

Effect of phosphorus deficiency on erythrocytic morphology and function in cows

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The aim of this study was to evaluate the influence of phosphorus (P) deficiency on the morphological and functional characteristics of erythrocytes in cows. Forty Holstein-Friesian dairy cows in mid-lactation were randomly divided into two groups of 20 each and were fed either a low-P diet (0.03% P/kg dry matter [DM]) or a control diet (0.36% P/kg DM). Red blood cell (RBC) indices results showed RBC and mean corpuscular hemoglobin decreased while mean corpuscular volume increased significantly ($p < 0.05$) in P-deficient cows. Erythrocyte morphology showed erythrocyte destruction in P-deficient cows. Erythrocytes' functional characteristics results showed total bilirubin and indirect bilirubin concentrations and aspartate transaminase and alanine transaminase activity levels in the serum of P-deficient cows were significantly higher than those in control diet-fed cows. Activities of superoxide dismutase and glutathione peroxidase in erythrocytes were lower, while the malondialdehyde content was greater, in P-deficient cows than in control diet-fed cows. Na^+/K^+ -ATPase and Mg^{2+} -ATPase activities were lower in P-deficient cows than in control diet-fed cows; however, Ca^{2+} -ATPase activity was not significantly different. The phospholipid composition of the erythrocyte membrane changed and membrane fluidity rigidified in P-deficient cows. The results indicate that P deficiency might impair erythrocyte integrity and functional characteristics in cows.

Keywords: cow, erythrocyte, function, morphology, phosphorus deficiency

Introduction

Phosphorus (P), which is essential for animal health, has a number of important biological functions, such as providing structure and strength to bones, cell walls, and phosphate buffer systems. Living cells use phosphate (PO_4^{-3}) to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. Moreover, ATP is important for phosphorylation, a key regulatory event in cells. Fifteen to twenty percent of P is found in the fluids and soft tissue of cows, while the rest is primarily located in the skeleton [8]. Low P intake by cows in early lactation might produce hypophosphatemia as a result of the sudden and increasing loss of P through milk [13]. Conditions such as downer cow syndrome, postparturient hemoglobinuria, inappetence, reduced milk production, and decreased fertility have been associated with hypophosphatemia [2,13]. In cattle, hypophosphatemia occurring at the onset of lactation is widely believed to be associated with periparturient recumbency and the downer cow syndrome [7]. After calcium (Ca), P is the

second major component of bone mineral; P participates in the process of glycolysis and oxidative phosphorylation and maintains the integrity of cellular structures [9]. In addition, P compounds containing the phosphate ion are components of DNA, RNA, ATP, and also of phospholipids, which form all cell membranes.

The common symptoms of downer cow syndrome and periparturient recumbency include hypophosphatemia and postparturient hemoglobinuria in dairy cows, while the symptoms of hemoglobinuria only occur in the initial days of lactation. Hemoglobinuria and acute intravascular hemolysis can cause serious consequences in dairy cows. It has been reported that severe hypophosphatemia causes a downward trend in ATP concentration in erythrocytes, which can lead to hemolytic anemia and hemoglobinuria in cows [19]. Ogawa [18] showed that glycolytic disorder and ATP depletion, which result from P deficiency, appear to be primary and essential steps leading to hemolysis in postparturient hemoglobinemia in cows. Erythrocytes are the most abundant cell type in blood. In addition, erythrocytes have an important role in host defense

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against bacteria and viruses through the cell membrane immune system [17]. Hemolytic anemia can result in many health problems, such as heart failure, arrhythmias, enlarged heart, and fatigue. Although the effects of P deficiency on hematological parameters have been studied [18,23], a limited number of reports have focused on systematic study of the effects of P deficiency on erythrocytes. Thus, the mechanism involved in the effects of P deficiency on erythrocytic function remains to be elucidated. The aim of this study was to obtain data for the subsequent establishment of the relationship between the level of P in the diet of cows and the morphofunctional state of erythrocytes. The assessed data included the P content in the blood serum of cows, erythrocyte indices (*e.g.*, red blood cell [RBC]), mean corpuscular hemoglobin (MCH), and mean corpuscular volume [MCV] indices), the structure of erythrocyte membranes, the phospholipid composition and fluidity of erythrocyte membranes, antioxidant enzyme activities in erythrocytes, and the activities of sodium-potassium adenosine triphosphate (Na^+/K^+ -ATPase), magnesium adenosine triphosphate (Mg^{2+} -ATPase) and calcium adenosine triphosphate (Ca^{2+} -ATPase) in erythrocyte membranes.

Materials and Methods

Experimental design

Cows were treated humanely and the experiment received prior ethical approval in accordance with Northeast Agricultural University policies (approved protocol No. SRM-15). All procedures were performed in an animal facility accredited by the Center for Accreditation of Laboratory Animal Care and Use China Health Science Foundation. Forty Holstein-Friesian dairy cows (2–3 years old) in mid-lactation (on average 90 days post calving), weighing 705 ± 20 kg and producing 28 ± 3 kg milk per day, were included in this study and were randomly divided into two groups (20 cows per group). Cows were housed in a tie-stall system on rubber mats covered with sawdust in a temperature-controlled building. No visual signs of pica or coprophagia were observed. The study consisted of a two-week acclimation period and a four-week P-amended diet supply. Cows were fed the same base total mixed ration (TMR), based on corn silage, grass seed straw, and beet pulp throughout the entire study period. The base TMR was formulated to meet nutrient requirements for lactating dairy cows, with the exception of its dietary P content [3]. A NaH_2PO_4 supplement was added to the base TMR (control diet) to obtain a dietary P content of 0.36% P/kg of dry matter (DM) during the acclimation period. Cows fed a P-deficient diet did not receive the NaH_2PO_4 supplement. The P-deficient diet contained 0.03% P/kg DM and was fed to one group of cows during the P depletion period. During that period, the other group of cows received the control diet. Cows had free access to feeding and water throughout the acclimation and P depletion periods. Cows

were milked twice per day before feeding.

Samples of blood were collected by jugular venipuncture at the end of the P depletion period. To obtain serum, the blood-containing vial was placed on a slanted surface for 10 to 15 min to allow for clotting. In addition, other samples of blood were collected in ethylenediaminetetraacetic acid (used for hematological examination) and heparin sodium (used to separate erythrocytes) anticoagulation tubes. Centrifugation of blood was performed at $2,450 \times g$ for 20 min at 4°C . The discarded leucocytes and plasma, as well as the remaining erythrocytes, were resuspended in phosphate-buffered saline (PBS), washed twice, and then resuspended in PBS.

Concentrations of serum P

Concentrations of serum P were measured in serum by using the phosphomolybdate method (Pars Azmun kit; Pars Azmun, Iran) and an auto-analyzer (model 7020; Hitachi, Japan).

RBC indices

Complete blood count analyses were accomplished by using an auto-animal hematology analyzer (LQ06 ABACUS Junior 5 VET; Diatron, Austria) and following the manufacturer's instructions. Parameters such as RBC, MCH, and MCV were obtained.

Freeze-substitution transmission electron microscopy (TEM)

Liquid nitrogen (-190°C), used as a cooling medium, produced rapid cooling and frozen erythrocyte samples. To reduce ice crystals, the frozen samples were irradiated with microwaves (maximum power 500 W, frequency 2.4 GHz) for 50 min. The irradiated samples were fixed in 1% osmium tetroxide and were kept at -80°C overnight; then raised to room temperature and dehydrated in a graded series of ethanol within 12 h. After warming, the samples were washed with absolute acetone and embedded in Epon 812. Finally, erythrocyte morphology was examined under a transmission electron microscope (JEM-100CX; Nihon Densi, Japan).

Assay of aminotransferase activities in serum

Aminotransferase activities were analyzed by using coupled kinetic assay methods [16]. The aspartate transaminase (AST) assays used a coupled enzymatic reaction (AST kit 000000020; Biosino Bio-Technology and Science, China). Initially, aspartate and α -ketoglutarate were converted to glutamate and oxaloacetate, which were then converted by malate dehydrogenase to provide malate and NAD^+ . The conversion of the NADH chromophore to the NAD^+ product, measured at 340 nm by using a visible spectrophotometer (V-1600; Cany Precision Instruments, China), was proportional to the level of AST enzyme in the sample. Alanine transaminase (ALT) assays (ALT kit 000000010; Biosino Bio-Technology and Science) were similar to the AST assays. Alanine and α -ketoglutarate

were first converted by ALT enzyme to glutamate and pyruvate. Then, the pyruvate reacted with a hydrazine reagent to produce a colored product. The concentration of ALT in each sample was determined by measuring the absorbance at 510 nm by using a visible spectrophotometer (V-1600; Cany Precision Instruments).

Determination of serum parameters

The concentrations of total bilirubin (T-Bil) and indirect bilirubin (I-Bil) were analyzed by using diagnostic kits (T-Bil kit, 000000300 and I-Bil kit, 000000310; Biosino Bio-Technology and Science).

Determination of antioxidant enzyme activities in erythrocyte

Superoxide dismutase (SOD) activity in erythrocytes was determined based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide [20]. One unit of SOD activity is defined as the amount of enzyme that causes a reduction of half the maximum inhibition of NBT; this reduction is obtained by measuring the absorbance at 550 nm (Nanjing Jiancheng Bioengineering Institute, China). Glutathione peroxidase (GSH-Px) activity in erythrocytes was measured by using a spectrophotometer (7230 G; Shanghai Jinghua, China) at 37°C and 412 nm absorbance according to the method described by Rotruck *et al.* [21]. Formation of malondialdehyde (MDA), as an indicator of lipid peroxidation, was determined by using a thiobarbituric acid assay (MDA Detection Kit A003; Nanjing Jiancheng Bioengineering Institute) [5]. Erythrocyte protein content in the samples was measured by applying the method described by Bradford [1].

Preparation of erythrocyte membranes and phospholipid extraction

Packed RBCs were hemolyzed by mixing in 40 volumes of phosphate buffer. White creamy intact ghost membranes were obtained after 3 to 4 identical washes, as described by Steck and Kant [24]. Phospholipids were extracted from the obtained erythrocyte ghost membranes by using a chloroform, methanol, and water system as described by Shafiq-ur-Rehman [22].

Phospholipid composition of erythrocytic membrane

Phospholipids are asymmetrically distributed in a bilayer membrane of erythrocytes, with amino-containing phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) on the inner cytoplasmic surface and choline-containing phosphatidyl choline (PC) and sphingomyelin (SM) on the exterior extracellular surface. Thin-layer chromatography (TLC) of solvent extracts of native and [¹⁴C]cyanate-treated erythrocyte membrane lipids, as well as PS, PE, PC, and SM was performed [30]. TLC plates (20 × 20 cm) were spotted with sample (typically 5–10 μL) by using a 5 μL Hamilton syringe and were developed in a solvent system (appropriate for the lipid to be investigated) to

within 0.5 cm of the top of the plate. The TLC plate was then air dried and stained for either phospholipid or total lipid visualization [30]. Phospholipids were visualized by spraying the plates with a phospholipid-specific stain, according to the procedure proposed by Trepanier [30].

Activities of Na⁺/K⁺-ATPase, Ca²⁺-ATPase, and Mg²⁺-ATPase in erythrocyte membrane

The Na⁺/K⁺-ATPase, Ca²⁺-ATPase, and Mg²⁺-ATPase activities in erythrocyte membranes were detected by using biochemical methods described in the instructions of reagent kits (Na⁺/K⁺-ATPase, catalog No. A070-2; Ca²⁺+Mg²⁺-ATPase, catalog No. A070-3) purchased from the Nanjing Jiancheng Bioengineering Institute, China. Each absorbance of Na⁺/K⁺-ATPase, Ca²⁺-ATPase, and Mg²⁺-ATPase was measured at 636 nm by using a microtiter plate reader (Varioskan Flash; Thermo Scientific, USA).

Erythrocyte membrane fluidity

Quantitative measurement of membrane fluidity was performed by applying the fluorescence polarization technique with 1,6-diphenyl-1,3,5-hexatriene (DPH) as the fluorescence probe. Erythrocyte membrane preparations (50 μg protein) were suspended in 50 mmol/L DPH solubilized in tetrahydrofuran and incubated at 37°C for 30 min. Fluorescence polarization of samples held at 25°C was determined by using a Hitachi fluorescence spectrophotometer (F-4500; Hitachi) equipped with rotating polarized filters. Samples were excited at 360 nm, while the emission intensity was read at 435 nm. Polarization (*P*) and fluorescence anisotropy (*γ*) were calculated by using the following equation: $P = I_{VV} - I_{VH-G} / I_{VV} + I_{VH-G}$, where *I_{VV}* and *I_{VH}* are the intensities measured parallel and perpendicular to the vertical axis of the excitation beam, and *G* is the efficiency ratio of the detection system for vertically and horizontally polarized light and is equal to I_{VH} / I_{HH} , where *I_{HH}* is the horizontal components of emission from horizontally polarized exciting light. The *γ* value was calculated by using the formula $\gamma = 2P / (3 - P)$ [12,15].

Statistical analysis

Data were analyzed by using SPSS for Windows (ver. 13; SPSS, USA) and GraphPad Prism (GraphPad Software, USA). Comparisons of means were conducted by using a two-tailed unpaired Student's *t*-test when the main effect was significant. Data are expressed as means ± SDs, and significance level was set at *p* < 0.05.

Results

In the control diet group, all cows remained healthy and no clinical symptoms were observed. However, 11 cows fed the P-deficient diet had clinical symptoms such as clinically

Table 1. Hematological parameters in cows that were fed a control diet or a phosphorus-deficient diet

Group	RBC ($10^{12}/L$)	MCH (pg)	MCV (fL)
P-deficient diet	$4.34 \pm 0.38^*$	$9.67 \pm 1.58^*$	$56.05 \pm 3.19^*$
Control diet	6.25 ± 0.20	15.24 ± 2.45	46.61 ± 3.33

Asterisks indicate significant differences ($p < 0.05$) between cows fed a control diet and those fed a P-deficient diet. Data presented as a mean \pm SD ($n = 20$). RBC, red blood cell; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

Table 2. Serum parameters in cows which were fed a control diet or a phosphorus-deficient diet

Group	T-Bil ($\mu\text{mol}/L$)	I-Bil ($\mu\text{mol}/L$)	AST (IU/L)	ALT (IU/L)
P-deficient diet	$2.73 \pm 0.47^*$	$2.47 \pm 0.26^*$	$179.2 \pm 23.24^*$	$49.6 \pm 7.6^*$
Control diet	0.16 ± 0.05	–	66.2 ± 4.3	19.02 ± 4.6

Asterisk in panels indicated that there were significant differences ($p < 0.05$) between cows fed control diet and those fed a P-deficient diet. Data presented as mean \pm SD ($n = 20$). T-Bil, total bilirubin; I-Bil, indirect bilirubin; AST, aspartate transaminase; ALT, alanine transaminase.

apparent muscle weakness or recumbency, jaundice, and reductions in feed intake and milk production.

Effect of P deficiency on serum P concentration

The concentration of P in serum was significantly lower in the P-deficient diet group (0.48 ± 0.37 mmol/L) than that in the control diet group (2.02 ± 0.16 mmol/L).

Effect of P deficiency on hematological parameters

As shown in Table 1, compared to the control diet group, cows in the P-deficient diet group had significantly lower RBC and MCH levels and a significantly higher MCV index (all $p < 0.05$).

Effect of P deficiency on erythrocyte morphology

The characteristic distribution of membrane-associated particles on the erythrocyte protoplasmic face and the etched surface of the cell revealed a texture that reflected the arrangement of the underlying particles (panel A in Fig. 1). The surface of the erythrocytes and the manometer range showed that the proteins of the erythrocyte membranes in the P-deficient diet group underwent a marked change in both structure and function. In the P-deficient group, the internal bilayer membrane surfaces were smooth and largely devoid of membrane-associated particles; occasionally, small clusters of particles could be seen (panel B in Fig. 1).

Effects of P deficiency on serum parameters

Serum T-Bil and I-Bil concentrations in the P-deficient diet group were greater ($p < 0.05$) than those in the control diet group. Similarly, there were significantly higher ($p < 0.05$) activity levels of both AST and ALT in the P-deficient diet group than in the control diet group (Table 2).

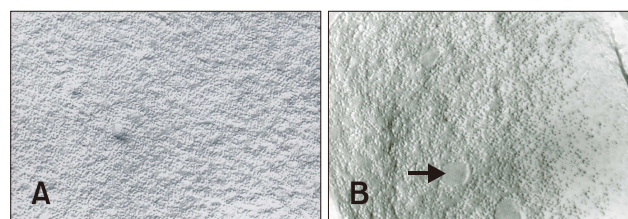


Fig. 1. Erythrocyte morphology in cows which had been fed the control diet (A) or a phosphorous-deficient diet (B). Arrow indicates the internal bilayer membrane surfaces were smooth and largely devoid of membrane-associated particles. 10,000 \times .

Antioxidant enzyme activity changes in erythrocytes

To examine whether P deficiency could result in oxidative stress, we assessed SOD and GSH-Px activities and MDA content in cows' erythrocytes (Table 3). SOD activity in the erythrocytes of the P-deficient group was significantly lower ($p < 0.05$) than that in the control diet group. Similarly, GSH-Px activity was significantly lower ($p < 0.05$) in the P-deficient diet group than in the control group. In contrast, the MDA content in the P-deficient diet group's erythrocytes was approximately 22% greater than that in the control diet group ($p < 0.05$).

Effects of P deficiency on phospholipid composition of erythrocyte membrane

In the present study, the composition of phospholipids in the control group's erythrocytic membranes was 54.9% PE, 6.35% PC+PS, and 38.75% SM as determined by using TLC (Fig. 2). The P-deficient group's erythrocyte membrane phospholipid composition showed a significantly lower PE level (39.48%) and significantly higher PC+PS (12.85%) and SM (46.68%)

Table 3. Antioxidant function in erythrocyte incows which were kept on control diet and a diet with deficiency of phosphorus

Group	SOD ($\mu\text{mol/L}$)	GSH-Px ($\mu\text{mol/L}$)	MDA (nmol/mL)
P Deficiency Diet	1,961.34 \pm 86.38*	157.67 \pm 12.58*	8.05 \pm 0.19*
Control Diet	2,785.88 \pm 115.5	202.23 \pm 11.45	6.61 \pm 0.33

Asterisks indicate significant differences ($p < 0.05$) between cows fed a control diet and those fed a P-deficient diet. Data presented as mean \pm SD ($n = 20$). SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

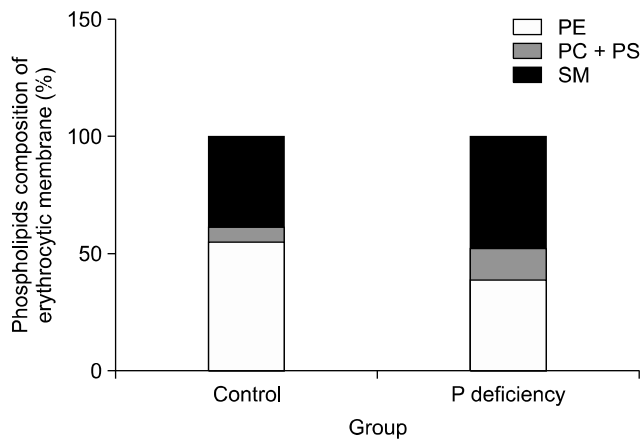


Fig. 2. The content of phosphatidyl ethanolamine (PE), phosphatidylcholine (PC), phosphatidyl serine (PS) and sphingomyelin (SM) in membranes of erythrocytes in cows that were fed a control diet or a phosphorus-deficient diet.

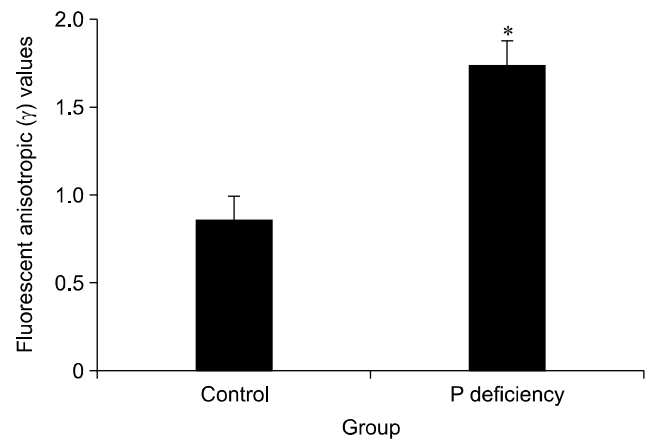


Fig. 4. Erythrocyte membrane fluidity in cows that were fed a control diet or a phosphorous-deficient diet. Asterisk indicates that a significant difference ($p < 0.05$) between cows fed a control diet and those fed a P-deficient diet. Data presented as a mean \pm SD ($n = 20$).

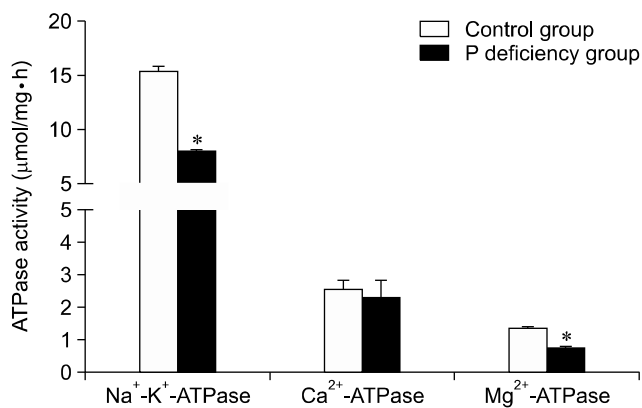


Fig. 3. The activities of Na^+/K^+ -ATPase, Ca^{2+} -ATPase, and Mg^{2+} -ATPase in membranes of erythrocytes in cows that were fed a control diet or a phosphorous-deficient diet. Asterisks indicate significant differences ($p < 0.05$) between cows fed a control diet and those fed a P-deficient diet. Data presented as a mean \pm SD ($n = 20$).

levels (all $p < 0.05$).

Effect of P deficiency on Na^+/K^+ -ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase activities in erythrocyte membranes

Fig. 3 shows that Na^+/K^+ -ATPase activity in erythrocyte membranes of the P-deficient group was significantly inhibited ($p < 0.05$), reaching a level approximately 48.5% lower than that in the control diet group. Similarly, the Mg^{2+} -ATPase activity in erythrocyte membranes also showed a significant inhibition in the P-deficient group; however, the activity levels of Ca^{2+} -ATPase in the two groups were not significantly different.

Effects of P deficiency on erythrocyte membrane fluidity

The markedly high γ values in the P-deficient erythrocyte membranes clearly indicate that the erythrocyte membranes in the P-deficient diet group would be more rigid than those in the control group ($p < 0.05$; Fig. 4).

Discussion

Postparturient hemoglobinuria is a condition that, worldwide, most commonly affects individual high-yield lactating dairy cows. It is characterized by development of peracute

intravascular hemolysis and anemia with a potentially fatal outcome. While the exact cause is unknown, P deficiency, or hypophosphatemia, has been suggested as a major predisposing factor. Some studies [6,26] have shown that P deficiency can induce a variety of functional and structural derangements, such as muscle weakness, decreased hepatic oxygenation, liver function abnormalities, red blood cell rigidity and hemolysis, and cerebral and platelet dysfunction. Because transaminase enzymes are important in the production of various amino acids, significantly increased transaminase activity can be an indicator of liver damage; bilirubin can be elevated due to continuous hemolysis. In the present study, we found that the P deficiency increased the activities of AST and ALT in cows, illustrating that P deficiency could induce liver function damage in cows, and such damage might be the result of erythrocyte damage. We also observed that, while serum P concentrations were significantly lower in the P-deficient diet group than in the control diet group, clinical signs such as anemia, tachycardia, weakness, hemoglobinuria with dark brown or red urine, and a drop on milk production quickly occurred in the P-deficient group. The freeze-substitution TEM results indicated a significant difference in the erythrocyte shape in the two groups. In addition, RBC indices revealed macrocytic anemia, which meant that erythrocytes were swelling and becoming hemolyzed, in cows fed the P-deficient diet.

Severe intracellular P depletion in erythrocytes is known to increase their osmotic fragility, possibly predisposing them to intravascular hemolysis. There are many factors associated with maintaining the normal function of erythrocyte membranes. Preventing lipid peroxidation is one of the most important factors, and lipid peroxidation is thought to be affected by dietary P levels [29]. Only a few preliminary studies have discussed the roles of P in animal antioxidant systems. Ogawa [19] indicated that inadequate P in the plasma impairs the function and viability of erythrocytes by hindering ATP production and causing oxidative stress. High production of reactive oxygen species results in oxidative stress, which is a deleterious process that can be an important mediator of damage to erythrocyte structures. In order to elucidate the basic oxidative functional responses of erythrocytes in cows under P deficiency, we measured erythrocyte SOD, GSH-Px activity, and MDA content. We observed that erythrocyte SOD, GSH-Px activity, and MDA content were significantly affected by P deficiency. Since SOD and GSH-Px have a major role in the process of eliminating free radicals [31], it appears that erythrocyte SOD and GSH-Px were downregulated in cows fed a low-P diet in order to reduce free radicals. This led to an accumulation of the final product of lipid peroxidation; finally, damaging the erythrocyte membranes in cows fed a P-deficient diet.

P deficiency decreases the life span of erythrocytes, an effect

presumably resulting from energy depletion. ATP has a significant role in the functioning of a cell [28]. ATP is considered by biologists to be the energy currency of life, as ATP transports chemical energy within cells for metabolic purposes [25]. The structure of ATP includes triphosphate, which contains P. In rats and humans, P depletion can induce cellular Ca toxicity and decrease the cytosolic ATP concentration [14]. High intracellular Ca interferes with mitochondrial oxidation and ATP synthesis and impairs Ca signaling in the cell, leading to a reversible loss of cell function in different cell types. Thus, P deficiency may likely affect ATP concentrations in erythrocytes [27]. ATPase works via two high-energy electrons that are used in most photosynthesis procedures with NADPH; they are converted into two separate molecules and carried between the chloroplast inner membranes. The ATPase controls the course of those two molecules and carries them to different sections in the inner membrane thylakoids. ATPase, by using the energy in the proton gradient established by the electron transport chain, synthesizes ATP from ADP and inorganic phosphate. ATP depletion further impairs the function of the Na^+/K^+ -ATPase, leading to a loss of cell K^+ , a phenomenon that fosters erythrocyte "apoptosis" [11]. Ca^{2+} -ATPase is responsible for the transport of Ca^{2+} to extracellular space. Both of those enzymes require Mg^{2+} ions for the active transport of cations [32]. In the present study, we observed 48.5% and 46.7% decreases in activities of Na^+/K^+ -ATPase and Mg^{2+} -ATPase, respectively, in erythrocyte membranes in cows fed the P-deficient diet. Although there were no obvious changes in Ca^{2+} -ATPase in the P-deficient group, there was a slight decline in Ca^{2+} -ATPase in that group.

Erythrocyte membrane fluidity may be influenced by lipid components, such as cholesterol and phospholipid [4]. Phospholipids are asymmetrically distributed in the erythrocyte membrane bilayer, with PS and PE occurring on the inner cytoplasm and PC and SM on the exterior extracellular surfaces. Structurally, these phospholipids provide molecular strength to maintain membrane stability, proper polarization, and special arrangements for optimal activity of membrane-bound enzymes [10]. In the present study, the P deficiency increased the PC+PS and SM contents and decreased the PE content in the erythrocyte membranes in cows. Those results illustrate that a P deficiency can alter the membrane phospholipid milieu by affecting erythrocyte membrane organization and structure, which may have a role in the modulation of fluidity in P deficiency.

The results of the present study support the hypothesis that a reduction of inorganic P in the feed, and subsequently the sera, of cows directly affects RBC function resulting in significant decreases in the number of RBC and the level of MCH and an increase in MCV when compared with those parameters in the control diet group. Those effects are reflected in the decrease in the activity level of some ATPases. Reducing the concentration

of P also affects the fluidity of erythrocyte membranes and the concentration of phospholipids (PE, PC + PS and SM) in those membranes. In addition, a P reduction alters the oxidative stress parameters in erythrocytes, indicated by a significant decrease in SOD and GSH-Px activities and an increase in MDA content. These data confirm that the reduction of P levels in cows may affect the life expectancy of their erythrocytes.

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Conflict of Interest

The authors declare no conflict of interests.

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