Developmental Changes in Serum Ferritin Concentration of Dairy Calves

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ABSTRACT
Serum ferritin concentration of nursing calves was measured by a two-site immunoradiometric assay to assess developmental changes and to evaluate relationships of serum ferritin with iron-related blood characteristics. Serum ferritin concentration of calves was low at birth and elevated slightly at 3 days of age. From 1 wk of age onward, serum ferritin concentration of untreated calves and calves fed only whole milk for 4 wk decreased and remained low throughout the nursing period. This finding almost coincided with hematological characteristics. However, that nearly normal hematological measures and weight gain persisted and that they did not develop any anemic symptom indicate that serum ferritin concentration is more sensitive than other hematological characteristics to iron depletion. However, serum ferritin concentration of calves administered 130 mg of ferrous fumarate (40 mg iron) daily from 3 to 22 days of age or injected with 4 ml of iron-dextran intramuscularly (400 mg iron) at 3 days and 2 wk of age increased sharply just after treatment and persisted high for 2 to 6 wk of age. However, there was large variation between animals for serum ferritin concentration. Thus, it seems likely that serum ferritin concentration is an index for monitoring prelatent iron deficiency of calves.

INTRODUCTION
Nursing calves fed only whole milk often develop anemia within a few weeks (3, 5, 20, 26). In the veal industry especially, where milk diet is commonly fed and rapid growth occurs, iron deficiency anemia is common (2, 4, 15, 17). Also, where iron deficient, susceptibility of calves to some pathogenic agents increases (13, 21). Administration of iron compounds to newborn calves is effective for calf growth and hematological aspects (5, 12, 25, 27, 30, 31). Thus, economic damage from iron deficiency is considerable. In the earlier studies, characteristics in blood for monitoring iron nutritional status were hemoglobin (Hb), hematocrit (Ht), red blood cells (RBC), serum iron (SI), and total iron-binding capacity (TIBC). There were close relationships among measurements in blood and storage iron in calves (29).

More recently, ferritin concentration in blood serum has attracted attention as a useful survey tool to monitor iron status, because ferritin in blood serum is directly proportional to iron stores under normal condition (32). In previous papers (7, 18) authors reported a highly sensitive two-site immunoradiometric assay measuring ferritin concentration in blood serum of dairy cows and bulls to assess their iron nutritional status. Also, our study on serum ferritin of nursing piglets indicated that circulating ferritin is an indicator for assessment of storage iron (8). Although there is considerable evidence on blood characteristics of nursing calves, only limited data are available on serum ferritin levels of calves.

Objectives of this study were to assess developmental changes in serum ferritin of calves and to evaluate relationships of serum ferritin with iron-related blood characteristics.

MATERIALS AND METHODS

Animals and Samples
Twenty male and 20 female Holstein calves were allotted into 4 groups of 10 calves by weight and sex. They were housed in individual calf pens on concrete and bedded with straw for the 13-wk experiment. Pens were cleaned every morning. Water was available ad libitum. The feeding program for calves is in Table 1. Calves were full fed colostrum three times a day for 1 wk and thereafter fed whole milk twice a
TABLE 1. Feeding program for calves.

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<tr>
<th>Weeks of age</th>
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<tr>
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<td>Calf starter, kg/day</td>
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<td>Concentrate, kg/day</td>
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<tr>
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<td>Calf starter</td>
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<td>Concentrate, kg/day</td>
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<td>.4</td>
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<td>Orchardgrass hay</td>
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a Fed colostrum ad libitum.

b Oral administration of 130 mg of ferrous fumarate (40 mg iron) daily.

c Injected iron-dextran (400 mg iron) intramuscularly.

day. Groups 1, 2, and 3 were on similar feeding programs. They were fed calf starter after 1 wk of age. Relative to the other groups, group 4 received additional whole milk and no starter until 4 wk of age. All calves were fed orchardgrass hay for ad libitum consumption after 1 wk of age. They were weaned at 9 wk of age and fed concentrate after weaning. Group 1 received an oral dose of 130 mg of ferrous fumarate (40 mg iron) daily from 3 to 22 days of age. Group 2 received 4 ml of iron-dextran intramuscularly (400 mg iron) at 3 days and 2 wk of age. This iron level was based upon dietary requirement of calves of 40 mg/day per head reported by Matron et al. (17), Roy et al. (28), and Hansard (10). Iron content of the rations was 283 ppm for calf starter, 179 ppm for concentrate, and 190 ppm for orchardgrass hay dry matter.

Observation and sampling of calves were at birth, 3 days, 1, 2, 3, 4, 6, 8, and 13 wk of age. Blood samples were collected by jugular puncture into nonheparinized and heparinized tubes. The RBC were counted by TOA microcell-counter (Model CC-108, TOA Iyo Denshi, Kobe) and Hb, Ht, and plasma total protein (TP) were determined as in (7). Serum was extracted from whole blood and frozen at −30°C for later analysis of SI, TIBC, and serum ferritin. The SI and TIBC were determined as in (7). However, SI and TIBC of calves administered iron-dextran intramuscularly was not measured because of difficulty of separation of Fe from iron-dextran complex.

**Two-Site Immunoradiometric Assay for Bovine Serum Ferritin**

Serum ferritin concentration was determined by a two-site immunoradiometric assay as in (7). Bovine ferritin was isolated from bovine spleen by the method of Penders et al. (24) and purified by high speed liquid chromatography.
(HLC-803A, Toyo Soda, Tokyo). The antisera against purified bovine ferritin was obtained from rabbit immunized by repeat subcutaneous injections of purified ferritin mixed with Freund's complete adjuvant. Gamma globulin fraction was salted out with Na_2SO_4, followed by affinity chromatography on a ferritin-coupled CNBr-activated Sepharose 4B column. A part of purified anti-bovine spleen ferritin antibody was coupled on silicone rubber strings (3 mm in diameter, 4 mm long). The other part of purified antibody was labeled with radioactive ^{125}I. Iodination of purified anti-ferritin antibody was according to the method of Niitsu et al. (23). Ferritin standard solutions and two- or threefold diluted serum samples were sandwiched between two antibodies, and radioactivity of antibody-antigen-antibody linkage of silicone rods was counted by an auto gamma scintillation counter (LKB Wallac, Turku). All samples were analyzed at least in triplicate. A standard curve for this assay showed a positive relation with ferritin concentration over a range of .1 to 500 ng/ml.

RESULTS AND DISCUSSION
Changes in body weight of calves are shown in Table 2. Daily gains of calves in groups 1, 2, 3, and 4 were .84, .75, .82, .78 kg. No difference was significant among daily gains of four groups (P<.05).

Hematological changes of calves are shown in Figures 1 and 2. The RBC, Hb, and Ht of untreated calves and calves fed only whole milk declined from birth to 1 wk of age. From 1 wk of age onward, RBC gradually increased, whereas Hb and Ht remained low. However, calves did not show any anemic symptom. The Hb and Ht increased gradually from 6 wk of age. These results are similar to (2, 4, 15, 17). Thus, it seems likely that the neonatal anemia of calves does not develop severely in a short period, as compared with that of piglets (6, 8). Under the present condition, severe iron deficiency may develop when calves are reared on exclusive milk diet for a long time.

However, hematological measurements of calves administrated iron orally or intramuscularly increased sharply just after treatment and reached maximum at 2 to 4 wk of age. The RBC, Hb, and Ht were higher (P<.01) for treated calves than for untreated calves during this period. According to ferrokinetic studies on calves (11, 14, 22), maximum uptake by red blood cell of radioactive ^{59}Fe has been 12 to 16 days after iron injection. Our findings almost coincided with the ferrokinetic data. However, no difference was significant between oral and injected groups for RBC, Hb, and Ht. Mean corpuscular volume (MCV) declined slightly from birth to 8 wk of age. The MCV was higher for iron treated calves than for untreated calves between 1 wk and 6 wk of age. Mean corpuscular hemoglobin (MCH) declined gradually from birth to 6 wk of age. The MCH was not affected so sharply as MCV by iron treatment. Mean

<table>
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<th>TABLE 2. Changes in body weight of calves.</th>
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<td><strong>Groups</strong></td>
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<td>Group 1^a</td>
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<td>Group 2^b</td>
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<tr>
<td>Group 3^c</td>
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<td>Group 4^d</td>
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</table>

^aFed calf starter after 1 wk of age, weaned at 9 wk of age, and received an oral dose of 130 mg of ferrous fumarate (40 mg iron) daily from 3 to 22 days of age.

^bFed calf starter after 1 wk of age, weaned at 9 wk of age, and received 4 ml of iron-dextran intramuscularly (400 mg iron) at 3 days and 2 wk of age, respectively.

^cFed calf starter after 1 wk of age, weaned at 9 wk of age.

^dFed calf starter after 4 wk of age, weaned at 9 wk of age.

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corpuscular hemoglobin concentration (MCHC) was relatively low at birth and elevated at 3 days of age, and thereafter it remained almost unchanged throughout the experiment. Erythrocyte of calves show microcytic and normochronic changes during the nursing period. Changes in TP, SI, and TIBC are shown in Figure 3. The TP and serum transferrin concentration increased rapidly from birth to 1 wk of age and thereafter plateaued. Although intense and
immediate change of TP occurs as a result of intact absorption from the small intestine, elevation of serum transferrin is derived from endogenous sources (16). Developmental changes in TIBC are similar to those reported by Martinsson et al. (16). The TIBC was slightly higher in calves fed only whole milk than in untreated calves and calves administered iron orally from 2 to 4 wk of age. The SI was relatively low at birth and 3 days of age. The SI of untreated calves and calves fed only whole milk increased gradually from 1 wk of age and was relatively constant after 4 wk of age. However, SI of calves administered iron orally increased temporarily with a peak at 1 wk of age ($P < .01$). This finding is consistent with that
of Getty et al. (9), but after 2 wk of age, SI of treated calves was not different from that of untreated calves.

Developmental changes in serum ferritin concentration in blood serum of dairy calves from birth to 13 wk of age are presented in Figure 4. Serum ferritin concentration of calves was low at birth and elevated slightly at 3 days of age. This is similar to that of nursing piglets (8,19). From 1 wk of age onward, ferritin concentration in blood serum of untreated calves decreased and remained below 10 ng/ml throughout the experiment. Similarly, serum ferritin concentrations of calves fed only whole milk was less after 1 wk of age compared with untreated animals, and serum ferritin almost disappeared from circulation from 2 to 4 wk of age. Ferritin concentration in blood serum of calves administered iron orally or intramuscularly increased rapidly in a short period and persisted high for 2 to 6 wk of age. However, there was a large variation between animals in serum ferritin concentration. Thereafter, it declined linearly to 13 wk of age. No difference in serum ferritin concentration was observed between treated and untreated animals at 13 wk of age. This indicates that active iron absorption occurs in intestine of neonatal calves and rapid growth accelerates in iron depletion of storage sites following low ferritin concentration in blood serum. This finding also indicates that most iron administered orally or injected is utilized
by red blood cells with only a small amount taken up by storage sites, because erythropoiesis of nursing calves is active (1, 11, 14, 22). Thus, it seems that calves respond well to oral administration in the neonatal period. Although untreated calves and calves fed only whole milk had low serum ferritin throughout the nursing period, nearly normal range of hematological measures and weight gain persisted. Accordingly, serum ferritin concentration is more sensitive than other hematological characteristics to iron depletion. Thus, it seems likely that serum ferritin concentration is an index for monitoring prelatent iron deficiency of calves.

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REFERENCES


