Effect of Supplemental Lactoferrin with Ferrous Iron on Iron Status of Newborn Calves

SHIN-ICHI KUME and SHINOBU TANABE
Department of Animal Nutrition, National Institute of Animal Industry, Tsukuba 305, Japan

ABSTRACT

Data for 18 male and 18 female calves, born from primiparous and multiparous cows, were collected to determine the effect of supplemental lactoferrin and FeSO₄ for 5 d after parturition on the Fe status of calves. Dietary treatments were 1) untreated, 2) 40 mg of Fe/d as FeSO₄, and 3) 40 mg of Fe as FeSO₄ plus 5 g of lactoferrin/d. Blood hematocrit and hemoglobin of calves born from primiparous cows at d 1 of age were lower than those of calves born from multiparous cows, but not different from those of their dams. Blood hematocrit and hemoglobin of male calves at d 1 of age were lower than those of female calves. Plasma Fe of primiparous cows at parturition was lower than that of multiparous cows, but plasma Fe of calves was not affected by parity of dam and sex of calf. Blood hematocrit and hemoglobin of untreated calves decreased from 1 to 10 d of age. Blood hematocrit and hemoglobin of calves treated with Fe or Fe plus lactoferrin increased from 2 to 10 d of age. Blood hematocrit and hemoglobin of calves treated with Fe plus lactoferrin were higher than those of calves treated with Fe at d 6 of age. Plasma Fe of calves treated with Fe or Fe plus lactoferrin increased temporarily at d 2 of age. Plasma Fe of calves treated with Fe plus lactoferrin at d 2 of age was lower than that of calves treated with Fe, but at d 6 and 10 of age, plasma Fe of calves treated with Fe plus lactoferrin were higher. (Key words: parity, lactoferrin, iron, calves)

Abbreviation key: Hb = hemoglobin, Hct = hematocrit, Lf = lactoferrin.

INTRODUCTION

Iron deficiency anemia of calves at birth has adversely affected calf growth rate and health (4, 5, 9, 10, 12, 16). Parity of dams and twinning are factors that affect the low blood hematocrit (Hct) and hemoglobin (Hb) of newborn calves at parturition (1, 7, 8, 14), but various factors affect the occurrence of anemia. Adams et al. (1) suggested that calves with lower hematological variables had higher mortality rates because of the difficulty calves had in maintaining normal body temperature. Additionally, low colostral Fe was insufficient to maintain blood Hct and Hb of newborn calves, which decreased as age increased (7).

Lactoferrin (Lf), an Fe-binding protein, may act as an Fe source for newborn calves (6, 11, 17), and the administration of Fe-saturated Lf can prevent anemia in rats (6). However, in the previous experiment (8), blood Hct and Hb of calves were unaffected by the administration of 20 mg of Fe/d as Fe-saturated Lf for 5 d after parturition, but the same amounts of Fe as FeSO₄ improved blood Hb. Although ferrous Fe may be more effective than Fe-saturated Lf as an Fe source for newborn calves in the previous experiment (8), further study is needed to clarify the functions of Lf for the erythropoiesis of newborn calves, because erythropoiesis of nursing calves is active (10).

The objectives of this study were to clarify the effect of the parity of dams or calf sex on the mineral status of calves and their dams and to compare the efficiency of supplemental Lf and FeSO₄ on the Fe status of calves during the first 10 d after birth.

MATERIALS AND METHODS

Data from 18 male and 18 female Holstein calves born from primiparous and multiparous cows were collected at the National Institute of Animal Industry (Tsukuba, Japan). Cows were managed in individual tie stalls and in a paddock during the dry period. The mean length of the dry period for multiparous cows was 4.4 mo. Cows were fed 3 to 4 kg/d of concentrate and appropriate amounts of Italian ryegrass silage, corn silage, and Italian ryegrass hay in individual tie stalls to meet recommendations (2) for TDN, protein, and minerals for approximately 3 wk before expected calving date. The quantity of iron in concentrate, Italian ryegrass silage, corn silage, and Italian ryegrass hay was 154, 637, 793, and 399 ppm (DM basis), respectively.
Thirty-six calves were assigned to three groups by parity of dam and sex of calf. Calves were separated from the dams at parturition and housed in individual pens. Each calf received approximately 1 kg of colostrum at parturition and, thereafter, 2.5 kg of milk twice a day, and calf starter pellets and mixed hay were offered. Calf starter and mixed hay contained 170 and 420 ppm of Fe (DM basis), respectively. Calves were separated of age, calves were fed approximately 2.5 kg of whole milk twice a day, and calf starter pellets and mixed hay were offered. Calf starter and mixed hay contained 170 and 420 ppm of Fe (DM basis), respectively.

Dietary treatments were 1) untreated, 2) 40 mg of Fe/d as FeSO₄, and 3) 40 mg of Fe as FeSO₄ plus 5 g of Lf/d. Lactoferrin was prepared from fresh skim milk (Morinaga Milk Industry Co., Ltd., Zama, Japan) and contained 17 mg of Fe/100 g; the extent of Fe saturation was estimated to be 13%, and 5 g of Lf contained only 0.85 mg of Fe. Treated calves were fed Fe sources mixed with morning colostrum from 1 to 5 d of age.

Blood samples of cows were obtained within 12 h after parturition. Blood samples of calves were collected at 0830 h on d 1, 2, 6, and 10 of age before morning colostrum was fed. The mean time of first blood sampling of male and female calves born from primiparous and multiparous cows was 21 to 22 h after parturition. Most calves received colostrum twice before the first blood sampling. Blood was collected via jugular vein puncture into heparinized vacuum tubes. Colostrum samples of dams were collected at parturition and at approximately 96 h postpartum. Blood samples of calves were collected at parturition and at 96 h postpartum. Blood Hct and Hb, plasma macrominerals, and colostrum composition were determined as previously described (7). Plasma Fe, Zn, and Cu were determined by atomic absorption spectrophotometry after dilution with distilled water.

The general linear models procedure of SAS (13) was used to analyze the effect of parity on blood and colostrum composition of cows at parturition or at 96 h postpartum and to analyze the effect of parity of dam and sex of calf on blood composition of calves at d 1 postpartum. Data of calves from 1 to 10 d of age were analyzed by least squares ANOVA using the general linear models procedure of SAS (13). The model was as follows:

\[ Y_{ijk} = \mu + T_i + P_j + S_k + C_{(ijkl)} + D_m + TP_{ij} + TS_{lk} + TD_{lm} + e_{ijklm} \]

where

- \( \mu \) = overall mean;
- \( T_i \) = effect of treatment;
- \( P_j \) = effect of parity of dam;
- \( S_k \) = effect of sex of calf;
- \( C_{(ijkl)} \) = random variable of calf nested within treatment, parity of dam, and sex of calf;
- \( D_m \) = effect of sampling day;
- \( TP_{ij}, TS_{lk}, \) and \( TD_{lm} \) = interactions; and
- \( e_{ijklm} \) = residuals.

An ANOVA was performed, and the differences were tested by least significant difference. Significance was declared at \( P < 0.05 \) unless otherwise noted.

**RESULTS AND DISCUSSION**

**Fe Status of Newborn Calves**

The mean BW at birth of calves born from primiparous cows was significantly lower than the mean BW of calves born from multiparous cows, but BW was not different between sexes (Table 1). The gestation length of cows was not significantly different between primiparous and multiparous cows, but the mean BW of primiparous and multiparous cows was 575 and 665 kg at approximately 1 mo before parturition. No metabolic or reproductive disorders occurred for cows around parturition, except for the occurrence of dystocia in a few cows, but the health status of calves was good at birth.

Blood Hct and Hb (\( P < 0.01 \)) of calves born from primiparous cows were significantly lower at d 1 of age than those of calves born from multiparous cows, although Hct and Hb of the dams were not different. Blood Hct and Hb of male calves at d 1 of age were significantly lower than those of female calves. Except for plasma inorganic P of cows, differences in blood composition of cows by sex of calf were nonsignificant.

Plasma Fe (\( P < 0.001 \)) of primiparous cows was lower than that of multiparous cows, but plasma Fe of calves did not significantly differ by parity of dam or sex of calf. Plasma Mg (\( P < 0.01 \)) of primiparous cows at parturition was lower than that of multiparous cows, but plasma inorganic P and Cu were higher. Plasma Ca, inorganic P, Mg, Zn, and Cu of calves did not differ significantly by parity of dam or sex of calf.

Male calves developed low blood Hct and Hb at d 1 of age in the present experiment. In the previous study (7), blood Hb of female calves was higher than that of males at d 1 of age, but was not significantly different between sexes at d 6 of age. Thomas et al. (16) reported that female calves had higher blood Hb than male calves from birth to 75 d of age. Thus, sex of calf might be a factor for the low blood Hct and Hb of calves at parturition.

From 19,497 calvings in 10 large dairies, Berry et al. (3) reported that calf mortality within 48 h of
TABLE 1. Least squares means of blood hematocrit (Hct), hemoglobin (Hb), and plasma concentrations of minerals of calves at d 1 of age and of their dams at parturition.

<table>
<thead>
<tr>
<th>Animal, no.</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mo</td>
<td>27.9f</td>
<td>59.4a</td>
<td>4.2</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>282</td>
<td>281</td>
<td>1</td>
</tr>
<tr>
<td>Hct, %</td>
<td>33.0</td>
<td>33.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>11.4</td>
<td>11.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>10.1</td>
<td>9.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Fe, ppm</td>
<td>0.85f</td>
<td>1.39e</td>
<td>0.09</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>0.54</td>
<td>0.50</td>
<td>0.04</td>
</tr>
<tr>
<td>Cu, ppm</td>
<td>1.06a</td>
<td>0.93b</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**Parity of dam**

<table>
<thead>
<tr>
<th>Primiparous</th>
<th>Multiparous</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>42.2b</td>
<td>45.0a</td>
</tr>
<tr>
<td>Multiparous</td>
<td>33.1b</td>
<td>37.2a</td>
</tr>
</tbody>
</table>

**Sex of calf**

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.7d</td>
<td>11.2e</td>
<td>0.4</td>
</tr>
<tr>
<td>9.8b</td>
<td>11.1a</td>
<td>0.2</td>
</tr>
</tbody>
</table>

a,bMeans within dams or calves of same row with different superscript letters differ (P < 0.05).
b,cMeans within dams or calves of same row with different superscript letters differ (P < 0.01).
c,dMeans within dams or calves of same row with different superscript letters differ (P < 0.001).

*At birth.

Supplemental Lf for Calves

Colostrum mineral concentrations of primiparous and multiparous cows at parturition and at 96 h postpartum are shown in Table 2. The mineral variations in colostrum were similar to those reported previously (7, 8). Although colostrum is the main source of minerals for newborn calves after birth, colostral Fe is much lower than recommended intakes for newborn calves (7, 8). Colostral Fe was not determined in the present experiment, but colostral Fe varied with parity or breed and decreased as time postpartum increased (17).

Blood Hct (P < 0.001) and Hb (P < 0.001) of untreated calves decreased from 1 to 10 d of age (Figure 1). Plasma Fe of untreated calves was decreased (P < 0.01) by d 6 of age. In the present experiment, Fe intakes from the colostrum of untreated calves were estimated to be about 3 to 5 mg/d during the 1st wk of life. Because 40 mg of Fe/d has been reported (8, 9, 10) to be the dietary requirement for newborn calves, colostral Fe was much lower than that required for calves. Thus, insufficient colostral Fe decreased blood Hct and Hb of untreated calves during the first 10 d after birth, a result that is in agreement with our previous reports (7, 8).
TABLE 2. Least squares means of colostrum yield and colostrum composition of cows at parturition and at 96 h after parturition.

<table>
<thead>
<tr>
<th>Variable and time</th>
<th>Parity</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows, no.</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colostrum yield, kg</td>
<td>0 h</td>
<td>1.9b</td>
<td>3.1a</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>11.6b</td>
<td>16.5c</td>
<td>0.7</td>
</tr>
<tr>
<td>Protein, %</td>
<td>0 h</td>
<td>14.6</td>
<td>16.4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>246</td>
<td>222</td>
<td>14</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>0 h</td>
<td>132</td>
<td>139</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>205</td>
<td>192</td>
<td>11</td>
</tr>
<tr>
<td>P, mg/dl</td>
<td>0 h</td>
<td>119</td>
<td>112</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>246</td>
<td>222</td>
<td>14</td>
</tr>
<tr>
<td>Mg, mg/dl</td>
<td>0 h</td>
<td>36.5</td>
<td>36.3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>10.9</td>
<td>11.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Fe, ppm</td>
<td>0 h</td>
<td>1.8</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>0.8</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn, ppm</td>
<td>0 h</td>
<td>23.9</td>
<td>21.7</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>6.0</td>
<td>5.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Cu, ppm</td>
<td>0 h</td>
<td>0.34</td>
<td>0.34</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>96 h</td>
<td>0.31</td>
<td>0.27</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*abMeans within same row with different superscript letters differ (P < 0.05).

cdMeans within same row with different superscript letters differ (P < 0.001).

Blood Hct of calves treated with Fe decreased (P < 0.01) at d 2 of age, and blood Hct of calves treated with Fe or Fe plus Lf increased (P < 0.001) from 2 to 10 d of age (Figure 1). Blood Hb of calves treated with Fe (P < 0.001) or Fe plus Lf (P < 0.001) increased from 1 to 10 d of age. However, blood Hct and Hb (P < 0.01) of calves treated with Lf plus Fe were significantly higher than those of calves treated with Fe at d 6 of age. Plasma Fe of calves treated with Fe (P < 0.001) or with Fe plus Lf (P < 0.001) increased temporarily at d 2 of age. Plasma Fe of calves treated with Fe at d 2 of age was higher (P < 0.01) than that of calves treated with Fe plus Lf, but, at d 6 and 10 of age, plasma Fe of calves treated with Fe was significantly lower.

Miyata et al. (10) indicated that most Fe, whether administered orally or injected, was utilized by red blood cells, and only a small amount was assimilated by storage sites; also, neonatal calves responded well to oral administration. The administration of 40 mg of Fe/d as FeSO₄ from 1 to 5 d of age elevated blood Hct and Hb of calves during the first 10 d after birth in our previous report (8) and in the present experiment. These results suggest that Fe supplementation at ≥40 mg of Fe/d just after parturition is a good way to improve low blood Hct and Hb of calves at birth.

Additionally, the accelerated improvement of erythropoiesis might be needed to prevent high mortality of anemic newborn calves, because oral or in-

Figure 1. Blood hematocrit (Hct; SEM = 0.4), hemoglobin (Hb; SEM = 0.1), and plasma Fe (SEM = 0.1) of untreated calves (●), calves treated with 40 mg of Fe/d as FeSO₄ (■), calves treated with 40 mg of Fe/d and 5 g of lactoferrin/d (▲) from 1 to 5 d postpartum.
jected Fe for newborn calves did not improve low
blood Hct or Hb by 1 wk after administration (4, 5, 8,
9, 10, 12, 16). Colostral Lf functions as a source of Fe
for the neonate and is a potent antimicrobial factor in
the alimentary tract of the calf (17). We suggested
(8) that ferrous Fe is more effective in elevating
blood Hct and Hb than is Fe-saturated Lf in newborn
calves. However, blood Hct and Hb of calves at d 6 of
age were improved by the supplemental Lf with fer-
rus Fe in the present experiment.
Nagasako et al. (11) reported that ferrous iron
was easily changed to the insoluble ferric state, but
the solubility of ferrous Fe was stabilized by the
presence of Lf, and the hyper Fe-binding activity of Lf
might have some physiological role in Fe absorption.
In the previous study (8), plasma Fe of calves treated
with Fe-saturated Lf was constant just after adminis-
tration, but plasma Fe of calves treated with Fe plus
Lf remained higher at d 6 and 10 of age in the present
experiment. The mechanism of Lf that was responsi-
ble for the increased Fe absorption in newborn calves
was not evident, but supplemental Lf, except Fe-
saturated Lf, might bind Fe at sites other than its
chelate-binding sites (11) and might increase the Fe
absorption form the guts or the shift to blood Hb
rather than the storage tissues. These results suggest
that supplemental Lf with ferrous Fe is more efficient
in accelerating the shift of Fe into the blood Hb at an
early stage after administration by the stabilization
of ferrous Fe in the gastrointestinal tract or plasma of
newborn calves.

The health status of calves was not adversely af-
ected by the administration of Fe plus Lf or Fe in
the present study. Plasma Ca was not significant among
treatments, but plasma Ca of calves treated with Fe
or Fe plus Lf was significantly higher at d 2 of age
than at d 1 of age (Figure 2). No significant differ-
ences existed in plasma inorganic P or Mg at d 2, 6,
and 10 of age. Plasma Zn of calves treated with Fe
plus Lf at d 2 of age was higher ($P < 0.01$) than that
of calves treated with Fe, but plasma Zn of calves
treated with Fe at d 10 of age was significantly higher
than that of untreated calves (Figure 3). Plasma Cu
of calves treated with Fe or Fe plus Lf was higher ($P
< 0.001$) than that of untreated calves from 1 to 10 d
of age. In the present experiment, no consistent trend
was found for the changes of plasma Ca, inorganic P,
Mg, Zn, or Cu by the administration of Fe or Fe plus
Lf.

Teraguchi et al. (15) reported that Lf ad-
inistered to mice that had been fed milk suppressed
the initial proliferation of fecal bacteria, which re-
quired Fe for growth. The functions of abundant Lf in
colostrum might be a regulation of Fe absorption and
a bacteriostatic effect for newborn calves, but Lf con-
ten ts in the colostrum of primiparous cows were
lowest (17); calves born from primiparous cows de-
veloped low Hct and Hb at parturition. Further study is

Figure 2. Plasma Ca (SEM = 0.3), inorganic P ($P_i$; SEM = 0.3),
and Mg (SEM = 0.06) of untreated calves (●), calves treated with
40 mg of Fe/d as FeSO4 (■), and calves treated with 40 mg of Fe
plus 5 g of lactoferrin/d (▲) from 1 to 5 d postpartum.

untreated calves than did calves born from multiparous cows and were bulls had lower blood Hct and Hb at d 1 of age compared to calves that were heifers. Insufficient colostral Fe decreased blood Hct and Hb of calves during the first 10 d of life. The administration of 40 mg of Fe/d as FeSO₄ or 40 mg of Fe plus 5 g of lactoferrin/d from 1 to 5 d of age improved blood Hct and Hb of calves, but blood Hct and Hb of calves treated with Lf plus Fe were higher than those of calves treated with Fe at d 6 of age. Plasma Fe of calves treated with Fe plus Lf remained higher at d 6 and 10 of age. Thus, supplemental Lf with ferrous Fe might be a more efficient means to accelerate the shift of Fe into the blood Hb at an early stage after Fe administration.

ACKNOWLEDGMENTS

The authors thank Y. Fukuwatari and Morinaga Milk Industry Co., Ltd. for the supply of Lf and T. Kojima, R. Matsuda, N. Kobayashi, and staff of National Institute of Animal Industry for technical help and assistance in sample collection.

REFERENCES


CONCLUSIONS

Calves born from primiparous cows and calves that were bulls had lower blood Hct and Hb at d 1 of age than did calves born from multiparous cows and calves that were heifers. Insufficient colostral Fe decreased blood Hct and Hb of calves during the first 10 d of life. The administration of 40 mg of Fe/d as FeSO₄ or 40 mg of Fe plus 5 g of lactoferrin/d from 1 to 5 d of age improved blood Hct and Hb of calves, but blood Hct and Hb of calves treated with Lf plus Fe were higher than those of calves treated with Fe at d 6 of age. Plasma Fe of calves treated with Fe plus Lf remained higher at d 6 and 10 of age. Thus, supplemental Lf with ferrous Fe might be a more efficient means to accelerate the shift of Fe into the blood Hb at an early stage after Fe administration.

Figure 3. Plasma Zn (SEM = 0.06) and Cu (SEM = 0.04) of untreated calves (●), calves treated with 40 mg of Fe as FeSO₄ (▲), and calves treated with 40 mg of Fe plus 5 g of lactoferrin/d (▲) from 1 to 5 d postpartum.