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ORIGINAL PAPER

DETERMINATION OF SERUM IRON LEVELS AND HEMOGLOBIN CONCENTRATION IN PRE-WEANING CALVES*

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Abstract

The aim of this study was to evaluate the serum iron (Fe) levels and hemoglobin (Hb) values in Holstein-Friesian calves with different types of milk diet. In total, 20 dairy calves (10 from each farm) were used for the experiment on two dairy farms. One farm provided the calves with a milk replacer twice a day from day 3 until weaning (farm 1), and the other one fed the calves native milk twice a day for three weeks before the milk replacer was administered (farm 2). Blood samples were collected three times, first at the age of 2-7 days, second time at the age of 30 days and last time at the age of 56 - 60 days, for the determination of iron and hemoglobin levels. Our experiment included monitoring of the occurrence of diarrheal and respiratory diseases in all calves. Values of serum iron and hemoglobin during the first sampling on farm 1: Fe 33.18 μmol l^{-1} (±29.39) and Hb 83.7 g l^{-1} (±11. 35); farm 2: Fe 8.38 μmol l^{-1} (±7.90) and Hb 90. 4 g l^{-1}(±8.36). Second sampling on farm 1: Fe 37.35 μmol l^{-1} (±23.40) and Hb 106 g l^{-1} (±9.30); farm 2: Fe 5.62 μmol l^{-1} (±3.42) and Hb 80.3 g l^{-1} (±16.59). Last time on farm 1 the results were: Fe 42.69 μmol l^{-1} (±13.50) and Hb 101.0 g l^{-1} (±6.88); farm 2: Fe 32.79 μmol l^{-1} (±16. 07) and Hb 89.9 g l^{-1} (±6. 71). No respiratory diseases were observed on either farm during this study, diarrheal diseases occurred five times on farm 1 and nine times on farm 2. The results show that iron deficiency and lower hemoglobin concentration are more prevalent in animals fed whole milk.

Keywords: calves, milk diet, iron, hemoglobin.

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INTRODUCTION

Iron (Fe) is an abundant essential element with a wide variety of functions in the organism. It occurs in multiple forms: in hemoglobin (Hb) and myoglobin, in proteins involved in electron transfer (cytochromes a, b, c), and in enzymes catalysing in redox reactions. In the organism, iron is mainly absorbed through the duodenum, but only 2-15% of this iron is absorbed. Its absorption is affected by the age of an animal, live weight, amount of iron in the organism, and its availability in the feed. Absorption is adversely affected by the concentration of phosphates, zinc, copper, manganese and phytic and oxalic acid in the feed. Absorption of non-hem iron is increased by ascorbic acid (Spears 2003, Soetan et al. 2010, Wysocka et al. 2020).

In enterocytes, iron is degraded by the enzyme hemoxygenase and is released into the circulation by the transmembrane protein ferroportin in the form of $Fe^{2+.}$ Furthermore, iron is oxidized by the membrane enzyme hefestin to Fe^{3+}, which is bound in the blood to the transport protein transferrin. Iron is then stored in cells in the form of ferritin and hemosiderin. The largest storage of excess Fe is in the liver, but some iron is also stored in spleen. On the surface of hepatocytes, there are receptors for transferrin which allow its transport in and out of cells. There is no effective mechanism for excretion of excess iron from the internal environment of the organism, therefore strict regulation of its absorption is important. Excretion of unabsorbed iron occurs with bile into the intestine and then into the faeces, and to a small extent also with urine and milk (Benito et al. 1998, Sedláčková et al. 2009, Radwińska, Żarczyńska 2014).

The occurrence of iron deficiency and related anemias is a common problem in piglets. On pig farms, iron preparations are applied preventively at the age of 2-5 days. However, in dairy calves, this problem is often overlooked. The body's iron requirements for neonatal calves are higher than for adults because they have to double their weight at the age of two months. In calves fed native milk, iron deficiency is more common because it is well known that milk contains only low concentrations of this element. Calves are born with a small storage of iron, which is enough for about 3-4 weeks of life. Calves with iron deficiency might have anemia, decreased growth rate and immunodeficiency, which may further cause other diseases (Mohri et al. 2004, Brun-Hansen 2006, Illek, Šoch 2015).

The aim of the study was to monitor the dynamic concentration of iron in the serum of calves during different types of dairy diet in relation to the value of hemoglobin, weight gain of calves and the incidence of respiratory and diarrheal diseases.

MATERIALS AND METHODS

20 Holstein-Friesian calves at the age from 2 to 7 days kept on 2 different farms (10 on each) were chosen for our experiment. After parturition, calves were moved into a profylactorium, where they stayed for one day. Calves were fed by 2-3 l of colostrum until 2 h after being born and another 2 l after 2 h (by a stomach tube). On the first farm, calves were then fed native milk to 3 days of age, after which native milk was replaced by 2-3 l of milk replacer twice a day (farm 1). On the second farm, calves were then fed 2-3 l of native milk twice a day to 21 days of age, and then they received milk replacer for 2 weeks (farm 2). On both farms, calves were fed milk replacer twice a day until 5 weeks of age. In the 6th week, the calves received milk replacer once a day. Calves were weaned in the 7th week of life, i.e. from the 49th day, when the calves received only water and a starter. From the age of 5 days, starter was supplied to calves *ad libitum*. The calves were housed in individual pens with access to water and starter.

Blood samples were collected from the external jugular vein three times: first at the age of 2-7 days, second time at the age of 30 days and last time at the age of 56 - 60 days. Blood samples were drawn into tubes containing a clot activator for serum analyses of iron and into vacutainers containing K2 EDTA for hematological analysis. Serum levels of iron were measured in a laboratory by Atomic Absorption Spectrometry (ASS) at wavelength 248.3 nm. Blood levels of hemoglobin were measured with a BC-2800 Auto Hematology Blood Analyser.

Calves were weighed at the same time as blood collection was made. During the experiment, the occurrence of diarrheal and respiratory diseases was monitored in all calves.

Measured values were then compared with the physiological range of 18.0 - 30.0 μmol l^{-1} Fe and 90.0 - 140.0 g l^{-1} Hb.

Statistical analysis was performed by calculating the average of values on individual farms, determining the standard deviation and correlation in the spreadsheet program Microsoft Excel©.

RESULTS

Our results of the serum iron concentration (Tables 1, 2) were compared with the physiological range and three groups were created. On farm 1, deficiency occurred during first collection in 50% – 5 pcs, at the second collection in 30% – 3 pcs and at the last one in 0% of samples. On farm 2, the incidence of iron deficiency was higher, in the first sampling it was in 80% – 8pcs, second time in 30% – 3pcs and third time it was 20% – 2 pcs of the samples (Table 3).

Table 1

Iron concentration on farm 1

Farm 1	Iron concentration (μmol l^{-1})		
	1st sampling	2nd sampling	3rd sampling
1	3.48	81.73	36.5
2	8.0	55.49	53.98
3	5.69	7.25	37.22
4	1.67	4.4	25.56
5	83.34	25.43	40.15
6	62.67	39.22	35.6
7	10.36	13.08	65.36
8	67.98	43.56	48.0
9	42.47	52.82	23.1
10	46.09	50.49	61.39
Average of values	33.18	37.35	42.69
Standard deviation	29.39	23.40	13.50

Table 2

Iron concentration on farm 2

Farm 2	Iron concentration (μmol l^{-1})		
	1st sampling	2nd sampling	3rd sampling
1	5.64	5.48	31.0
2	2.4	0.9	5.3
3	9.31	9.1	29.86
4	23.56	8.05	64.29
5	5.73	4.76	25.09
6	2.49	3.62	23.63
7	2.16	3.91	48.14
8	5.18	13.29	40.67
9	3.55	4.35	16.42
10	23.71	2.74	43.52
Average of values	8.38	5.62	32.79
Standard deviation	7.90	3.42	16.07

The measured hemoglobin values on both farms are listed in Tables 4 and 5. At the first collection, a decreased value of hemoglobin was observed on farm 1 in 60% – 6 pcs of samples. In the second sampling, it was 0% of samples and in the third also 0%. On the second farm, hemoglobin values were decreased in 50% – 50 pcs of samples in the first collection, 70% – 7 pcs in the second and 40% – 4 in the third (Table 6).

Table 3

Concentration of serum iron levels and occurrence of its deficiency

Physiological range of iron: (18.0-30.0 μmol l^{-1})	Fe					
	1st sampling		2nd sampling		3rd sampling	
	farm 1	farm 2	farm 1	farm 2	farm 1	farm 2
<17.9	5	8	3	10	0	2
18.0 to 30.0	0	2	1	0	2	3
30.1 and <	5	0	6	0	8	5
Deficiency (%)	50	80	30	100	0	20

Table 4

Hemoglobin values on farm 1

Farm 1	Hemoglobin concentration (g l^{-1} Hb)		
	1st sampling	2nd sampling	3rd sampling
1	82	99	103
2	90	121	115
3	80	92	93
4	70	99	95
5	84	114	96
5	96	106	108
7	66	106	100
8	91	95	96
9	92	118	108
10	106	110	96
Average of values	85.7	106	101
Standard deviation	11.35	9.30	6.88

Table 5

Hemoglobin values on farm 2

Farm 2	Hemoglobin concentration (g l^{-1} Hb)		
	1st sampling	2nd sampling	3rd sampling
1	86	81	98
2	93	92	84
3	99	91	90
4	81	84	99
5	90	88	85
6	80	74	96
7	79	33	90
8	102	91	93
9	102	84	76
10	92	85	88
Average of values	90.4	80.3	89.9
Standard deviation	8.36	16.59	6.71

Table 6

Values of hemoglobin levels and occurrence of its deficiency

Physiological range of hemoglobin: (90.0-140.0 g l^{-1})	Hb					
	1st sampling		2nd sampling		3rd sampling	
	farm 1	farm 2	farm 1	farm 2	farm 1	farm 2
< 89.9	5	5	0	7	0	4
90.0 to 140.0	5	5	7	3	10	6
140.1 and <	0	0	3	0	0	0
↓	60%	50%	0%	70%	0%	40%

The results showed that since the beginning of our experiment, there was a difference in weight gain between farms (Tables 7, 8). The average total weight gain was higher on farm 1. The difference in weight gain between the two farms was 1 kg at the end of the experiment (Table 9). Correlation between iron and hemoglobin values is shown in Table 10.

Table 7

Weight gain on farm 1

Farm1	Weight gain (kg)		
	age of 2-7 days	age of 30 days	age of 56 - 60 days
1	45.5	56.5	73
2	38	51	58
3	45	57.5	71
4	43	56.5	74
5	46.5	59.5	79.9
5	41	54.5	67
7	45	55	70
8	47	60.5	82
9	49	57.5	75
10	40	52	72
Average of values	44	56.05	72.19
Standard deviation	3.26	2.86	6.34

No respiratory diseases were observed on either farm during this study. Diarrhea occurred 5 times on farm 1. On farm 2, seven cases of diarrhea were recorded in the second month of life and two cases before weaning.

The iron level of milk fed to calves on farm 2 was 26.2 mg kg^{-1} DM of milk, and the iron level in milk replacer was 100 mg kg^{-1} DM of milk replacer.

Table 8

Weight gain on farm 2

Farm2	Weight gain (kg)		
	age of 2-7 days	age of 30 days	age of 56 - 60 days
1	44	57.5	69
2	46.5	60	72
3	48	58.5	72
4	36	49.5	59
5	40.5	50	63
6	34.5	47	55
7	36	48	58
8	36	58.5	72
9	25.5	45.5	55
10	25.5	51	65
Average of values	37.25	52.55	64
Standard deviation	7.39	5.20	6.65

Table 9

Average weight gain on both farms

Average weight gain (kg)	Age of 2-7 days	Age of 30 days	Age of 56 - 60 days
Farm 1	44	56.1	72.2
Farm 2	37.3	52.6	64

Table 10

Correlation between iron and hemoglobin values

Correlation: Fe x Hb (-1 to 1)	1st sampling	2nd sampling	3rd sampling
Farm 1	0.548	0.316	-0.026
Farm 2	-0.112	0.250	0.601

DISCUSSION

Results of the serum iron levels show that feeding calves only with native milk results in a higher incidence of serum iron deficiency. During the third collection, the calves were already fed with milk replacer, therefore the serum iron levels were higher. An important source of iron for newborn calves is lactoferrin from colostrum, which contains this protein in a higher dose than milk does (1.5-5 mg ml^{-1} compared to 0.02-0.75 mg ml^{-1}) – Knowles et al. (2000), Allan et al. (2018). On both farms, iron levels were low after

birth (50% and 80%), thus it is likely that the lactoferrin content in colostrum was insufficient. Reif et al. (2019) examined serum iron levels, hemoglobin concentration and hematocrit level in 118 heifers calves. They found that serum iron deficiency was detected in 44.9% of cases, hematocrit and hemoglobin were more or less significantly lower in 43.2% and 17.8% of the animals, respectively. The lowest serum iron concentrations occurred mostly at the age of 5 to 6 weeks.

About 60% of iron in the organism is bound to hemoglobin, which is a complex molecule of the protoporphyrin heme and globin. Consequently, hemoglobin will be the last place to show the retention of that element and therefore should not be used as an indicator of its deficiency. Hemoglobin is bound in erythrocytes and allows the transfer of respiratory gases. The lifespan of erythrocytes in calves is 110-120 days (Mizuno 1959). After birth, there is a physiological decrease in hematocrit (PCV), Hb and erythrocyte counts. This can be caused by the intake of colostrum, which osmotically dilutes the volume of blood (Knowles et al. 2000). The decrease in erythrocyte levels is also due to the fact that intrauterine erythrocytes have a shorter lifespan (Harvey 1997). According to the calculation of the correlation coefficient (Table 10), we can state that the degree of dependence of the serum iron level and hemoglobin content is different, but is mostly greater than or equal to 0.0, and we can therefore say that iron deficiency in the body causes a decrease in the hemoglobin level. In the study conducted by Benesi et al. (2019), it was found out that from 385 Holstein calves at the age of 30 days, anemia was found in 14.5% of samples (55/385). Lower serum iron levels with a predominance of hypochromatic/normochromatic microcytic anemia were found in most anemic samples. They discovered that anemia occurs mostly within 14 days after birth (78.2%), 40% cases were from birth to 7 days of age and 38.2% in the second week. A study done by Illek and Šoch (2015) found that a significant decrease in iron and hemoglobin levels occurred between the ages of 20 and 30 days and therefore the best time for administering iron is 10-15 days after birth. In our experiment, decreased serum iron levels occurred mostly at the age of 5 to 6 weeks.

In a study of Mohri et al. (2006), significant differences were found between control and experimental group who has oral supplementation of 150 mg per day of ferrous sulphate for 28 days. Experimental group had higher PCV, total gain, mean daily gain and weight gain during first month of life. In this experiment they did not find any significant difference in the incidence of the disease of calves. Calves injected with 1 g of Fe-dextran per animal on the second day after birth showed significantly higher total and daily weight gain. However, no significant differences were observed between MCV, MCHC and iron levels (Mohri et al. 2010).

Joerling and Doll (2019) conducted a study comparing blood parameters used to determine anemia in calves. They found that out of 40 calves, 19 had low serum iron levels. In the determination of ferritin, 14 calves appeared

to be deficient. Their research showed that the use of serum iron and hemoglobin levels has limited suitability for determining the deficiency of this element. They suggest that the determination of ferritin be preferred because iron in the body is subject to physiological processes and its decrease can be caused, for example, by inflammation. We did not determine this parameter in our experiment, but it could be the subject of further research.

Iron is an element that significantly interferes with a wide range of processes in the body and it is therefore important to address the issue of its deficiency not only in calves. The occurrence of anemia in the youngest categories of calves can be prevented by administering iron preparations and should not be overlooked. In conclusion, we can confirm that feeding calves only with native milk is not sufficient to maintain proper iron and hemoglobin levels until weaning.

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