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ORIGINAL PAPER

CHANGES IN RED BLOOD CELL CHARACTERISTICS OVER THE COURSE OF SUBCLINICAL PHOSPHORUS DEFICIENCY IN DAIRY COWS*

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Abstract

Phosphorus deficiency is a common problem on dairy farms. It most often occurs in a subclinical form or follows an atypical course for this disease. The diagnosis of P (phosphorus) deficiency is only possible based on laboratory tests. The research objective was to explore the changes in red blood cell parameters over the course of subclinical phosphorus deficiency in dairy cows, and the possibility of using such information in early diagnosis. The study was performed on 35 cows (group I) with atypical clinical symptoms (pale mucous membranes and periodic problems with appetite) and subclinical phosphorus deficiency, and a group of 15 animals without clinical symptoms and with the correct concentration of inorganicnic phosphorus (Pi) in the blood. The results of the haematological and biochemical tests were statistically analysed using the Mann-Whitney rank test, Statistica 13 (StatSoft Poland). Before the treatment, a decrease in blood Pi concentration (1.34±0.21 mmol l^{-1}) and the significantly lower values of red blood cell parameters were observed in the study group as compared to the animals from the control group. The cows were diagnosed with hypochromic normocytic anaemia. Animals from group I were supplemented with phosphorus preparation (7.8 g P 100 ml^{-1}, dipotassium phosphate, magnesium phosphate) for 3 weeks. After this period, the phosphorus concentration in the blood was recuperated and the red blood cell parameters (RBC 6.69±1.02 x10^{12} L^{-1}, Hgb 94.33±11.93 g L^{-1}, Hct 0.29±0.04 l L^{-1}) increased significantly as compared to the first blood sampling (RBC

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5.54±0.59 x10^{12} L^{-1}, Hgb 78.33±10.61 g L^{-1}, Hct 0.24±0.03 l L^{-1}). Haematology examination is of prognostic significance regarding the occurrence of subclinical phosphorus deficiency in dairy cows and should be included in the panel of herd screening tests.

Keywords: haematology, phosphorus deficiency, anaemia, dairy cows.

INTRODUCTION

Phosphorus is an essential element in animals, necessary for the maintenance of proper bodily functions. It takes part in the storage, processing and release of energy and also in enzymatic reactions, in addition to which it is a vital component of cell membranes and the skeletal system (Zhang et al. 2017). A decrease in the concentration of this element in blood (hypophosphataemia) is currently one of the most common problems on high-production dairy farms, especially during the periparturient and early lactation period. It may occur within a frequency range of 10-50%, and in some cases it affects up to 70% of the animals in a herd (Cohrs, Grünberg 2018, Singh 2018, Grünberg et al. 2019). In its clinical form, Pi deficiency causes postpartum downer cow syndrome and postpartum haemoglobinuria (Goff 2006, 2008, Kaya et al. 2008, Grünberg et al. 2015). In this form, it is usually easy to recognize due to the characteristic symptoms. Postpartum haemoglobinuria is associated with haematuria, pallor of the mucous membranes, decreased appetite and lower milk production. In the case of postpartum downer cow syndrome, the animals are lying down or have difficulty standing up (especially on their hind legs), they experience lameness and joint pain, in the lower parts of their limbs (Correa et al. 1993, Smith et al 1997, Metre, Callan 2003, Ménard, Thompson 2007). The symptoms are different in the subclinical form or in an atypical course of phosphorus deficiency. Periodic appetite disturbances, decreased milk production or progressive pallor of the mucous membranes are then observed in cows. Animal owners may associate these disorders with other diseases (digestive disorders, invasion by intestinal parasites, rumen acidosis, etc.). Pi deficiency may develop unnoticed over a long period of time, and only the occurrence of lameness, difficulty standing up or isolated cases of haemoglobinuria in the herd may necessitate a more extensive diagnosis or specifically aimed treatment. The subclinical form is rarely described in the literature (Gartner et al. 1982, Malafaia et al. 2018).

The effect of phosphorus deficiency on the red blood cell system has only been widely described in relation to the development of postpartum haemoglobinuria. In the available literature (Resum et al. 2017, Zhang et al. 2017, Deeba, Bashir 2019, Rahmati et al. 2021), there are studies reporting that severe hypophosphataemia may lead to intravascular haemolysis and may even be the cause of haemoglobinuria in cows. The pathomechanism causing the intravascular destruction of erythrocytes in the course of hypophosphataemia is unknown (Ok et al. 2009, Rahmati et al. 2021). Inorganic phosphorus (Pi) is responsible for the integrity of cell membranes, and its deficiency leads to a decrease in the phospholipid content and may result in damage to erythrocytes. Furthermore, it is hypothesized that a decrease in the phosphorus concentration in the body leads to a reduction in the production of ATP (adenosine triphosphate) in cells, which in turn reduces the cell's ability to defend itself against oxidative stress (Resum et al. 2017). Cell damage may also be related to an insufficient concentration of intracellular ATP, which is necessary to maintain the integrity of cell membranes. In cases of severe hypophosphataemia, the intracellular concentrations of phosphorus and intracellular ATP decrease, resulting in intravascular haemolysis (Adams et al. 1993, Resum et al. 2017). It seems that similar changes in the red blood cell system to those described in haemoglobinuria, but expressed to a much lesser degree, may be expected over the course of subclinical or atypical phosphorus deficiency.

The aim of the study was to investigate the red blood cell parameters over the course of subclinical phosphorus deficiency in dairy cows. The results of the laboratory tests of the studied animals before and after phosphorus supplementation will be compared with those obtained from healthy animals.

MATERIALS AND METHODS

The study was carried out on a farm with a tethered housing system located in the central Lublin region, on a group of 50 HF (Holstein-Friesian) breed, aged 3 to 6 years, with an average milk yield of above 9000 litres per lactation and a BCS of 3/5 (the average body weight cows between 500-550 kg). The cows were between the 30th and 60th day of their lactation period. The farm used the TMR feeding system, and the feed dose included maize silage, maize grain, haylage, grass silage, hay, straw, pellets, on-farm grown grains, feed additives with a protein content of 18% mineral, and vitamin premix and supplements. The feed doses were designed on the basis of milk yield, the current physiological period, the age and body weight of the cows. Cows in the control and study groups received the same rations of TMR. The animals were under constant veterinary care. Before blood sampling, detailed inspection and clinical examination of the animals were carried out, while the feeding method and the quality of the feed provided as well as the milk yield were also assessed. Only animals without parasitic and infectious disease, injuries or any kind of bleeding were included in the study. Also, any animals with a history of dystocia and calving-related disorders were disqualified. The cows that qualified for the experimental group and received supplementation showed only varying degrees of pale conjunctiva, a periodic lack of appetite and lower (by 5 to even 20%) milk production when compared to the previous lactation period.

Blood was collected from the cows by sampling from the external jugular vein before treatment and three weeks later. After the results of the first blood sampling were obtained, the cows were divided into two groups, based on a clinical examination and the concentration of Pi in the blood.

The study group of 35 cows (group I) included only the animals with minor disorders, such as pale mucous membranes and periodic problems with appetite. Moreover, in the medical interview, the owner indicated a lower milk production than in the previous lactation period. These symptoms started between 7 and 14 days after calving, and they continued with varied severity until the beginning of the study. Laboratory tests showed a reduction in the Pi levels of the blood. These animals were then supplemented with phosphorus (7.8 g P 100 ml^{-1} per cow, dipotassium phosphate, magnesium phosphate) for 3 weeks.

A control group (15 cows, group II) included the animals that did not show any clinical symptoms and changes in the laboratory tests.

The haematology examination included the determination of the following parameters: RBC – red blood cell count, Hgb – haemoglobin, Hct – haematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration and the leukocyte (WBC) and platelet count (PLT) using an automatic Horiba scil Vet abc Plus analyser. The serum parameters, such as the total concentration of calcium (Ca), inorganic phosphorus (Pi), magnesium (Mg), total bilirubin (TB), total protein (TP) urea (Urea) and the activity of gamma-glutamyl transpeptidase (GGTP) were determined using a Horiba automatic analyser ABX Pentra 400. The concentrations of copper (Cu) and iron (Fe) in the serum samples were determined using the method of flame atomic absorption spectrometry (FAAS) by Avanta PM, GBC, Australia.

The results of the haematological and biochemical tests were statistically analysed using the Mann-Whitney rank test, Statistica 13 (StatSoft Poland). The calculations were performed at a significance level of $p<0.05$ and $p<0.001$.

RESULTS AND DISCUSSION

Certain symptoms in the study group developed between the 7th and 14th day after calving and persisted with varied severity until the start of the study. In the first period of the occurrence of symptoms in the cows, additional faecal parasitological tests were performed and the animals were dewormed, yet the described symptoms persisted. The results of extended laboratory tests (Tables 1, 2) indicated a decrease in the concentration of inorganic phosphorus (Pi) in the serum and changes in some haematological parameters were observed. After three weeks, the tested animals

Table 1

Mean values of haematological parameters in cows

Parameter (unit)	Bovine reference intervals (Baumgartner 2005)	Group I (n=35)		Control group (n=15)
		before treatment	after treatment	
RBC x10^{12} L^{-1}	5 - 7	5.54±0.59^{A,a}	6.69±1.02^{B,b}	7.98±0.55^{B,c}
Hgb (g L^{-1})	105 - 140	78.33±10.61^{A}	94.33±11.93^{B}	112.40±10.83^{C}
Htc (l L^{-1})	0.30 - 0.40	0.24±0.03^{A,a}	0.29±0.04^{A,B,b}	0.33±0.03^{B,c}
MCV (fl)	40 - 60	43.08±2.84^{A}	41±2.50^{A}	50.70±3.33^{B}
MCH (fmol)	0.9 - 1.5	0.86 ±0.06^{A}	0.88±0.07^{A}	1.08±0.08^{B}
MCHC (mmol L^{-1})	16 - 21	20.38±0.35^{a}	20.33±0.51^{a}	19.65±0.73^{b}
WBC x10^{9} L^{-1}	6.2 - 9.5	5.49±1.22^{A}	6.91±2.10^{A,B}	8.31±0.75^{B}
PLT x10^{9} L^{-1}	200 - 800	294.75±113.97^{A}	416.7±202.76^{A,B}	540.20±73.31^{B}

A, *B*, *C* – the significance of the differences between mean values in the experimental group vs the control group at p<0.001, *a*,*b*,*c* – the significance of the differences between the mean values in the groups vs the control group at p<0.05 (using the Mann-Whitney *U* rank test), X±SD – the mean± standard deviation.

Table 2

Results of the biochemical tests of the cows

Parameter (units)	Bovine reference intervals (Meyer, Harvey 2004)	Group I (n=35)		Control group (n=15)
		before treatment	after treatment	
Pi (mmol L^{-1})	1.5 - 2.9	1.34±0.21^{A}	1.74±0.39^{B}	1.63±0.09^{B}
Ca (mmol L^{-1})	1.98 - 2.5	2.52±0.10	2.57±0.04	2.57±0.09
Mg (mmol l^{-1})	0.7 - 1.1	0.96±0.14	0.95±0.05	0.99±0.07
Fe (µmol L^{-1})	21.5-38.5	38.23±6.11^{Aa}	31.21±4.57^{ABb}	28.44±4.44^{Bb}
Cu (µmol L^{-1})	12-20	14.53±3.15	11.68±1.55	12.89±0.60
GGTP (U L^{-1})	20 - 48	25.50±5.55	31.75±22.23	17±6.16
TB (µmol L^{-1})	1.7 – 5.1	2.05±0.68	2.39±1.02	1.71±0.51
TP (g L^{-1})	59 - 77	73.91±8.12^{a}	65.38±6.91^{b}	72.17±2.48^{ab}
Urea (mmol L^{-1})	1.66 - 7.47	1.85±0.23	2.12±0.36	2.1±0.45

A, *B* – the significance of differences between the mean values in the groups vs the control group at p<0.001, *a*,*b* – the significance of differences between the mean values in the groups vs the control group at p<0.05 (using the Mann-Whitney *U* rank test), X±SD – the mean± standard deviation.

showed better utilization of their feed, an increase in milk production, although they did not regain their full milk production yield when compared to the previous year. Following supplementation with phosphorus, no pallor of the mucous membranes was observed upon clinical examination.

The concentration of phosphorus in the study group in the first sampling (1.34±0.21 mmol L^{-1}) was significantly lower compared with both the control group (1.63±0.09 mmol L^{-1}) and the second blood sampling (1.74±0.39 mmol L^{-1}). At the same time, this result was higher than the Pi concentrations reported by other authors in cases of postpartum haemoglobinuria or downer cow syndrome (Metre, Callan 2003, Ménard, Thompson 2007, Zhang et al. 2017, Albayati, Luaibi 2020). This indicates that we are dealing with a subclinical form of the deficiency of this element. There are no available literature data concerning this form of Pi deficiency and information indicating the occurrence of laboratory disturbances related to internal homeostasis in the course of this disease. The supplementation resulted in a significant increase in the Pi concentration as compared with the reference values, which exceeded the mean value of the concentration in the control group. This outcome is consistent with the average concentrations of Pi in healthy animals found in the literature. The biochemical tests also indicated high average iron (Fe) contents for both the first and second blood sampling, which were significantly higher than in the healthy animals (Table 2). During phosphorus supplementation, there was a significant reduction in Fe levels in the blood. In addition, the biochemical tests did not reveal any significant changes that could affect the clinical condition of the animals. Our observations show that subclinical phosphorus deficiency may be far more important for the occurrence and slow progression of haematological changes than the deficits of other elements.

Before the treatment, significantly lower values of red blood cell parameters were found in the haematological examination of animals with Pi deficiency as compared with healthy animals, although the RBC and MCV parameters were within the standard reference limits (Baumgartner 2005). On the other hand, MCHC was the only red blood cell parameter that did not change (Table 1). These results indicate the occurrence of hypochromic normocytic anaemia. Similar results were obtained by Zhang et al. (2017), who experimented with limiting the supply of phosphorus in the cows' diet. It was observed that the Pi deficiency reduced the viability of erythrocytes, which in turn could potentially reduce the level of ATP in red blood cells. These authors assumed the existence of mechanisms similar to those described in postpartum haemoglobinuria. Zhang et al. (2017) found that reducing the amount of phosphorus in the cow's diet reduces the RBC and MCH values in the blood. In our research, similar values were obtained despite the appropriate concentration of phosphorus in the diet of animals. In 25% of cows, the red blood cell count decreased below normal (Baumgartner 2005), while in the remaining animals it was close to the lower reference norm. These values were significantly lower compared to those found in healthy animals (Table 1). In over 80% of the study cows, a reduction in the MCH index was found (Baumgartner 2005). However, Grünberg et al. (2015) claimed that a deficiency of phosphorus in a diet did not affect the parameters of red blood cells.

After the clinical symptoms had disappeared and the blood phosphorus level was corrected, the values of the red blood cell parameters increased. There was a significant increase in the number of red blood cells as compared to the first blood sample collection. Grünberg et al. (2015) also detected a significant increase in RBC after phosphorus supplementation with feed. In addition, in the study animals, a significant increase in haemoglobin and haematocrit levels was observed, but these parameters were still below the standard reference limits (Baumgartner 2005). The authors obtained similar results during the restoration of the normal phosphorus concentration in the blood in cows with postpartum haemoglobinuria (submitted). In the available literature, there are no data on changes in red blood cell parameters after correction of Pi deficiency over the course of PPH (postparturient hemoglobinuria). Unfortunately, most authors only compare the results produced by sick animals to those of the control group (Mahmood et al. 2013, Zhang et al. 2017, Albayati, Luaibi 2020).

Changes in the leukocyte count were also observed during the research. In cows with phosphorus deficiency, a significant decrease in the number of WBCs was noted compared to the results produced by healthy animals. After the blood Pi concentration was normalized, the number of leukocytes increased significantly when compared to the first blood sample collection, and this was also confirmed by other authors (Forrester, Moreland 1989).

Changes in the platelet count were also observed during the research. In cows with phosphorus deficiency, a significant decrease in the number of PLTs was noted compared to the results produced by healthy animals. After normalizing the Pi concentration in the blood, the platelet count increased significantly compared to the first blood sample collection, although it was within the reference range (Forrester, Moreland 1989, Baumgartner 2005).

In summary, it may be concluded that subclinical phosphorus deficiency reduces red blood cell parameters and it may also result in the occurrence of hypochromic normocytic anaemia. A haematological examination is of prognostic significance regarding the occurrence of a subclinical form of phosphorus deficiency in dairy cows. The pallor of the mucous membranes of the natural body orifices in animals should be a recommendation for haematological examination. The results outlined above indicate the validity of including a haematological blood test in the panel of screening tests in dairy herds.

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