Ferritin in Blood Serum of Dairy Cows

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ABSTRACT
Concentration of ferritin in blood serum of dairy cows was measured by a two-site immunoradiometric assay to assess changes in the iron nutritional status during gestation, parturition, and lactation. Although anemia did not occur in pregnancy of dairy cows, there were slight decreases of red cell counts, hemoglobin, and hematocrit in the early stage of lactation. Ferritin concentration remained relatively constant in late gestation (35 ng/ml), but deviations were considerable. Ferritin rose gradually from 3 days prepartum with a sharp elevation after parturition. At 1 to 2 wk postpartum, it had increased to about twice amounts in late gestation. During the subsequent 8 wk postpartum, it fell gradually and thereafter maintained almost unchanged (40 ng/ml). Both iron in blood serum and total iron-binding capacity declined from 2 wk prepartum to the end of gestation but showed a rise beginning about 2 wk after parturition. Because changes in iron-related proteins just before and after delivery may be results of inflammatory reactions accompanying delivery, ferritin concentration is not a good index for diagnosis of iron deficiency in lactating cows just after parturition.

INTRODUCTION
Ferritin in blood serum is in equilibrium with tissue ferritin, and it is directly proportional to iron stores under normal conditions (11). Walters et al. (21) demonstrated that 1 ng/ml of serum ferritin represents about 8 mg of storage iron. Similarly, correlation was high negative between logarithm of ferritin concentrations and iron absorption rates (2, 4, 9, 20). Accordingly, ferritin is a useful survey tool for monitoring of iron stores.

A highly sensitive immunoradiometric assay measuring ferritin concentration (1, 8, 12, 15, 23) is used to study diagnosis of iron deficiencies in humans. Ferritin concentration in rats and dogs also has been studied extensively (3, 10, 18, 22). However, investigations of ferritin concentration in dairy cows have not been reported. Although iron deficiency seldom occurs in dairy cows, the iron nutritional status of dairy cows may be influenced by their physiological conditions: gestation, parturition, and lactation.

The objective of our study was to assess changes in nutritional status of dairy cows by measuring ferritin concentration in blood serum by two-site immunoradiometric assay by antibody-coated silicone rods.

EXPERIMENTAL PROCEDURE

Animals and Samples
The experiment was with 13 lactations from 10 Holstein cows. Ten cows were used with 3 of them used twice, in first and second lactations. They were placed in individual stalls with gum matted floors and brought to a concrete yard near the barn for exercise once a day. All cows were fed concentrate diet, hay cubes, beet pulp, and timothy hay to meet the dietary requirements for maintenance, production, and reproduction (16). Experimental rations had ratios of concentrate to roughage (dry matter) 60:40 to 30:70. Block salt and water were provided free choice. The iron content of a ration was 170 ppm for concentrate, 570 ppm for hay cubes, 395 ppm for beet pulp, 105 ppm for timothy hay, and 400 ppm for block salt.

Observations and samplings of the cows were at 5, 4, 3, 2, and 1 mo, 2 and 1 wk, and 1 to 3 days before parturition; at parturition; then 3 days, 1, 2, 3, 4, 6, and 8 wk and 3, 4, 5, 6, and 7 mo after parturition. Blood samples were col-
lected by jugular puncture into nonheparinized and heparinized tubes. Red blood cells (RBC) were counted by a TOA microcell-counter (Model CC-108). Hemoglobin (Hb) and hematocrit (Ht) were determined as in (6). Serum was extracted from whole blood and frozen at −20°C for later analyses for the following constituents: iron (SI), total iron-binding capacity (TIBC), protein (SP), and ferritin. The SI, TIBC, and SP were determined as in (6).

Measurements of Bovine Serum Ferritin

Preparation of Bovine Ferritin and Antibody. Bovine ferritin was prepared from spleen. A part of the spleen was homogenized with four parts of distilled water, and the homogenate was heated at 70 to 75°C for 10 min. After centrifugation at 1,500 × g for 20 min, the supernatant was passed through a Millipore filter (pore size, .45 μm) and ultracentrifuged by an automatic preparative ultracentrifuge (65P, Hatchi, Tokyo) as described by Penders et al. (17). The precipitate then was dissolved in 15 mM phosphate-buffered saline (PBS buffer, pH 7.4) and chromatographed successively on a column of high-speed liquid chromatograph (HLC – 803A, Toyo soda, Tokyo). Ferritin fraction was concentrated by collodion bag (SM 13200, Sartorius Membrane Filter, Göttingen, West Germany). Purity of the specimens was examined by electrophoresis on 6% polyacrylamide gel (pH 8.3). Thus, ferritin preparation was electrophoretically homogenous. Concentrations of purified ferritin were estimated by the method of Lowry et al. (14).

A .5 mg sample of purified spleen ferritin dissolved in 1 ml of PBS buffer was mixed with the same volume of Freund's complete adjuvant (Iatron, Tokyo). The mixture then was injected subcutaneously into the back of adult male white rabbits followed 3 wk later by a second dose of 1 ml. Serum was collected 6 wk after initial injection. Sera obtained 2 wk after second injection had adequate activity for quantitative immunoreaction and was stored at −30°C.

Preparation of Antibovine Spleen Ferritin Antibody. The IgG fraction from the antisera was separated by fractionation with Na2SO4, followed by chromatography on a ferritin-coupled CNBr-activated Sepharose 4B column. Bovine spleen ferritin was coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden). After a complex had been formed between bound ferritin and antibody, the antibody was separated from the immune complex by 3M potassium thiocyanate. It immediately was dialyzed against PBS buffer and concentrated by collodion bag.

Antibovine Spleen Ferritin Antibody Coupled to Silicone-Rubber Strings. Silicone rubber strings (3 mm in diameter, Kunii Co., Ltd., Tokyo, Japan) were cut into pieces 4 mm long, carefully washed with .1% sodium dodecyl sulfate overnight, extensively rinsed with hot water (60°C), and incubated with antibovine spleen ferritin antibody (8 μg/ml) in sodium phosphate buffer (.25 M/liter, pH 7.5) for 30 min at room temperature in a beaker. The antibovine spleen ferritin antibody coupled silicone rubber strings were washed three times with .25 M sodium phosphate buffer, then three times with 10 mM/liter sodium phosphate buffer containing 5.8 g of NaCl, .2 g of MgCl2, 1 g of Na3, and 1 g of bovine serum albumin per liter (pH 7.0). They were stored in the buffer at 4°C until use.

Iodination of Antiferritin Antibody. Antibovine spleen ferritin antibody (20 μg/.03 ml) was incubated with 1 mCi/.01 ml of Na125I, .05 ml of .5 M sodium phosphate buffer and 20 μg/.01 ml of chloramine-T at room temperature for 1 min. After the reaction was stopped by 100 μg/.05 ml of sodium metabisulfite,.005 ml of 1 M KI was added to the reaction mixture. The labeled antibody was separated from free iodine-125 by Sephadex G-75 column equilibrated in TEN buffer (.02 M Tris-HCl, pH 7.6, .01 M EDTA, .1 M NaCl) containing 1% bovine serum albumin.

Two-Site Immunoradiometric Assay for Bovine Serum Ferritin. The schematic procedure of this assay system is in Figure 1. The silicone rubber strings loaded with antibovine ferritin antibody were incubated with 50 μl of the sample serum and a series of seven concentrations of bovine spleen ferritin, from .1 to 500 ng/ml, in a final volume of .25 ml of incubate buffer (1 g of bovine serum albumin, 1 ml of normal rabbit serum, and 200 mg of sodium azid per liter of PBS). After tubes were shaken at 37°C for 3 h in horizontal shaker, the silicone rubber strings then were washed three
times with 1.0 ml of the incubation buffer. A .2 ml sample of $^{125}$I-labeled antibody (approximately 20,000 cpm) was placed in the tubes and again incubated in the rotating shaker at $4^\circ$C for 18 h. The silicone rubber strings then were washed four times with 1.0 ml of .9% NaCl. Radioactivity of the tubes was measured by an LKB Wallac autogamma scintillation counter. A standard curve for this assay system showed a positive relation to ferritin concentration over a range of .1 to 500 ng/ml (Figure 2). All samples were analyzed at least in triplicate.

RESULTS AND DISCUSSION

Changes in the hematological measures are in Figures 3 and 4. Although RBC counts, Hb, and Ht in late pregnancy tended to remain constant, they temporarily were elevated at parturition. From 3 days postpartum to 3 wk postpartum, RBC counts, Hb, and Ht decreased gradually, followed by a slight rise during the subsequent 5 wk. Accordingly, they were lower in early lactation than in late gestation periods. Serum protein was relatively low in late pregnancy, but immediately after parturition it increased sharply and then remained nearly constant. Stage of gestation and lacta-
Figure 3. Changes in red cells (RBC), hemoglobin (Hb), hematocrit (Ht), and serum protein (SP) of dairy cows before, at, and after parturition (P). Mean ± standard error.

Figure 4. Changes in mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) of dairy cows before, at, and after parturition. Mean ± standard error.

Figure 5. Changes in serum iron (SI), total iron-binding capacity (TIBC), and unsaturated iron-binding capacity (UIBC) of dairy cows before, at, and after parturition. Mean ± standard error.

Figure 6. Changes in serum ferritin of dairy cows before, at, and after parturition. Mean ± standard error.

Anemia in pregnancy was observed in several animals, principally due to a rise in plasma volume greater than the rise in red cell volume in late gestation, but in contrast to human females, all reported cases of anemia were moderate (7). Because increase in blood volume during gestation of dairy cows is almost proportional to plasma volume and body weight in-
creases, no anemia develops during gestation (13, 19). In our study, no decline of blood measures was observed as parturition approached. In addition, TIBC as well as SI declined progressively from 2 wk before delivery. Ferritin concentration remained relatively constant throughout the gestation period. Accordingly, it seems likely that anemia does not occur in pregnancy of dairy cows if dietary iron is supplied.

At parturition, RBC counts, Hb, and Ht concentrations were elevated greatly with no significant change in serum protein. This phenomenon may be caused by rapid mobilization of red cells from storage sites in spleen and bone marrow effected by the physiological stress of delivery. The TIBC declined parallel with SI during gestation, showing the lowest 3 days to 1 wk postpartum. This coincides with the rapid increase in ferritin just before and after delivery. Because inflammation causes an inverse relationship between ferritin and TIBC and SI (5), changes in the iron-related proteins may be the result of inflammatory reactions in uterus and udder accompanying delivery. Because the reproductive tract of dairy cows requires at least 30 days to return to a normal state for rebreeding, the circulating ferritin concentration is not a good index for a diagnosis of iron deficiencies in lactating cows just after parturition. Accordingly, ferritin concentration is not a measure for the size of iron stores in the early stages of lactation.

After parturition, the slight decline of the hematologic measures paralleled increases of TIBC and UIBC, but red cell indices were not affected, showing normocytic and normochronic cells. This may indicate that there was a slight iron imbalance in early stage of lactation. From the ferritin determination, however, no iron deficiency was observed in late gestation and lactation periods of the dairy cows. Ferritin of dairy cows is lower than in human and rats but shows little difference between cows and bulls (Y. Miyata, K. Furugouri, and K. Shijimaya, unpublished data).

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REFERENCES


