Suitability of oral administration of monosodium phosphate, disodium phosphate, and magnesium phosphate for the rapid correction of hypophosphatemia in cattle

Imke Cohrs¹ | Walter Grünberg¹,²

¹Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hanover, Germany
²Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands

Background: Hypophosphatemia is commonly associated with disease and decreased productivity in dairy cows particularly in early lactation. Oral supplementation with phosphate salts is recognized as suitable for the rapid correction of hypophosphatemia. Little information is available about the differences in efficacy between salts used for oral phosphorus supplementation.

Objectives: Comparison of efficacy of oral administration of NaH₂PO₄, Na₂HPO₄, and MgHPO₄ in treating hypophosphatemia in cattle.

Animals: 12 healthy dairy cows in the fourth week of lactation in their second to fifth lactation.

Methods: Randomized clinical study. Phosphorus deficient, hypophosphatemic cows underwent a sham treatment and were afterwards assigned to 1 of 3 treatments—NaH₂PO₄, Na₂HPO₄, or MgHPO₄ (each provided the equivalent of 60 g of phosphorus). Blood samples were obtained immediately before and repeatedly after treatment.

Results: Treatment with NaH₂PO₄ and Na₂HPO₄ resulted in rapid and sustained increases of plasma phosphate concentrations ([Pi]). Significant effects were apparent within 1 hour (NaH₂PO₄: P = 0.0044; Na₂HPO₄: P = 0.0077). Peak increments of plasma [Pi] of 5.33 mg/dL [5.26–5.36] and 4.30 mg/dL [3.59–4.68] (median and interquartile range) were reached after 7 and 6 hours in animals treated with NaH₂PO₄ and Na₂HPO₄, respectively, whereas treatment with MgHPO₄ led to peak increments 14 hours after treatment (3.19 mg/dL [2.11–4.04]).

Conclusions and Clinical Importance: NaH₂PO₄ and Na₂HPO₄ are suitable to rapidly correct hypophosphatemia in cattle. Because of the protracted and weaker effect, MgHPO₄ cannot be recommended for this purpose. Despite important differences in solubility of NaH₂PO₄ and Na₂HPO₄ only small plasma [Pi] differences were observed after treatment.

KEYWORDS: dairy cow, hypophosphatemia, magnesium phosphate, oral, sodium phosphate, treatment

Abbreviations: AUC-Pi, area under the concentration-time curve of plasma phosphate; C, CONTROL (denomination of treatment group); [Ca], concentration of calcium; [Mg], concentration of magnesium; MgP, group treated with MgHPO₄ (denomination of treatment group); NaP, group treated with NaH₂PO₄ (denomination of treatment group); Na₂P, group treated with Na₂HPO₄ (denomination of treatment group); P, phosphorus; Pi, phosphate; [Pi], concentration of phosphate; Pi-Cmax, peak concentration of plasma phosphate; Pi-Cmax-model, peak concentration of plasma phosphate derived from a model; ΔPi-Cmax, peak concentration of plasma phosphate increment; ΔPi-Cmax-model, peak concentration of plasma phosphate increment derived from a model; Pi-Tmax, time to peak concentration of plasma phosphate; Pi-Tmax-model, time to peak concentration of plasma phosphate derived from a model; [TP], total protein concentration.

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1 | INTRODUCTION

Hypophosphatemia in dairy cows is thought to be associated with an increased risk of disease and impaired productivity particularly in early lactation. It not only has been linked to decreased feed intake and productivity but is also believed to cause or contribute to periparterm conditions of the dairy cow such as the downer cow syndrome or postparturient hemoglobinuria. The incidence of periparturient hypophosphatemia even in clinically healthy dairy cows is considerable. Metabolic profiling studies reported incidences of subnormal plasma phosphate concentrations ([Pi]) on the day of calving of above 50% and of 10%-15% in the first two weeks of lactation. The relevance of this periparturient hypophosphatemia remains disputed as it is frequently not associated with clinical signs such as recumbency and postparturient hemoglobinuria. It is the reason for which phosphorus (P) supplementation is recommended and practiced particularly in fresh cows.

Treatment recommendations published over the past decades often favor IV bolus administration of solutions containing phosphate (Pi) salts. This approach however has raised concerns for several reasons. First in most countries of the world there are no commercial Pi-containing solutions available that are labeled for the parenteral use in farm animals, making this approach an off-label treatment. Products containing P in a form different and not convertible to Pi seem to be unsuitable for the correction of hypophosphatemia. Secondly bolus infusion of Pi-salt solutions results in a pronounced but short lived increase of plasma [Pi] above the reference range. The poor solubility of Pi may result in complexing of Pi with divalent cations such as calcium or magnesium in plasma thereby causing a decline of the plasma calcium ([Ca]) and magnesium concentration ([Mg]), which presents a concern particularly in early lactation. Thirdly the plasma [Pi] returns to baseline values within two to three hours after administration, making this treatment unsuitable to be used as stand-alone treatment of hypophosphatemia.

Oral treatment of P-depleted cows with Pi salts slightly delays onset of action when compared with IV treatment but the treatment is easy to administer and has as an effect lasting for 12–24 hours. Currently, a variety of Pi-containing salts are recommended and used for the oral treatment of hypophosphatemia in cattle, but studies comparing these salts in their efficacy to correct hypophosphatemia are scant. Therefore, the objective of this study was to compare different Pi-salts commonly used in the field in their treatment efficacy for the correction of hypophosphatemia in dairy cows. The salts to be compared were monosodium dihydrogen phosphate (NaH₂PO₄), disodium monohydrogen phosphate (Na₂HPO₄), and magnesium phosphate (MgHPO₄). We hypothesized that the efficacy for the correction of hypophosphatemia of these salts is primarily driven by their solubility characteristics. We therefore anticipated most rapid and pronounced effects for the most soluble NaH₂PO₄ followed by Na₂HPO₄ while a weaker and more delayed effect was expected for the poorly soluble MgHPO₄.

2 | MATERIALS AND METHODS

2.1 | Animals, housing, and feeding

The national and institutional guidelines for the care and use of experimental animals were followed and all experimental procedures were approved by the Utrecht University Institutional Animal Care and Use Committee (AVD108002016616).

Twelve clinically healthy Holstein Friesian cows in their fourth week of lactation were used for this study. Cows were between 3 and 6.5 years old and in their second to fifth lactation. All animals were healthy on the basis of physical examination despite of pronounced hypophosphatemia. Cows were housed in tie-stalls with rubber bedding covered with saw dust in a temperature controlled environment.

Prior and during the experimental treatment enrolled cows were fed a Total Mixed Ration that was markedly phosphorus deficient, but that otherwise met currently used nutrient recommendations for dry and lactating dairy cows for a period of ~8 weeks. The dry cow ration that was offered for 4 weeks before the expected calving date contained ~0.13% P in DM and the ration fed after calving contained ~0.18% P in DM. The dietary P content was thus ~50% below requirement during the late dry period and at least 40% below the recommended daily dietary supply after parturition. Cows were fed and milked twice daily between 0600 and 0700 hours and 1800 and 1900 hours. The daily milk yield during the experimental treatment period ranged between 25 and 32 kg/day, daily feed intake ranged between 13 and 18 kg DM.

2.2 | Experimental study

All cows received a sham treatment serving as control and were thereafter assigned to 1 of 3 experimental treatments by randomization. The wash-out period between control and treatment was between 24 and 48 hours. Treatments consisted in a single oral administration of either plain water (sham treatment, group C, n = 12) or the equivalent of 60 g P in the form of monosodium dihydrogen phosphate (302 g NaH₂PO₄ × 2H₂O, group NaP, n = 4), disodium monohydrogen phosphate (345 g Na₂HPO₄ × 2H₂O, group Na₂P, n = 4) or magnesium hydrogen phosphate (338 g MgHPO₄ × 3H₂O, group MgP, n = 4). The salt was dissolved in 2 L of warm (~38°C) water and was administered by oro-gastric tube. Treatments were administered at standardized times between 0800 and 0830 hours. Sham treatment always preceded the experimental treatment. This approach was used because of the high individual variability of the treatment effect of PO administered Pi observed in earlier studies and the resulting difficulty to determine an adequate wash-out period for the treatment. For blood sampling a 16 G catheter (Angiocath; Becton Dickinson, Heidelberg, Germany) connected to an extension set (Discofix C-3, 10 cm; Braun Melsungen AG, Melsungen, Germany) was placed aseptically in a jugular vein the evening before the sham treatment.

Blood samples were collected immediately before treatment or sham administration (0) and again 30, 60, 90, 120, 180, 240, 300, 420, 720, and 1440 minutes after treatment. Blood was collected in 10 mL
tubes containing lithium heparin as anticoagulant and kept at room temperature until processed. Samples were centrifuged within 10 min at 1600g for 15 minutes. Plasma was then harvested and stored at −20°C until analyzed.

2.3 | Biochemical analysis

The plasma [Pi], [Mg], and [Ca] were analyzed photometrically (ammonium molybdate, xylidyl blue, and Arsenazo III, respectively) on an automated analyzer (ABX Pentra 400; Horiba, Europe GmbH, Langenhagen, Germany). Total protein concentration ([TP]) was measured by refractometry using a temperature corrected refractometer.

2.4 | Data analysis

For each animal, the maximum plasma [Pi] (Pi-max) and the time to maximum plasma [Pi] (Pi-T-max) were derived from its individual concentration – time plot. The area under the curve of plasma [Pi] (AUC-Pi) was calculated using the trapezoidal rule. The peak increment of plasma [Pi] was determined for each study animal by subtracting the baseline plasma [Pi] from the peak concentration of the electrolyte in question (ΔPi-Cmax). The same approach was used for [Mg] in animals of group MgP.

Plasma [Pi] at T1 was furthermore corrected for the change in plasma [TP] on a percent basis occurring between T0 and T1 as described earlier.12 This approach was used to assess changes of plasma volume that may have occurred during the observation and may have affected the plasma [Pi] or [Mg].

To improve the precision of the estimation of ΔPi-Cmax and Pi-Tmax the plasma [Pi] for the treatment curves were modeled as log-normal distribution such that f = y0 + y1e^{-0.5*(ln(x)/b)^2/a} using a plotting software package (Sigma Plot 12.5; Systat Software, San Jose, California). ΔPi-Cmax-model and Pi-Tmax-model were then derived from the respective functions for each treatment group.

2.5 | Statistical analysis

Results are expressed as mean and SD or as median and interquartile range (IQR) for variables that were not normally distributed. Normality of distribution was checked by Shapiro-Wilk’s test for normality distribution and by checking normal probability plots. Log transformations were used wherever appropriate to obtain normal distribution. Repeated measurements ANOVAs were conducted to determine treatment, time, and interaction of time and treatment effects using an autoregressive model with Cow ID as subject. Post hoc tests were used whenever F-test was significant. Bonferroni-corrected P values were used and a P < .05 was considered statistically significant. A statistical software package was used for all analyses (SAS 9.2; SAS Institute, Cary, North Carolina).

3 | RESULTS

All cows tolerated the assigned oral treatment well and remained healthy without any apparent adverse effects.

The plasma [Pi]-time curves stratified by group are presented in Figure 1. All cows included in the study presented pronounced hypophosphatemia immediately before treatment (NaP: 1.27 mg/dL [0.41 mmol/L], Na2P: 1.38 mg/dL [0.45 mmol/L], MgP: 1.44 mg/dL [0.47 mmol/L], C: 1.30 mg/dL [0.42 mmol/L]), which is notably beneath reference range of 4–8 mg/dL or 1.4–2.6 mmol/L.13 Baseline plasma [Pi] did not differ between groups.

The ANOVA revealed a significant treatment effect, with all treatment groups differing from control and MgP differing from NaP and Na2P. Although the plasma [Pi] in group NaP was numerically higher than in Na2P at various time points the difference was not significant at any time. Time effects were apparent in all groups except control.

The plasma [Pi] tended to increase within 30 minutes of treatment in groups NaP and Na2P with significantly increased values relative to baseline 60 minutes after treatment (NaP: P = .0044; Na2P: P = .0077).

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**FIGURE 1**  Plasma phosphorus concentration–time curve (median and IQR) stratified by treatment groups: group NaP (treatment with NaH2PO4, solid line; n = 4), group Na2P (treatment with Na2HPO4, dashed dotted line; n = 4), group MgP (treatment with MgHPO4, dotted line; n = 4), and group C (control group, dashed line; n = 12). Time points marked with* differ significantly from T0. Values marked with different lowercase letters at a specific time point differ significantly between groups (P < .05; Bonferroni corrected). Data of groups NaP and MgP are slightly offset with respect to time to improve readability. Reference range for plasma phosphorus in cattle: 4–8 mg/dL.13 C, control, MgP, treatment with MgHPO4, NaP, treatment with NaH2PO4, Na2P, treatment with Na2HPO4.
The Pi-\(T_{\text{max}}\) was 420 and 360 minutes after treatment with a peak increment of 5.33 mg/dL (1.72 mmol/L) and 4.30 mg/dL (1.39 mmol/L) in group NaP and Na2P, respectively (Table 1). The plasma [Pi]-time curve of group MgP revealed a very flat increase with values differing from baseline only after 300 minutes and a Pi-\(T_{\text{max}}\) of 860 minutes with a maximum plasma [Pi] increment of 3.19 mg/dL (1.03 mmol/L). As expected no time effect on plasma [Pi] was observed in the control group. The median plasma [Pi] reached the reference range between 120 and 180 minutes after treatment in groups NaP and Na2P and after 720 minutes in group MgP. The plasma [Pi] remained markedly increased compared with baseline values until the end of the observation period at 24 hours post treatment in all three treatment groups.

Analysis of the protein corrected plasma [Pi] and comparison with uncorrected plasma [Pi] did not reveal any effect attributable to plasma volume changes (data not shown).

Table 1 summarizes the peak increment of plasma [Pi] (Pi-\(\Delta C_{\text{max}}\)), Pi-\(T_{\text{max}}\), and the AUC-Pi, values determined from the modeled plasma [Pi]-time curves are shown in Table 2. The AUC-Pi was the largest in group NaP but differed only numerically from group Na2P. The AUC-Pi of group MgP was 50% of group NaP.

Treatment with MgHPO4 led to significant differences between group MgP and C (240–720 minutes) and MgP and Na2P (420–720 minutes) in plasma [Mg] (Figure 2).

Plasma [Ca] neither showed a significant time nor treatment effect (data not shown).

### Discussion

After oral treatment of markedly phosphorus deficient cows with 1 of 3 different Pi containing salts (NaH2PO4, Na2HPO4, or MgHPO4) the increase in plasma [Pi] was most pronounced in group NaP, followed by Na2P. The increment of plasma [Pi] in group MgP was considerably flatter and delayed in time. We suggest that solubility characteristics of the salts used plays a central part in oral availability for resorption.

The main objective of the present study was to compare the efficacy of different Pi containing salts for the treatment of hypophosphatemia in dairy cows. The addressed question is relevant as the expectation of an oral treatment with Pi-salts to rapidly correct acute hypophosphatemia in cows is inherently different from the use of Pi-salts used as feed additives to supplement P-deficient diets. An oral treatment for the correction of hypophosphatemia that is suitable to replace or at least complement parenteral P-supplementation should have a rapid onset of action and a sustained effect lasting for at least 24 hours. For example, earlier studies revealed that dicalcium phosphate, a salt widely used for the supplementation of P-deficient ruminant rations, is poorly suited for the rapid correction of hypophosphatemia.6,9 Another interesting observation is that the preference of different Pi containing salts varies by geographic regions for no apparent reason. While monosodium phosphate is widely used for the treatment of hypophosphatemia in the Americas, in Europe disodium phosphate is widely marketed for the oral treatment of cattle.2,5,14

Baseline plasma [Pi] determined in this study were consistent with pronounced hypophosphatemia and without difference between treatment groups, confirming that the dietary P-depletion protocol used before application of the experimental treatment was appropriate to induce hypophosphatemia. A rapid and sustained effect on the plasma [Pi] was observed for both sodium phosphate salts with significant effects detectable within 1 hour, peak concentrations occurring after six to seven hours and an effect sustained for over 24 hours (Figure 1 and Table 1). In both groups, the low end of the reference range of plasma [Pi] was reached within 2–3 hours and maintained for 24 hours after treatment. Differences between group NaP and Na2P were not significant suggesting a similar effect of NaH2PO4 compared with Na2HPO4. This is remarkable as both salts differ considerably in their solubility characteristics. Na2HPO4 has a relatively low solubility of 7.7 g/dL water15 compared with NaH2PO4 with 85 g/dL water at 20°C.15 The low sample size of four animals per treatment group in this study likely resulted in insufficient statistical power to reveal the moderate difference between the treatments with NaH2PO4 and Na2HPO4.

The treatment effect with NaH2PO4 reported in this study differs from results of other studies conducted in cattle having used similar doses of NaH2PO4.9,10 In these studies, a less pronounced effect on
the plasma [Pi] was reported with increases in the range of only 3 mg/dL (1 mmol/L), while peak concentrations were reached sooner, between 4 and 5 hours after treatment. It is probable that this difference between studies is attributable to the much more pronounced hypophosphatemia in this study that was achieved through a considerably longer duration of dietary P-depletion. The degree of stimulation of enteral Pi-absorption is associated with the degree of P-deprivation. Stronger stimulation of enteral P uptake is therefore likely to have contributed to higher peak plasma [Pi] values and could also explain delayed peaks through prolonged absorption.

Discrepancies between this and other studies also exist for the effect of Na2HPO4. An older study used Na2HPO4 to treat dairy cows but reported no effect. The lack of effect of oral Na2HPO4 reported in that study might however be attributable to the use of dairy cows that were not P-deprived, which might have resulted in considerably lower absorption rates of dietary P.

The oral treatment with MgHPO4 in this study was markedly less effective in the timely correction of hypophosphatemia. A significant effect on the plasma [Pi] was apparent only five hours after treatment, with peak concentrations occurring 14 hours after treatment and peak increments of plasma [Pi] that were considerably lower than in the groups treated with sodium phosphate salts. We attribute this protracted effect on the plasma [Pi] to the low solubility of MgHPO4 (0.025 g/dL water at 20°C). MgHPO4 has been reported to precipitate to quanite (NH4MgHPO4) in the rumen of sheep, a reaction if also occurring in cattle could have further contributed to impaired bioavailability of this compound. There are similar poor effects on the plasma [Pi] for dicalcium phosphates another phosphate salt with very low solubility in water. Both, dicalcium phosphate and MgHPO4 must be considered unsuitable for rapid correction of hypophosphatemia through oral treatment as drench or bolus and are probably more suited as feed additives to supplement P-deficient diets on a long term.

Treatment with MgHPO4 in this study led to increases in plasma [Mg] in the range of 0.3 mg/dL (0.12 mmol/L) compared with the group C. It should be noted that control and treatment groups had plasma [Mg] within the reference range at all times (1.7-3.0 mg/dL; 0.7-1.23 mmol/L). The delayed effect on plasma [Mg] matches the slope of the plasma [Pi] in the animals treated with MgHPO4 which corroborates the assumption that the absorption of this salt is hampered by its very limited solubility and possibly precipitates in ruminal fluid.

Supplementation of large oral doses of P was reported to impair the apparent absorption of Mg in animals that were not P-deficient. It has thus been suggested that in states of limited availability of dietary Mg excessive supply of oral P might increase the risk for hypomagnesemic tetany. In contrast, in states of low dietary P-supply it was found that the supplementation of P can enhance trans-ruminal Mg-absorption in isolated rumen of sheep. In this study, treatment with sodium phosphate salts had no effect on the plasma [Mg], which suggests that a single treatment with oral monosodium- or disodium phosphate does not cause a relevant disturbance of the Mg balance in treated animals. This is in agreement with an earlier study investigating the effect of a single oral dose of different phosphate salts in hypophosphatemic dairy cows.

5 CONCLUSIONS

In conclusion a single oral treatment with NaH2PO4 or Na2HPO4 providing the equivalent of 60 g P is adequate for the timely correction of hypophosphatemia in cattle. The treatment has a rapid onset of action and a sustained effect. Oral administration of MgHPO4 has a considerably protracted effect on the plasma [Pi] and is therefore not recommended to be used as drench or bolus in animals requiring rapid correction of blood [Pi].

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CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
The national and institutional guidelines for the care and use of experimental animals were followed and all experimental procedures were approved by the Utrecht University IACUC (AVD108002016616).

ORCID
Imke Cohrs [http://orcid.org/0000-0001-9966-244X](http://orcid.org/0000-0001-9966-244X)

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