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Effects of Parenteral Supply of Iron on RBC Parameters, Performance, and Health in Neonatal Dairy Calves

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Abstract The present study was designed to evaluate the effects of parenteral iron on red blood cell parameters, performance, and health in dairy Holstein calves. Twenty neonatal calves were equally divided at random into two groups, one of which served as controls. Care was taken to ensure homogeneity of sex, age, and general health status of the animals. The controls received a normal diet and water ad libitum, while the study animals were injected with 1 g iron as Fe-dextran 2 days after birth. A daily record was kept of the calves' weight and growth parameters. At periods of 24–48 h after birth and at 14, 28, and 42 days of age, jugular blood was drawn from all the experimental and control animals to measure the packed cell volume, red blood count, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and iron levels. At the start of the experiments, there were no significant differences between these parameters between the two study groups ($p>0.05$). With time, significant differences were seen between most of the values measured ($p<0.05$) except for the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and iron level. Significant differences were seen for total weight gain and mean daily weight gain, which were higher in the iron-supplemented group ($p<0.05$).

Keywords Iron · Neonatal dairy calves · Hematological parameters · Health · Supplementation · Weight gain

Introduction

Iron is an essential component of hemoglobin, myoglobin, and several enzymes such as catalase, peroxidase, and cytochrome oxidase [1]. Iron requirement for domestic animals

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are influenced by age, growth rate, availability of dietary iron source, and the criteria of adequacy. The iron requirements of ruminants are not well established and most recommendations are estimate [2]. It is generally accepted, however, that the iron requirements of young animals are higher than those of mature ruminants are and thought to be about 100 ppm. Deficiencies are most likely to occur in neonates because cows' milk is low in iron (about 10 ppm). The iron reserves of the calf, which are primarily in the liver, are generally sufficient to prevent serious anemia if calves are fed dry feeds beginning at a few weeks of age. When calves are fed a milk diet exclusively for several weeks, then they may develop iron deficiency anemia, which can adversely affect growth and feed conversion [3]. Iron deficiency is associated with numerous clinical signs, including anemia, reduced growth, and increased occurrence of diseases. It was showed that Fe deficiency anemia is frequent problem in veal calves but not in calves fed roughage, concentrates, and mineral/vitamin supplements [4]. However, Bunger et al. [5] concluded that unrestricted feeding on concentrate rich in iron during colostral feeding period did not prevent Fe deficiency and believed that anemia developing in the first month of life was not physiological but was due to Fe deficiency. Numerous studies showed that the administration of iron to calves (orally or parenterally) provided an increase in hematological parameters and a better growth in calves [6–15]. On the other hand, there is a report that indicated iron supplementation (injection) had no effect on red blood cell (RBC) parameters and health of supplemented calves [16].

The general opinion in heifer replacement system in dairy herds is that iron level is sufficient in diet of neonatal calves, since calf starter contain concentrate, mineral, and vitamin supplement, and high-quality hay was added to diet as soon as possible. In this feeding system, activation of rumen is desirable to shift diet from milk to dry feed. This study was performed to clarify whether injection of 1,000 mg iron dextran after birth in neonatal dairy calves in heifer replacement system fed roughage and concentrate could promote healthy RBC parameters, health, and performance in non-anemic calves.

Materials and Methods

The study was conducted in a dairy herd with approximately 600 calves per year at Mashhad suburb (northeast of Iran). This herd consisting of pure bred of Holstein breed was totally confined in open shed housing without access to pasture. Dry cows were fed with alfalfa hay (20.08 kg), corn silage (11.4 kg), and concentrate (1.25 kg) containing barley, cotton seed, bran, beet root, and 1% DM supplement.

Cows were dried 2 months before expected time of parturition and transferred to a separate stall. As the time of parturition approached, the cows were moved to straw-bedded maternity pen. Prompt assistance was given to cows with dystocia. Following parturition, the umbilicus of each calf was treated with povidone iodine, and the calf was weighed and transferred to individual pen. Within the first hour of life, 2 kg of pooled herd colostrum was fed by nipple bottle. Colostrum feeding was continued every 2 h with 1.5 kg of colostrum for the next 6 h of life and every 12 h in second day of age. Then, herd milk was substituted for feeding (2 kg twice daily) until 30 days of life. After this time, calves were fed by milk replacer (Table 1) twice daily (a total of 4 kg per day) until 90 days of life. Calf starter includes concentrate (90% DM) and high quality alfalfa (10% DM), and water was offered free choice, which started from 48 h of life after transferring to individual pen. The constituents of calf starter are shown in Table 2. The calves were weaned at 80 days of life. The heifer calves were mainly used as herd replacements.

Table 1 Ingredient Composition of Milk Replacer (DM%)

Ingredients	Percentage
Milk product	80
Vegetable fat	15
Premix of vitamins, mineral, etc.[a]	5

[a] Each kg milk replacer contains vitamin A (55,000 IU), vitamin D_3 (4,500 IU), vitamin E (80 mg), vitamin C (120 mg), vitamin B_1 (16 mg), vitamin B_2 (10 mg), vitamin B_6 (8 mg), vitamin B_{12} (40 μg), vitamin K (6 mg), niacin (50 mg), panthothenic acid (25 mg), choline chloride (1,900 mg), probiotic (1.4×10^6 CFU), iron (110 mg), copper (17 mg), manganese (26 mg), zinc (150 mg), cobalt (2 mg), iodine (0.6 mg), and selenium (0.3 mg)

Twenty neonatal calves were equally divided at random into two groups, one of which served as controls. Care was taken to ensure homogeneity of sex, age, and general health status of the animals. The controls received a normal diet and water ad libitum while the study animals were injected with 1 g iron as Fe-Dextran (Oterop, Belgium) 2 days after birth. A daily record was kept of the calves' weight and growth parameters.

Ten milliliters of jugular blood was taken from all calves 24–48 h after birth and at 14, 28, and 42 days of age for measuring hematological parameters and iron concentration. Blood (2.5 ml) was anticoagulated with EDTA for hematological analysis (FL medical, Italy), and plain tubes supplied serum for analysis. The serum was harvested after centrifugation at 1,800×*g* for 10 min and stored at −20°C until analysis. Anticoagulated blood was analyzed shortly after collection for number of RBC, hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) by an automatic hematology cell counter (Abbott, Cell dyne 1700, USA). The amount of iron was measured by commercial kit [Pars Azmoon, Tehran, Iran (Ferene S method)] using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy.

For evaluation of growth and health, body weight of all of the calves was measured at birth, and each sampling time and days of treatment for each calf were recorded at the end of the study.

Statistical analysis was conducted using SPSS for windows (release 9, SPSS Inc, Chicago, USA). Age effect was examined using ANOVA. All analysis was corrected for repeated measurements and included age and group as fixed factors and calf as random factor. Parametric *t* test was used to investigate significant difference between groups for

Table 2 Ingredient Composition of Concentrate Mix Fed to Calves (DM%)

Ingredients	Percentage
Corn	50
Barley	15
Soybean meal	22
Beet pup	3
Wheat bran	3
Molasses	5.5
DCP	0.2
Limeston	0.9
Supplement[a]	0.4

[a] Each kg of supplement contains vitamin A (50,000 IU), vitamin D_3 (10,000 IU), vitamin E (0.1 g), calcium (196 g), phosphorus (96 g), sodium (71 g), magnesium (19 g), iron (3 g), copper (0.3 g), manganese (2 g), zinc (3 g), cobalt (0.1 g), iodine (0.1 g), and selenium (0.001 g)

Table 3 Effects of Parenteral Iron Supply on Measured Parameters

Parameters	Control	Test	SE	Group	Age	Group^{x} age
PCV (L/L)	0.32	0.33	0.01	NS	S	NS
RBC (10^{12}/L)	5.24	5.45	0.21	NS	S	NS
Hb (g/L)	88.6	98.3	4.3	NS	S	NS
MCH (pg)	17.11	18.25	0.63	NS	NS	NS
MCHC (%)	28.53	30.41	1.1	NS	NS	NS
Fe (μg/dL)	71.74	78.56	4.59	NS	NS	NS
Weight (kg)	43.38	46.73	1.31	NS	S	NS

NS non-significant effect ($p>0.05$), *S* significant effect ($p<0.05$)

total weight gain and total daily gain. Chi-square test was also used for comparison of disease occurrence between groups. $p\leq0.05$ was considered as significant.

Results

The results are summarized in Tables 3 and 4. Group had no significant effect on the amounts of PCV, RBC, hemoglobin, MCH, MCHC, weight, and iron concentration ($p>0.05$). Age (sampling time) had significant effects on the values of all measured parameters ($p<0.05$) except MCH, MCHC, and iron concentration. Significant interactions between sampling time and group were not seen for any of measured parameters ($p>0.05$).

Significant differences were seen for total and mean daily weight gain between trial groups. The test calves had a better weight gain in comparing to control group ($p<0.05$). Chi-square test revealed no significant difference for the percent of days of treatment between trial groups.

Discussion

Sufficient iron content in the diet is necessary for the production of RBC and Hb. A progressive reduction in quantities of HCT, RBC, and Hb occurs over the first weeks of life [14, 16–18]. Bomba et al. reported the reduction in RBC parameters and serum iron in calves fed milk replacer, hay, and grain mix. The authors believed that the most critical period of anemia was 3–5 weeks of age [19]. In a study on blood parameters among calves reared by different methods, calves that represented a practice common to the rearing for dairy herd replacement had significantly higher values of RBC parameters, iron, and saturation of transferrin and significantly lower TIBC level than other trial groups [20].

Table 4 Mean ± SE of Total Performance and Health Between Trial Groups

Parameters	Control	Test	*p* value
Total weight gain (kg)	8.17±3.66	13.00±0.56	0.021*
Total daily gain (kg)	0.194±0.036	0.309±0.013	0.021*
Treatment days	4.5±1.45	4.0±1.25	0.797

*$p<0.05$ significant difference

Various studies reported that administration of iron provided an increase in RBC parameters in calves [4, 7, 9, 10, 13, 14, 21].

The results of Bostedt et al. [13] indicated that the administration of 1,000 and 1,500 mg Fe-dextran provided a lasting peripheral iron concentration and an increase in hemoglobin concentrations. These effects were observed during 6 weeks of the study, but the control group exhibited a significant decrease in peripheral iron and hemoglobin concentrations over the same period. On the other hand, Heidarpour Bami et al. [16] suggested non-significant higher RBC parameters in calves received 1,000 mg iron dextran parenterally. In the present study, administration of iron in the test group similarly provided an increase in RBC parameters although the difference was not significant with control group. These changes could be attributed to the iron levels and husbandry system of calves. These results suggest that the iron level in the diet of the control group was not sufficient for perfect normal erythropoiesis although it was sufficient for prevention of anemia.

In the present study, the amounts of MCH and MCHC were higher in the test group than those in the control group, although the difference was not significant. In agreement with our results, no significant difference was reported for MCV and MCHC between control calves and calves supplemented with oral iron as ferrous sulfate at a rate of 150 mg/daily for 28 days [14]. Similar results were also reported for RBC indices following iron injection (1,000 mg) in neonatal dairy calves [16].

Concerning non-significant differences between RBC parameters and indices between test and control groups, it is important to consider the sample size effect. It seems that at the current sample size of ten for each group, the statistical tests were not sensitive enough to detect differences (beta error). To detect a difference based on desired effect size with standard levels of power and alpha error, the sample size needed to be higher than ten.

The occurrence of a progressive reduction in serum Fe concentration over the first few days of life was reported by several studies [22–24]. Various studies reported that the administration of iron (orally or parenterally) provided an increase in serum iron concentration in calves [6, 9, 13, 14, 19, 21, 25, 26]. In the present study, the administration of iron provided no significant changes in serum Fe concentration between trial groups. It may be due to a 12 to 13 days interval between iron injection and the first measurement of the concentrations in serum.

Mollerberg et al. [6] and Bostedt et al. [13] reported that there were no difference in the body weight between the iron-supplemented and control calves. In another study, there was no significant effect of iron injection in average daily gain from birth through 15 weeks in beef calves in comparing with those that were not received iron injection [8]. In agreement with the results of the present study, Geisser et al. [10] and Sakozy et al. [7] reported that, by the administration of iron, a better weight gain resulted. Better total and daily weight gains were also reported following oral supplementation of iron at a dose rate of 150 mg/day for 28 days in neonatal dairy calves [15]. Better total, weekly, and daily weight gain were reported in neonatal dairy calves after parenterally supplementation of iron dextran at a dose rate of 1,000 mg/calf [16]. There was a marked growth response to the administration of iron even in calves that were fed hay and grain in addition to milk [15, 16]. It seems that sufficient iron is needed for normal appetite, secretion of IGF-I and tri-iodothyronine, and glucose utilization [20, 27, 28].

Iron is a nutrient related to health and immunity. However, except for anemia prevention, the role of iron in maintaining health in calves is not still completely clear. In veal calves with sub-normal body iron level, higher prevalence of infectious diseases was reported [6, 9, 11]. Bunger et al. [9] reported that the prevalence of pneumonia and diarrhea and the frequency of treatments for these diseases were higher in the group of calves that were not

supplemented with iron than the calves that were given oral iron supplementation. On the other hand, Mohri et al. [15] suggested that oral iron supplementation at a dose rate of 150 mg/day for 28 days had no effect on health and days of treatments between trial groups. Similarly, Heidarpour Bami et al. [16] reported parenteral administration of iron (1,000 mg iron dextran) after birth had no significant effect on health of injected calves in comparing with control animals.

In the present study, better weight gain in calves that received iron injection indicated that RBC parameters alone were not sensitive and reliable indicators of iron sufficiency for health status in calves since anemia did not exist but higher iron supplementation resulted to better weight gain. Anemia may not be used to determine iron adequacy because use of dietary iron for hemoglobin synthesis can take precedence over demands for other iron compounds, as iron is preferentially shunted from other iron pools to hemoglobin synthesis. Thus, hemoglobin may be the last pool to show the effects of iron inadequacy [29].

Probably, in non-anemic calves, the levels of RBC parameters and gain could be affected by additional iron supplementation. It may be needed to reevaluate the appropriate amount of iron for maximum erythropoiesis, health, and performance for calves in heifer replacement system.

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