responded to the Cu plus Mg supplement with less postcalving Hb depression. In contrast, the Hb status of the older cows on this supplement was not improved above those that received the other supplements. Yet 55% of the first calf heifers and 50% of the cows in third or greater lactations receiving Cu plus Mg

Figure 5. Mean hemoglobin concentrations graphed by sample week, uterine infusion group, and milk production group. Weeks at which there are significant (P<.05) differences are marked by an asterisk. Numbers in parentheses indicate number of animals per group.
conceived before 80 d postpartum compared with an average of 16.5 and 25.2% in the other three supplement groups. Second lactation cows receiving Cu plus Mg did not show improved fertility or increased Hb. However, second lactation cows tended to have the highest plasma Mg. These results suggest that although plasma Mg and Hb are correlated with number of days to conception, improved fertility due to Cu plus Mg was not contingent on their increased concentrations in plasma. Also, the lactation group × supplement group interactions indicate that the animals responded to mineral supplementation differently depending on age and milk production. This suggests that age or milk production may alter the mineral concentration required in the diet. Although considerable research has and is taking place concerning Mg metabolism and grass tetany in the postpartum beef cow, the topic appears to have been largely ignored in dairy cattle. These data are indicative of the complexity of nutritional effects on reproduction and the importance of an adequate, balanced diet during late gestation and early lactation if maximum fertility is to be maintained.

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