

Blood disorders caused by hypophosphatemia in dairy cows

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ABSTRACT

Objective: To describe the main blood disorders caused by hypophosphatemia (low P level in the blood) in dairy cows.

Design/Methodology/Approach: Publications about blood disorders caused by hypophosphatemia in dairy cows were analyzed.

Results: In addition to a decrease in milk production and several reproductive and metabolic disorders, hypophosphatemia can cause alterations in blood cells, mainly in erythrocytes, as a consequence of the decrease of the phosphorus (P) needed to generate adenosine triphosphate (ATP) and the alterations of the cell membrane phospholipids.

Study Limitations/Implications: Few studies have described how P affects different blood cells or their components.

Findings/Conclusions: Hypophosphatemia has been associated with structural and functional alterations in blood cells.

Keywords: Hypophosphatemia, dairy cows, phosphorus deficiency, blood cell abnormalities, haemolysis.

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INTRODUCTION

Phosphorus (P) is one of the most important minerals for animal health. It fulfills many structural functions in the bones, the cell wall (Morse *et al.*, 1992; Zhang *et al.*, 2017), and in energy-dependent reactions, such as the development of high-energy phosphate ester bonds and the adenosine triphosphate (ATP) (Erickson and Kalscheur, 2020). The P in the cow's saliva influences the ruminal environment and acts as an enzyme cofactor, pH regulator, and medium osmolarity; in addition, it contributes to cellulose digestion and microbial synthesis (Ramírez-Pérez, 2005; Yosathai, 2014). The casein and the fat of the milk include P (Alvarez-Fuentes *et al.*, 2016); its concentration fluctuates between

0.7 and 1.2 g kg⁻¹ (NRC, 2021). Therefore, a high milk production requires more P (Grünberg, 2008).

P deficiency in the blood of highly productive dairy cows is associated with reproductive problems and milk production decrease (Yosathai, 2014), as well as mastitis, metritis, and infertility (Soldá *et al.*, 2017). These problems take place during transitional periods (Chiwome *et al.*, 2017). In addition, Eisenberg *et al.* (2019) reported blood disorders caused by hypophosphatemia—including intravascular haemolysis of the red blood cells and cellular immunosuppression—which have been related to metabolic disorders (Molefe and Mwanza, 2019). Consequently, this review aimed to describe the alterations in blood cells caused by hypophosphatemia in dairy cows.

MATERIALS AND METHODS

The data used in the study were gathered from Scopus, Google Scholar, and NCBI. The keywords used for the search (individually or combined) were: phosphorus, phosphorus deficiency, hypophosphatemia, blood cell disorder, erythrocytes, leucocytes, platelets, haemoglobin, haemolysis, and dairy cows.

Hypophosphatemia

Determining the phosphorus state and the requirements of the body of a cow is fundamental (Sharifi *et al.*, 2007), because inorganic phosphorus (IP) in the plasm is an indicator of its bioavailability in food (Montiel *et al.*, 2007). Recently, NRC (2021) reported that the normal P concentration in the plasm of dairy cows ranges from 1.3 to 2.6 mM (4 to 8 mg dL⁻¹). Therefore, hypophosphatemia takes place when the Pi plasm concentration is <2 mg dL⁻¹ (Rahmati *et al.*, 2021). Hypophosphatemia decreases food consumption (Cohrs and Grünberg, 2018), mainly among cows during the transitional period (Ménard and Thompson, 2007; Ramírez-Nava, 2009). This disorder impacts up to 70% of the animals in production units (Kaczmarek *et al.*, 2021). Hypophosphatemia results from a sudden and growing loss of P through the milk (Albornoz *et al.*, 2016). P deficiency in the diet hinders the balance of such loss (Najarnezhad *et al.*, 2016). The clinical signs of low P concentration in the blood include: anorexia, paresis, and haemoglobinuria (Barrios *et al.*, 2010). This disorder is also associated with different pathologies such as Pica, Downer cow syndrome, rickets, osteomalacia (Eisenberg *et al.*, 2014; Soldá *et al.*, 2017; Molefe and Mwanza, 2019), displaced abomasum, abomasal volvulus, hepatic lipidosis, metritis, mastitis (Grünberg *et al.*, 2005; Grünberg, 2008; Ismail *et al.*, 2011; Zhang *et al.*, 2017; Macías *et al.*, 2018), retained placenta, silent heat, irregular estrus, inactive ovary, and delayed sexual maturity (Yosathai, 2014). In addition, it causes metabolic diseases such as milk fever, ketosis, and postpartum haemoglobinuria (Resum *et al.*, 2017). Hypophosphatemia has also been related to both functional and structural cellular alterations, including haemolysis of erythrocytes and cellular immunosuppression (Eisenberg *et al.*, 2019).

Blood disorders caused by hypophosphatemia

Blood disorders caused by hypophosphatemia have been reported in different animal species (pigs, rats, and cattle) and humans (Jubb *et al.*, 1990; Eisenberg *et al.*, 2014;

Najarnezhad *et al.*, 2016); erythrocytes are the most studied blood cells (Nozad *et al.*, 2012; Zhang *et al.*, 2017). Erythrocytes require ATP to control cell volume, keeping their shape and plasticity (Grünberg *et al.*, 2015). Intravascular haemolysis occurs when there is a low P concentration in the blood ($0.4\text{--}1.5\text{ mg dL}^{-1}$) of post-partum dairy cows. This disorder is caused by the decrease of the glyceraldehyde 3-phosphate dehydrogenase (G-3-PD) enzyme, which plays a key role in glycolysis and ATP formation (Macías *et al.*, 2018). Intravascular haemolysis causes the explosion of erythrocytes by capillary pressure (Brechtbühl *et al.*, 2008), as a result of a deficient ATP intracellular concentration, which is fundamental to keep the integrity of the cell membrane (Abramowicz *et al.*, 2022). Erythrocyte haemolysis reduces the count of erythrocytes (RBC), haemoglobin (Hgb), hematocrit (Htc) (Kaczmarek *et al.*, 2021), and platelets (PLT) (Abramowicz *et al.*, 2022). In addition, Brechtbühl *et al.* (2008) and Abramowicz *et al.* (2022) reported normocytic normochromic/hypochromic anemia, while Noro and Wittwer (2011) and Zhang *et al.* (2017) mentioned haemoglobinuria. Noro and Wittwer (2011) and Kaczmarek (2021) recorded polychromasia, reticulocytes, anisocytosis, macrocytosis, spherocytes, and Heinz bodies in the erythrocytes.

There is a relationship between P and cell protection, because the membranes are made up of phospholipids (Soldá *et al.*, 2017). Zhang *et al.* (2017) studied the morphofunctional state of erythrocytes of cows under a deficient P diet. They recorded a variation in the phospholipid content (particularly phosphatidylcholine and phosphatidylserine). In addition, they reported a decrease in the antioxidant activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the erythrocytes. The reduction of ATP erythrocyte synthesis is another source of weakness in the membrane. Ultimately, this phenomenon diminishes the cell defenses against oxidative stress (Ok *et al.*, 2009). Hypophosphatemia also causes alterations in immune cells. Eisenberg *et al.* (2014) reported a decrease in the concentration and survival of granulocytes and B lymphocytes in dairy cows. Langova *et al.* (2020) and Libera *et al.* (2021) suggested that, as a result of P deficiency, mastitis or laminitis in dairy cows can be related to a decrease in the phagocytic activity of the granulocytes. Other studies have reported the opposite effect: hypophosphatemia can increase leukocyte and neutrophil concentration, during a haemolytic condition (Brechtbühl *et al.*, 2008; Noro and Wittwer, 2011). Table 1 shows the blood disorders and clinical signs recorded among dairy cows fed with a P-deficient diet.

Hypophosphatemia treatment and prevention

As a preventive measure, Noro and Wittwer (2011) recommend an appropriate P content in the diets, to meet the maintenance and milk production requirements, especially during the beginning of the lactation cycle. In addition, hematological analyses, such as hemogram (which considers the RBC, Htc, and Hgb parameters), are key to a timely prevention and detection of P deficiency (Abramowicz *et al.*, 2022). However, reverting the clinical signs of blood cell alterations (particularly in erythrocytes) is fundamental once hypophosphatemia is detected. Different treatments can be used to revert this situation, including intravenous therapies with phosphate salts, monosodium, disodium, and trisodium phosphates, and selenium, copper, manganese, and potassium (Cohrs and Grünberg, 2018; Rahmati *et*

Table 1. Blood disorders and clinical signs among dairy cows fed with a P-deficient diet.

Number of cows evaluated	Days in milk	P in diet (%)	Phosphatemia	Clinical signs	Blood alteration	Reference
72	28 days BC	1-TMR ¹ -0.30% P 2-TMR ¹ -0.31% P 3-TMR ¹ -0.43% P	1-0.9 mmol L ⁻¹ 2-1.4 mmol L ⁻¹ 3-1.9 mmol L ⁻¹	Haemoglobinuria Pica Syndrome ↓ MP	Anemia Hypophosphatemia	(Stockdale <i>et al.</i> , 2005)
8	100 days	LP-TMR ² 0.2% P DM AP-TMR ² + NaH ₂ PO ₄ 0.36% P DM HP-TMR ² + NaH ₂ PO ₄ 0.45% P DM	LP-0.07 mmol dL ⁻¹ AP-1.3 mmol dL ⁻¹ Hp-1.9 mmol dL ⁻¹	No clinical signs	↓ Gran y BL	(Eisenberg <i>et al.</i> , 2014)
10	100 and 200 days	LP-TMR ² 0.2% P DM AP-TMR ² + NaH ₂ PO ₄ 0.36% P DM HP-TMR ² + NaH ₂ PO ₄ 0.42% P DM	LP-1.5 mg dL ⁻¹ AP-4.1 mg dL ⁻¹ HP-5.5 mg dL ⁻¹	No clinical signs	Intravascular hemolysis Hypophosphatemia	(Grünberg <i>et al.</i> , 2015)
40	90 days	C-TMR ² + NaH ₂ PO ₄ 0.35% P DM LP-TMR 0.03% P DM	C 2.02 mmol L ⁻¹ LP 0.48 mmol L ⁻¹	Muscle weakness or recumbency Jaundice Anorexia ↓ MP	↓ RCB y MCH ↑ BNP, AST y ALT ↓ SOD y GSH-Px ↓ PC ↓ Na ⁺ /K ⁺ -ATPase and Mg ²⁺ -ATPase Hyperacute hemolysis Macrocytic anemia	(Zhang <i>et al.</i> , 2017)
36	42 days BC and 28 days AC.	AP-TMR ³ NaH ₂ PO ₄ 0.44 % P DM LP-TMR ³ 0.20% P DM	AP 5.1 mmol L ⁻¹ LP 1.4-2.3 mmol L ⁻¹	Haemoglobinuria Dystocia Anorexia ↓ MP	Anemia Intravascular hemolysis.	(Grünberg <i>et al.</i> , 2019a)
36	42 days BC and 28 days AC.	AP -TMR ³ + NaH ₂ PO ₄ 0.28-0.44 % P of MS LP -TMR ³ 0.15-0.20% P DM	AP 1.7 mmol L ⁻¹ LP 0.5 mmol L ⁻¹	Anorexia Haemoglobinuria Metritis Displaced abomasum	Intravascular hemolysis. Anemia Hepatic lipidosis Hypocalcemia Ketosis	(Grünberg <i>et al.</i> , 2019b)
18	42 days BC and 42 days AC.	AP -TMR ² NaH ₂ PO ₄ 0.28-0.44 % P of DM LP -TMR ² 0.15-0.20% P DM	AP 1.8 mmol L ⁻¹ LP 0.6 mmol L ⁻¹	Hemoglobinuria Anemia	Intravascular hemolysis. ↓ Gran y BL	(Eisenberg <i>et al.</i> , 2019)
30	42 days BC.	AP- TMR ⁴ 0.35%P DM LP- TMR 0.15% P DM	AP 1.7 mmol L ⁻¹ LP-1.4 mmol L ⁻¹	Recumbency Metritis and endometritis Mastitis Limp Anorexia Haemoglobinuria	Hypophosphatemia Intravascular hemolysis Hypocalcemia	(Wächter <i>et al.</i> , 2022)

↑, Increased; ↓, decreased; C, control; AC, after calving; BC, before calving; BT, before treatment; AT, after treatment; P, phosphorus; LP, Low phosphorus; AP, adequate phosphorus; HP, high phosphorus; TMR, total mixed ration; DM, dry matter; RBC, red blood cell; MCH, mean corpuscular hemoglobin; Gran, granulocytes; BL, B lymphocytes; BNP, total bilirubin; AST, alanine aminotransferase; ALT, aspartate aminotransferase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Na⁺/K⁺-ATPase, sodium-potassium pump; Mg-ATPase, magnesium pump; MP, milk production; NaH₂PO₄, sodium phosphate; TMR: ¹ 1-corn silage, barley grains, canola meal and hay. ² TMR plus pellets (commercial concentrate), ³ -TMR plus soybean meal. ² Corn silage, grass seed straw and beet pulp. ³ Corn silage, grass seed straw, beet pulp and soybean meal. ⁴ Corn silage, beet pulp, hay, straw and concentrate.

al., 2021). In addition, blood transfusions can immediately be carried out to balance the erythrocyte loss caused by haemolysis (Noro and Wittwer, 2011). Other treatments include the oral intake of calcium, magnesium, and iron solutions, mixed with phosphorus salts (Noro and Wittwer, 2011). These treatments can be supplied in the food or water (Rahmati *et al.*, 2021). Antioxidants (such as ascorbic acid) can also be used as a complement, to reduce the oxidative stress caused by the P deficiency in the cells (Soldá *et al.*, 2017).

CONCLUSIONS

Hypophosphatemia causes alterations in the erythrocytes and leucocytes of the blood cells of dairy cows. These alterations are both functional and structural. They impact the RBC, Htc, PLT, and leucocyte hematological parameters. Nevertheless, few studies have evaluated the disorder caused by hypophosphatemia in the different blood cells and their components in dairy cows.

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