Enteral administration of sodium phosphate, potassium phosphate and calcium phosphate as treatment for hypophosphatemia in lactating dairy cattle

Master thesis:
M.J.H.A. Idink
Supervisor: dr. W. Grünberg
Utrecht University, Faculty of Veterinary Medicine
Department of Farm Animal Health

Abstract

Onset of lactation and various periparturient diseases can result in hypophosphatemia in dairy cattle. For treatment of hypophosphatemia, oral supplementation is assumed to be the best method. Sodium phosphate salts are frequently used for oral treatment, but other phosphate salts might be equally effective and therefore interesting alternatives. Debate exists about the role of the reticular groove reflex after oral administration of NaH_2PO_4 containing solutions. Six healthy lactating cows were fed a phosphorus deficient diet in order to induce a hypophosphatemia in study animals. Each animal received four different treatments in randomized order, phosphate absorption was studied after treatment with either NaH_2PO_4, KH_2PO_4 or $Ca(H_2PO_4)_2$. Activation of the reticular groove reflex was evaluated with the acetaminophen absorption test. Oral and intraruminal administration of NaH_2PO_4, as well as intraruminal administration of KH_2PO_4 resulted in similar increases in plasma Pi concentrations. Intraruminal administration of $Ca(H_2PO_4)_2$ resulted in a numerically lower increment in plasma phosphate concentration than intraruminal administration of NaH_2PO_4 and KH_2PO_4. It is concluded that intraruminal administration of KH_2PO_4 is equally effective as oral and intraruminal administration of NaH_2PO_4, intraruminal administration of $Ca(H_2PO_4)_2$ however is less effective. No indication was found that oral administration of a 3.2 M NaH_2PO_4 solution induced closure of the reticular groove.

Introduction

Phosphorus has more known biologic functions in the body than any other mineral element, which makes it an important element for metabolic function and structural stability of any living organism. It is a component of phospholipids, phosphoproteïns, nucleic acids and is necessary for generation of adenosine triphosphate (ATP). It is an essential buffer for the acid-base balance. Together with calcium, it is a major component of bone which contains about 80% of the total body P content. Less than 1% of the total amount of P in the body is found in the extracellular space, where it is predominantly present as inorganic phosphate (Pi) (Goff, 2000; Grünberg, 2008). The body's P equilibrium is regulated through intestinal absorption, renal and salivary excretion and exchange of P between extracellular space and bone (Horst, 1986).

Because the majority of the soluble and thus biologically active P pool is located in the intracellular space, the plasma Pi concentration ([Pi]), reflecting the extracellular space, does not give a reliable representation of the total amount of P in the body. Pi can shift from the extracellular to the intracellular space, which reduces plasma Pi concentration while the total body Pi content remains equal. On the other hand, gradual changes in total body P can occur without explicit changes in plasma [Pi] (Grünberg, 2008; Grünberg et al., 2006). This means that a phosphate depletion is not always accompanied by hypophosphatemia and conversely, hypophosphatemia does not necessarily indicate a phosphate depletion.

Concerns about excessive amounts of P shed into the environment through feces have led to regulations limiting the amount of P allowed to be applied with manure onto agricultural soil. Lowering the P content in soil will consequently lead to a decrease in P content in forages used to feed dairy cattle. Reduced dietary P content is widely assumed to be without negative effects for the dairy cow during lactation (Van Krimpen et al., 2010). However, during periods with sudden changes in P homeostasis such as the onset of lactation, meeting P requirements are likely to become a growing challenge with this reduced availability of dietary P.

Hypophosphatemia is a common problem in dairy cows affected by clinical milk fever, abomasal disorders or fatty liver. Since hypophosphatemia is considered to be the consequence rather than the cause of these conditions, resolving the underlying disease will in most cases resolve hypophosphatemia as well. However, hypophosphatemia is believed to exacerbate feed intake depression and may furthermore impair the animal's well-being. Therefore, additional treatment of hypophosphatemia with Pi might improve recovery (Grünberg, 2008).

Treatment of hypophosphatemia can consist of either oral or intravenous administration of phosphate salts. For both oral and intravenous treatment, sodium phosphate salts were found to be best suited. For intravenous treatment, 30 g of NaH_2PO_4 dissolved in 300 mL distilled water proved to rapidly correct hypophosphatemia. The effect however was short-lived, plasma [Pi] returned to baseline values within two h (Cheng et al., 1998).

Oral treatment with phosphate salts has a prolonged effect on plasma [Pi] when compared to intravenous treatment, but has a lag time between treatment and increase in plasma [Pi]. Time to peak plasma [Pi] reported in the literature vary widely depending on the study and range from 1-4 h. Some studies found only minimal effects on plasma [Pi] after oral treatment but those were conducted on animals that were not hypophosphatemic (Cheng et al., 1998; Grünberg et al., 2013; Horner and Staufenbiel, 2004).

Peak plasma [Pi] within 1-2 h after oral administration is only plausible when significant amounts of Pi are absorbed from the reticulorumen or when the solution bypasses the reticulorumen, which would require closure of the reticular groove reflex. Even though it has been proven that the ruminal mucosa is capable of absorption of Pi, this does not happen in quantities that are biologically relevant (Beardsworth et al., 1989; Grünberg et al., 2013). Closure of the reticular groove is reported in young and adult cattle at

least incidentally after oral admission of various sodium salt solutions (Carruthers et al., 1994; Riek, 1954). A 1.0 M solution of NaH_2PO_4 however did not induce the reticular groove reflex (Grünberg et al., 2013).

For oral treatment, solubility is an important factor for efficacy of phosphate salts on Pi absorption because substances with a higher solubility are likely to transit through the ruminant forestomach system faster (Grünberg et al., 2013). Table 1 presents an overview of solubilities of a number of phosphate salts. Like sodium phosphate, potassium phosphate has a good solubility in water. In human medicine, potassium phosphate is frequently used for oral treatment of hypophosphatemia (Gaasbeek and Meinders, 2005; Weisinger and Bellorín-Font, 1998). For a combined treatment with Ca and Pi, $CaHPO_4$ is well researched but was found to be poorly effective (Cheng et al., 1998; Grünberg et al., 2013). $Ca(H_2PO_4)_2$, might be a good alternative as it is more soluble in water.

Salt	Solubility (g/100g H_2O)
NaH_2PO_4	94.9^{25}
Na_2HPO_4	11.8^{25}
Na_3PO_4	14.5^{25}
KH_2PO_4	25.0^{25}
K_2HPO_4	168^{25}
K_3PO_4	106^{25}
$Ca(H_2PO_4)_2$	1.8^{20}
$CaHPO_4$	0.02^{25}
$Ca_3(PO_4)_2$	0.00012^{20}
$Mg_3(PO_4)2$	0.00009^{20}
$NH_4H_2PO_4$	40.4^{25}
$(NH_4)_2HPO_4$	69.5^{25}
$(NH_4)_3PO_4$	18.3^{25}

Table 1: Solubility of various phosphate salts in water. Temperatures in °C is given in superscript. Data obtained from CRC Handbook of Chemistry and Physics, 90th edition (2010)

The main objective of the present study was to compare the suitability of oral treatment with KH_2PO_4 and $Ca(H_2PO_4)_2$ to the currently best established treatment with NaH_2PO_4 for correction of hypophosphatemia in cattle. Another objective was to evaluate if the reticular groove reflex could be activated with a 3.2 M NaH_2PO_4 solution after oral administration.

It was hypothesized that treatments with NaH_2PO_4 and KH_2PO_4 intraruminally would result in similar plasma Pi concentration-time curves but intraruminal treatment with $Ca(H_2PO_4)_2$ would result in a less steep curve and a lower peak plasma Pi concentration. Furthermore, it was hypothesized that oral treatment with a 3.2 M NaH_2PO_4 solution would not be able to consistently activate the reticular groove reflex.

Materials and methods

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 (DEC; permit no 2013.iii.03.033).

A total of six healthy, lactating, non pregnant, Holstein-Friesian cows were used during this experiment. All six cows included in this study were between 5 and 7 years old. All cows were between 99 and 196 days of their 3th, 4th or 5th lactation. They weighed between 554 and 717 kg (mean 611 ± 68.6). The mean milk production in 305 days in the previous lactation was 9850 ± 1490 kg. On physical examination all cows were found healthy. Cows were housed in individual tie stalls with rubber bedding, covered with sawdust, in a temperature controlled room.

Cows were fed ad libitum, fresh feed was offered two times a day between 06.00 and 07.00 h and between 18.00 and 19.00 h as a total mixed ration (TMR). The ration was based on maize silage, grass seed straw and beet pulp and was formulated to meet the NRC guideline recommendations, except for

the phosphorus content (NRC, 2001). The phosphorus content in the ration was 2.0 g/kg DM. This ration was fed during 4 weeks prior to the treatments with the aim to induce a phosphate depletion and hypophosphatemia. The TMR was mixed three times a week, on Monday, Wednesday and Friday and kept refrigerated until fed. Cows were milked twice daily between 06.00 and 07.00 h and between 18.00 and 19.00 h.

Experimental study

All cows received four treatments in randomized order with a 48 h washout period in between treatments. Treatments consisted of 302 g NaH_2PO_4 dihydrate, 263 g KH_2PO_4 or 244 g $Ca(H_2PO_4)_2$ monohydrate, all treatments contained the equivalent of 60 g P. Treatments were administered either orally with a 250 mL drench gun or intraruminally through a orogastric tube. Salts administered through a orogastric tube were dissolved in 1.5 L of warm water (38 °C). Salt administered with a drench gun was dissolved in 600 mL of warm water, in order to obtain a 3.2 M salt solution. No attempt was made to measure the amount of spillage during administration of the salt solution with a drench gun. Treatment groups were 1) intraruminal administration of a $Ca(H_2PO_4)_2$ solution (CaRu); 2) intraruminal administration of KH_2PO_4 salt solution (KRu); 3) intraruminal administration administration of a 1.0 M NaH_2PO_4 salt solution (NaRu) and oral administration of a 3.2 M NaH_2PO_4 salt solution into the buccal cavity (NaOr). Acetaminophen (AP) was added to the test solution in groups NaRu and NaOr at a dose of 50 mg/kg BW to evaluate the role of the reticular groove. Amounts of AP were between 28 and 36 g.

The evening before first treatment, all cows were fitted aseptically with a 16 G jugular venous catheter (Angiocath; Becton-Dickinson) with an extension set (Discofix C-3, 10 cm; Braun Melsungen AG) for blood collection. Three catheters had to be replaced during the whole study period. When catheters were dysfunctional during treatment days, blood was collected with a vacutainer (20G needle; Becton-Dickinson) from the jugular vein.

Treatments were administered between 8.00 and 8.30 h. Blood samples were collected immediately before treatment (T0) and at 30, 60, 90, 120, 180, 240, 300, 420, 720 and 1440 min after treatment. Before blood collection, catheters were flushed with 5 mL heparinized saline solution (40 IU Na-heparin/mL 0.9 NaCl solution) and 3 mL of blood was drawn from the catheter and discarded. Then another 10 mL was drawn into an empty syringe and transferred to a tube containing lithium-heparin as anticoagulant. Finally the catheter was flushed again with 5 mL of heparinized saline solution. Blood tubes were kept at room temperature until they were centrifuged at 1614 *g* for 10 min. Harvested plasma was used for determination of the total protein concentration by refractometry. The remaining plasma was transferred into another tube that was stored at -18 °C until analyzed.

Biochemical analysis

Plasma concentrations of inorganic phosphate (ammonium molybdate), magnesium (calmigate) and acetaminophen (AP, turbidimetric inhibition immunoassay) were determined spectrophotometrically. Total plasma Ca, K and Na concentration was determined by indirect potentiometry (ion selective electrodes). An automated analyser (DXC-600, Beckman Coulter Inc.) was used for the biochemical analysis. Total protein was determined by refractormetry.

Data analysis

For each treatment maximal plasma [Pi] (CPmax) and time until maximal plasma [Pi] (TPmax) for the time interval 0 to 420 min were determined from the plasma concentration time curve of the respective treatment group. The time interval from immediately before treatment until 420 min post treatment was chosen to circumvent a potentially confounding effect of the evening feeding that was provided 2 h before blood sampling at 720 min post treatment.

The increment in plasma [Pi] (CP_{INCR}) was calculated by subtracting plasma [Pi] at T0 from measured plasma [Pi] values. The same was done for increment in plasma AP (CAP_{INCR}) and K (CK_{INCR}). Increments rather than absolute values were used to neutralize the effect of differences in baseline values between groups. From plasma Pi concentration increment time curves, Cmax (CP_{INCR}max) and Tmax (TP_{INCR}max) were determined with values until 420 min post treatment for the same reason as described above. Areas under the plasma CP_{INCR} and CAP_{INCR} time curve for the first 240 and 420 min were calculated using the trapezoidal rule (Chiou, 1978).

Plasma volume changes were crudely estimated on the basis of total protein concentration ([TP]) changes in plasma relative to T0 (Fielding and Magdesian, 2011). Plasma volume changes for each sampling time 'i' were calculated using the equation: $Voldiff_i$ (%) = $[TP_i]/[TP_0]$ x 100%.
Absolute electrolyte concentrations as well as increments relative to T0 were corrected for plasma volume changes to identify a possible effect of plasma volume changes on the electrolyte concentrations using the equation: $Cvol_{electrolyte} = C_{electrolyte} / Voldiff \times 100$

Statistical analysis
Results are expressed as mean values with standard deviation. Normality of distribution was tested by Shapiro-Wilk's test for normality. For data that were not normal distributed medians and interquartile ranges were used. A statistical software package was used for analysis (SAS 9.2, SAS Institute Inc). Repeated measures analysis of variance (ANOVA) was used to determine time effects, treatment effects as well as treatment time interaction effects. Bonferoni corrected P-values were used to asses differences between and within treatments. Significance was assumed at $P < 0.05$.

Results
Baseline plasma [Pi] were 1.28 ± 0.31; 1.20 ± 0.32; 1.36 ± 0.46 and 1.16 ± 0.40 mmol/L for groups CaRu, KRu, NaRu and NaOr respectively and did not differ between groups. Baseline [Pi] were 0.88 ± 0.28; 1.40 ± 0.26; 1.39 ± 0.36 and 1.32 ± 0.31 mmol/L on first, second, third and fourth study days. Baseline [Pi] on day 1 were significantly lower than baseline [Pi] on day 2 and 3. To correct for this difference the increment of plasma [Pi] over time rather than the absolute plasma [Pi] was used for further analysis. Plasma phosphate increment time concentration curves for the different treatment groups are presented in figure 1. CP_{INCR}max, TP_{INCR}max, $AUCP_{INCR240}$ and $AUCP_{INCR420}$ are presented in table 2.

Treatment group	NaRu		NaOr		KRu		CaRu	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD
CP_{INCR}max (mmol/L)	1.22	0.42	1.09	0.40	1.13	0.30	0.87	0.15
TP_{INCR}max (min) median [IQR]	270 [240-420]		300 [120-420]		360 [240-420]		360 [300-420]	
$AUCP_{INCR240}$ (mmol x min/L)	156.88	65.21	145.46	68.62	144.30	66.34	64.20	45.50
$AUCP_{INCR420}$ (mmol x min/L)	339.73	131.04	296.98	124.65	321.80	119.40	182.55	74.08

Table 2. Maximal plasma phosphate concentration increment (CP_{INCR}max); time to maximal plasma phosphate concentration increment (TP_{INCR}max) and areas under the plasma Pi increment concentration-time curves for the first 240 min ($AUCP_{INCR240}$) and 420 min ($AUCP_{INCR420}$) after treatment stratified by treatment group. Data presented as mean values with their standard deviation or as medians and interquartile ranges (IQR). None of these values were found significantly different between treatment groups ($P<0.05$, Bonferoni corrected)
NaRu, NaH_2PO_4 administered into the rumen; NaOr, NaH_2PO_4 administered into the buccal cavity; KRu, KH_2PO_4 administered into the rumen and $Ca(H_2PO_4)_2$ administered into the rumen.

Increment in plasma [Pi] from 60 min post treatment in group NaOr and 90 min post treatment in groups KRu and NaRu were higher than baseline and remained above baseline concentration until the end of the observation period (1440 min). Plasma [Pi] was above baseline values in group CaRu from 180 min to 720 min post treatment.

In all treatment groups, a second peak is found at 720 min after treatment. $CP_{INCR}max$ and $TP_{INCR}max$ as well as the area under the plasma [Pi] increment for the first 240 and 420 min ($AUCP_{INCR240}$ and $AUCP_{INCR420}$) are presented in table 2.

In figure 2, concentration time curves are presented for plasma potassium concentration ([K]), plasma calcium concentration ([Ca]) and plasma sodium concentration ([Na]). In groups NaOr, KRu and CaRu average baseline plasma [K] value was just below the reference value of 3.9 mmol/L (3.63 ± 0.59; 3.70 ± 0.32 and 3.87 ± 0.37 mmol/L respectively). Baseline plasma [K] was higher in group NaRu (4.2 ± 0.39 mmol/L) compared to groups NaOr and KRu.

In group KRu, plasma [K] was above baseline values from 120 min to 420 min post treatment with a peak plasma [K] (CKmax) of 4.55 ± 0.176 mmol/L at 300 min post treatment, equivalent to an average

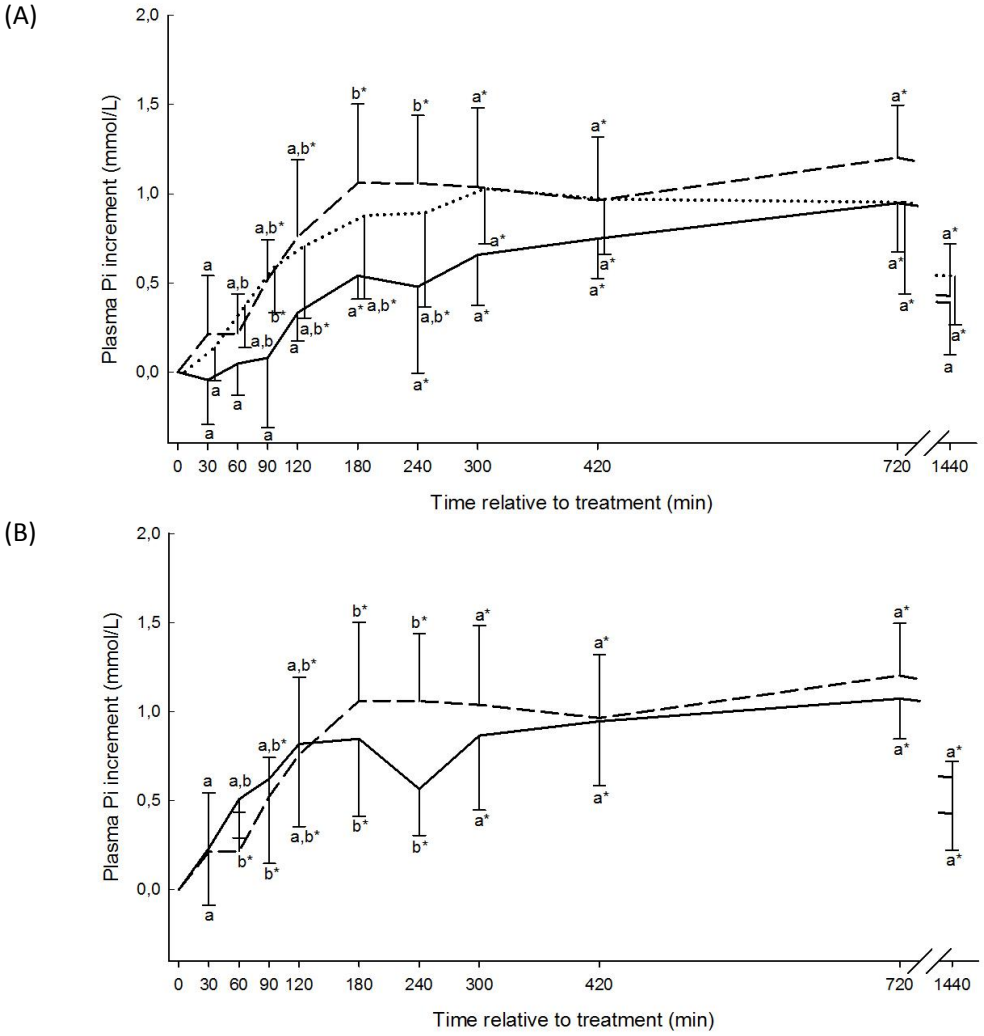

Fig 1: Course of plasma phosphate concentration relative to baseline after treatment with NaH_2PO_4 administered into the rumen (NaRu; – – – ; A and B); KH_2PO_4 administered into the rumen (KRu; ····· ; A); $Ca(H_2PO_4)_2$ administered into the rumen (CaRu; —— ; A) and NaH_2PO_4 administered into the buccal cavity (NaOr; —— ; B). Values are means with their standard deviation represented by vertical bars. *Mean value is significantly different from that at T0; $^{a, b}$ Mean value with unlike letters were significantly different between groups (P<0.05, Bonferoni corrected). Data of KRu are slightly offset with respect to time to improve readability.

maximum increment of 1.00 ± 0.33 mmol/L. A decrease in plasma [K] of 0.57 and 0.60 mmol/L was observed at 30 and 60 min post treatment in group NaRu. Volume corrected plasma [K] did not show this decline. Plasma [K] was significantly above baseline in group NaOr at 420 min post treatment.

No changes were found in plasma [Ca] within treatment groups. Between treatment groups, only NaOr was different from CaRu at 120 minutes after treatment but this was not found significant after correction for plasma volume changes. Plasma [Na] remained within reference ranges. Changes were only found in group NaOr, in which they were elevated from 60 to 240 min post treatment and at 720 min post treatment.

Concentration time curves for plasma magnesium concentration ([Mg]) are presented in figure 3 (A). No significant changes in plasma [Mg] were identified within or between groups. All values remained within reference range.

Volume changes were calculated from plasma protein concentrations. Volume changes time curves are presented in figure 3 (B). Significant differences were found between treatment groups but not within treatment groups when compared to T0. In group NaOr, plasma volume tended to go down and remained low until 1440 minutes post treatment.

Characteristics of acetaminophen absorption kinetics ($CAP_{INCR}max$, $TAP_{INCR}max$, $AUCAP_{INCR240}$ and $AUCAP_{INCR420}$) in groups NaRu and NaOr are presented in Table 3. Also, differences between these parameters in group NaRu and NaOr are presented. Plasma AP increment concentration time curves are presented in figure 3 (C).

Treatment group	NaRu		NaOr		
	Mean	SD	Mean	SD	Difference
$CAP_{INCR}max$ (mmol/L)	73.3 [a]	4.51	58.17 [b]	9.25	21%
$TAP_{INCR}max$ (min) median [IQR]	90 [90-90]		90 [60-120]		
$AUCAP_{INCR240}$ (mmol x min/L)	11863 [a]	998	8450 [b]	2115	29%
$AUCAP_{INCR420}$ (mmol x min/L)	18888 [a]	1290	13644 [b]	2621	28%
$CP_{INCR}max$ (mmol/L)	1.22 [a]	0.42	1.09 [a]	0.40	11%
$TP_{INCR}max$ (min) median [IQR]	270 [240-420]		300 [120-420]		
$AUCP_{INCR240}$ (mmol x min/L)	156.88 [a]	65.21	145.46 [a]	68.62	7%
$AUCP_{INCR420}$ (mmol x min/L)	339.73 [a]	131.04	296.98 [a]	124.65	13%

Table 3. Maximal plasma acetaminophen concentration increment ($CAP_{INCR}max$); time to maximal plasma acetaminophen concentration increment ($TAP_{INCR}max$); areas under the plasma acetaminophen increment concentration-time curves for the first 240 min ($AUCAP_{INCR240}$) and 420 min ($AUCAP_{INCR420}$) after treatment stratified by treatment group; maximal plasma phosphate concentration increment ($CP_{INCR}max$); time to maximal plasma phosphate concentration increment ($TP_{INCR}max$); areas under the plasma Pi increment concentration-time curves for the first 240 min ($AUCP_{INCR240}$) and 420 min ($AUCP_{INCR420}$) after treatment stratified by treatment group and difference between values from group NaRu compared to NaOr. Data presented as mean values with their standard deviation or as medians and interquartile ranges (IQR). [a, b] Mean value with unlike letters were significantly different between groups ($P<0.05$, Bonferoni corrected). NaRu, NaH_2PO_4 administered into the rumen and NaOr, NaH_2PO_4 administered into the buccal cavity.

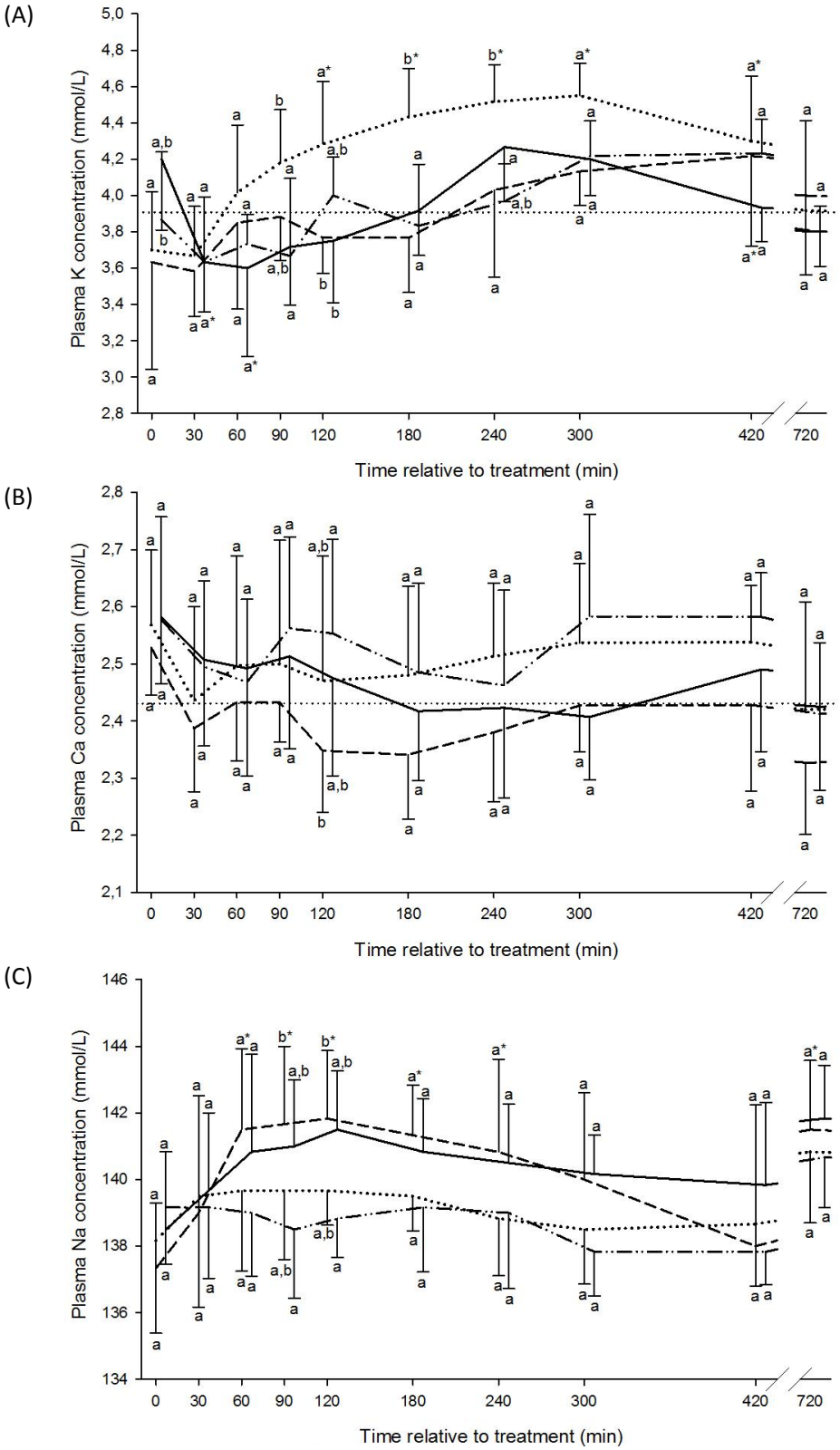

Fig 2: Course of plasma potassium concentration (A), plasma calcium concentration (B) and plasma sodium concentration (C) after treatment with NaH_2PO_4 administered into the rumen (NaRu; ——); KH_2PO_4 administered into the rumen (KRu; ·····); $Ca(H_2PO_4)_2$ administered into the rumen (CaRu; – · – ·) and NaH_2PO_4 administered into the buccal cavity (NaOr; – – –). Values are means with their standard deviation represented by vertical bars. Straight small dotted line are lower limits of the reference value for plasma potassium (A) and plasma calcium (B). *Mean value is significantly different from that at T0; [a, b] Mean value with unlike letters were significantly different between groups (P<0.05, Bonferoni corrected). Data of KRu and CaRu are slightly offset with respect to time to improve readability.

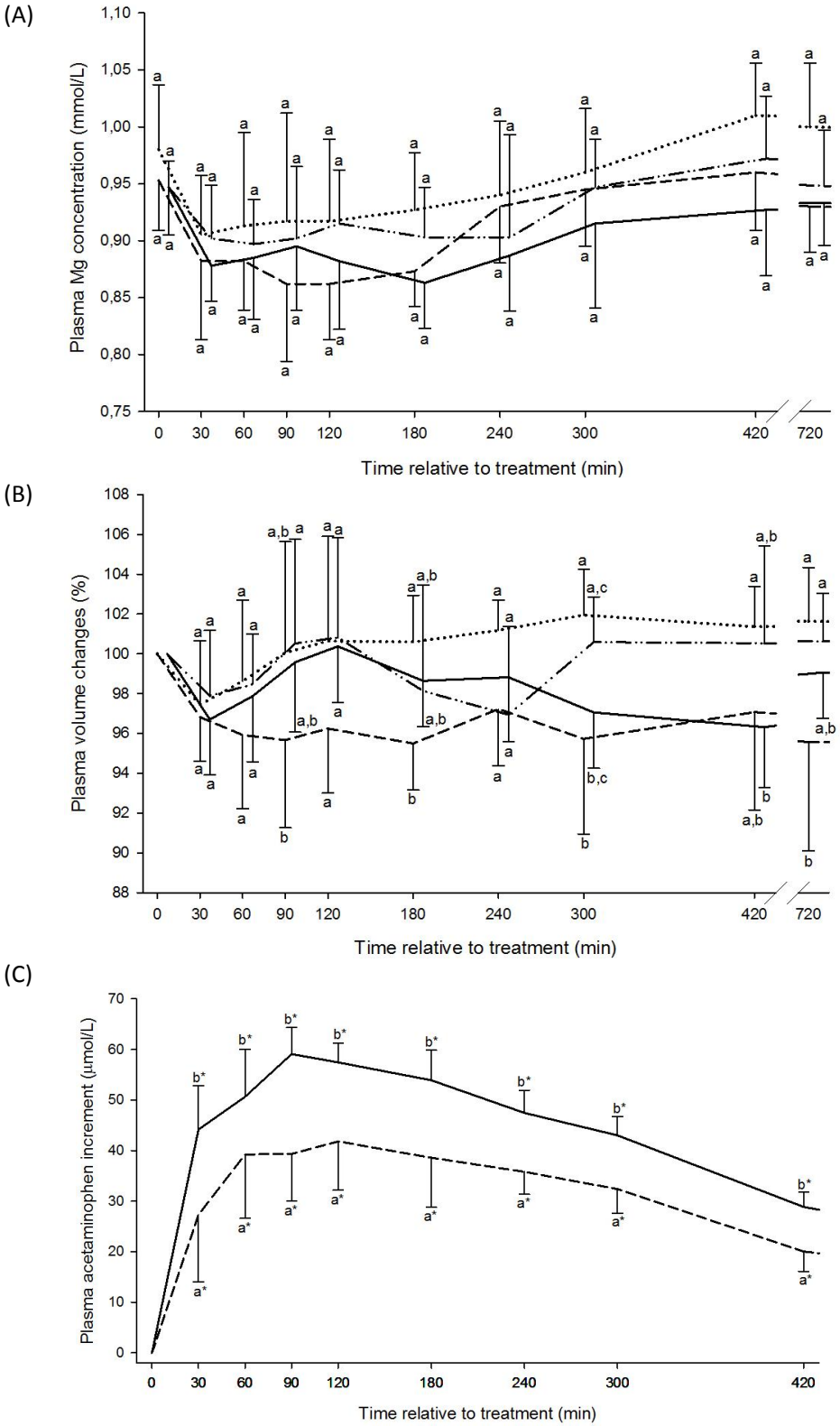

Fig 3: Course of plasma magnesium concentration (A), changes in plasma volume relative to T0 (B) and plasma acetaminophen concentration (C) after treatment with NaH_2PO_4 administered into the rumen (NaRu; ———); KH_2PO_4 administered into the rumen (KRu; ·····); $Ca(H_2PO_4)_2$ administered into the rumen (CaRu; – · – ·) and NaH_2PO_4 administered into the buccal cavity (NaOr; – – –). Values are means with their standard deviation represented by vertical bars. *Mean value is significantly different from that at T0; [a, b] Mean value with unlike letters were significantly different between groups (P<0.05, Bonferoni corrected). Data of KRu and CaRu are slightly offset with respect to time to improve readability.

Discussion

The main objective of this study was to compare the suitability of KH_2PO_4 and $Ca(H_2PO_4)_2$ for oral correction of hypophosphatemia in cattle to the currently best established treatment with NaH_2PO_4. Another objective was to evaluate the role of the reticular groove reflex after oral administration of NaH_2PO_4 in a 3.2 M solution.

Because enteral P absorption is dependent on the P status in ruminants and other species it is imperative to conduct P absorption studies with the aim of exploring the effect of different P salts in P deficient cattle (Horst, 1986). For this purpose, cows were fed with a P deficient diet for four weeks prior to experimental treatments to induce a phosphate depletion in study animals. The P content in the ration was 2.0 g/kg DM, current P recommendation for lactating dairy cattle is 3.2-4.2 g/kg DM (NRC, 2001). Mean baseline plasma [Pi] prior to first treatment was below reference values for all treatments, which is indicative that dietary Pi content and length of depletion phase were adequate to induce phosphate depletion in this study.

The fact that baseline values were significant higher on day 2 and 3 compared to day 1 and numerical higher on day 4 compared to day 1, suggests that the wash out period of 48h may not have been long enough for plasma [Pi] to return to baseline value, at least not for treatment on day 1. This was not anticipated because in other studies plasma [Pi] returned to baseline values within 24 h (Grünberg et al., 2013). Since treatments were randomly assigned to cows and treatment days, this probably did not interfere with treatment outcome. A possible explanation for the prolonged effect of the first treatment is that the duration of phosphate depletion in the present study animals was longer than in other studies.

A second peak in plasma [Pi] was observed at 720 min after treatment in all treatment groups. A probable explanation for this second peak might be interference with feed intake. One to two hours before this blood sample was taken, fresh feed was offered, serving as a new source of phosphate. We corrected for this by calculating AUC until 420 min rather than 720 min post treatment, which was the last blood sampling time before fresh feed was offered. With a control group, in which animals were treated with a solution without Pi, it would have been possible to identify changes in plasma [Pi] that were not the result of treatment.

Enteral administration of NaH_2PO_4 through a stomach tube was fit to rapidly increase plasma Pi levels in phosphate depleted cows from 90 min for at least 23 h. These results are in agreement with Grünberg et al. (2013) and Geishauser et al. (2010) who found an increase in plasma [Pi] from 90 to 720 min post treatment and 120 to 1440 min post treatment respectively with NaH_2PO_4. In the present study, administration of 263 g of KH_2PO_4 elevated plasma [Pi] within 90 min and it remained elevated for at least 23 h. There was no significant difference between groups NaRu and KRu which shows that KH_2PO_4 is equally effective for treatment of hypophosphatemia as NaH_2PO_4 administered by the same route. $Ca(H_2PO_4)_2$ elevated plasma [Pi] after 4 h and remained elevated for at least 8 h. Although this difference is not significant, $CP_{INCR}max$, $AUCP_{INCR240}$ and $AUCP_{INCR420}$ were numerically lower in group CaRu than in other groups (KRu and NaRu) which suggests that $Ca(H_2PO_4)_2$ is less effective for treatment of hypophosphatemic cows than NaH_2PO_4 and KH_2PO_4. Until now only $CaHPO_4$ was studied in other studies as a treatment for phosphate depleted animals. They all concluded that $CaHPO_4$ had a slow and limited effect on plasma Pi concentration (Cheng et al., 1998; Grünberg et al., 2013). In comparison to $CaHPO_4$, $Ca(HPO_4)_2$ induced a faster and higher increase in plasma [Pi] which makes it more suitable for treating phosphate depleted animals.

The combination of Ca and Pi is mainly interesting for treatment of hypocalcemia which can occur concomitantly with hypophosphatemia around calving (Goff et al., 2002). It should be noted that the absence of an effect of oral $Ca(H_2PO_4)_2$ treatment on plasma [Ca] in the present study does not indicate that similar results would be obtained in hypocalcemic cows. Enteral absorption of Ca is a tightly regulated process that will be up-regulated in hypocalcemic animals (Horst, 1986). Cows in the present

study were not hypocalcemic. To evaluate the effect of $Ca(H_2PO_4)_2$ on plasma [Ca] in hypocalcemic cows, it should be studied with hypocalcemic cows.

The mean plasma [K] in group KRu was increased from 2 h after treatment and stayed elevated for 5 h. The mean increment in plasma [K] was 1.00 mmol/L. Enteral absorption of K is dependent on diffusion and is not regulated (Leonhard-Marek et al., 2010). Nearly all of dietary potassium is passively absorbed across the intestinal tract. As a result of a higher gradient of [K] over the intestinal wall in hypokalemic animals, absorption might even increase. Although animals in this study were only mildly hypokalemic, an at least similar increase in plasma [K] can be expected when hypokalemic animals, with a higher electrochemical gradient between gut lumen and extracellular space, are treated with 263 g KH_2PO_4. This dose of KH_2PO_4 was equivalent to 75 g K which is equivalent to 143 g KCl. Current recommendations for oral K supplementation in severe hypokalemic cattle are 120-240 g KCl 2-3 times daily which indicates that the amount KH_2PO_4 administrated in this study would also be sufficient for treatment of hypokalemic cows (Sweeney, 1999).

High dietary K is reported to reduce the absorption of Mg (Field and Suttle, 1979; Greene et al., 1983). In the present study, a single administration of KH_2PO_4 did not result in decreased plasma [Mg]. Mg is absorbed mainly from the rumen, by two mechanisms. The first is dependent on the potential difference across the apical cell membrane. This explains the negative effect of elevated [K] on Mg absorption, because uptake of K reduces the apical membrane potential, and thus Mg absorption. The second mechanism is electro neutral and is activated at high [Mg] in ruminal fluid (Leonhard-Marek et al., 2010). It is possible that the dietary Mg content in the experimental diet, that was in agreement with current recommendations, was sufficient to compensate for the effect through this latter mechanism. Another possible explanation is that the effect was short-lived or too small to be detected in plasma.

Solubility of treatment solution is an important factor for efficacy of phosphate salts on Pi absorption after enteral treatment of cattle, because substances with a higher solubility are likely to passage the ruminant forestomach system faster (Grünberg et al., 2013). The results of this study support this statement because the salts with best solubility (NaH_2PO_4 and KH_2PO_4) appeared to have a faster and longer effect on plasma [Pi] than the less soluble $Ca(H_2PO_4)_2$.

Mg and Pi can precipitate together with ammonium (NH_4) as $MgNH_4PO_4$ at high concentrations of Mg and Pi in ruminal fluid in vitro. Precipitation of $MgNH_4PO_4$ also occurred in the rumen of sheep with high concentrations of Mg and NH_4 (Axford et al., 1982). High phosphorus content in feed reduces apparent Mg absorption in the rumen of pregnant heifers (Schonewille et al., 1994). In the present study, treatments did not result in reduced plasma [Mg]. This observation, together with the rapid increment in plasma [Pi] makes it less likely that large amounts of $MgNH_4PO_4$ precipitate in the rumen after a single administration of Pi containing solutions.

At T0, [K] in group NaRu was significant higher than in groups KRu and NaOr. A significant decrease of plasma [K] at 30 and 60 min post treatment compared to T0 was found in group NaRu. No explanation could be found for the higher baseline plasma [K] in that group, nor for the decrease after 30 min. However, it could be noted that this decrease brings plasma [K] in group NaRu closer to those from groups NaOr and CaRu.

Plasma [Na] increased significantly in group NaOr and numerically in group NaRu but remained within reference range, which was expected, because Na is highly osmotic and an increase in plasma [Na] will be quickly followed by an increase of water fraction in the plasma (plasma volume). This increased plasma volume however can cause dilution of other electrolytes and proteins in plasma.

Changes in extracellular volume were crudely assessed by determining changes in plasma protein concentration. As the total amount of protein in plasma is presumed to be more or less constant during the day, changes in [TP] are assumed to be the result of changes in plasma volume (Fielding and Magdesian, 2011). Electrolyte concentrations were corrected for changes in extracellular volume to

determine to which extent observed changes were due to hemodilution or hemoconcentration rather than to changes in amount of specific electrolytes.

In a study in which cows were treated with a NaH_2PO_4 solution, peak plasma [Pi] was found at 1 h post treatment (Cheng et al., 1998). In another study however, peak plasma [Pi] was found not earlier than 210 min post oral treatment (Grünberg et al., 2013). It was discussed that with a turnover rate of 10% of the liquid phase of the rumen content per h and an abomasal emptying rate of approximately 1 h in healthy lactating dairy cows, peak plasma [Pi] within 1 h after oral treatment with NaH_2PO_4 solution is only plausible if the liquid either bypasses the retuculorumen, as this occurs with closure of the reticular groove, or by transruminal Pi absorption in biologically relevant quantities. Absorption of P in the reticulorumen has been the subject of many studies and these studies indicate that P can be absorbed from the reticulorumen, but not in quantities that are biologically relevant (Beardsworth et al., 1989; Grünberg et al., 2013). Closure of the reticular groove is reported in young and adult cattle at least incidentally after oral admission of water and various salt solutions (Carruthers et al., 1994; Riek, 1954). The most obvious dissimilarity between the studies of Cheng et al. (1998) and Grünberg et al. (2013) seems to be the osmolarity of the solution, which were 1.0 M and 3.2 M respectively, although the exact route of administration used in the study of Cheng et al. is not reported in his paper. The objective of this study was to explore whether oral administration of a 3.2 M NaH_2PO_4 solution (similar to what was used by Cheng et al.) can activate the reticular groove reflex and thereby fasten enteral Pi absorption. For this purpose we used the acetaminophen absorption test (APAT).

Acetaminophen, a widely used analgesic and antipyretic drug, is not absorbed in the stomach but in the proximal small intestines in single stomached species (Clements et al., 1978). It is assumed that the contribution of AP absorption in the gastric system of ruminants is very limited as well and several studies reported that APAT is a reliable and minimal invasive test for evaluation of function of the reticular groove reflex and oroduodenal transition time (ODT) (Grünberg et al., 2013; Herrli-Gygi et al., 2008; Schaer et al., 2005; Sharifi et al., 2009). However, in the present study, differences between Cmax and Tmax of Pi and AP after oral and intraruminal treatment did not indicate that the reticular groove reflex was activated after treatment with a 3.2 M NaH_2PO_4 solution.

Since the results of Cheng et al (1998) could not be reproduced in the present study we cannot assume that oral treatment with a NaH_2PO_4 solution will be effective earlier than 3 h post treatment. Since there is no relevant absorption of Pi from the rumen and there is no consistent effect on the reticular groove, rumen motility appears to be an important factor in kinetics of oral Pi absorption.

An undetermined amount of test solution was lost during oral treatment. When assumed that spillage is zero for ruminal admission, spillage for oral admission could not have exceeded 28% based on difference in $AUCAP_{INCR420}$ in groups NaOr and NaRu. It is remarkable that difference in $AUCP_{INCR420}$ between groups NaOr and NaRu was 13%. We have no explanation for this difference. In another study where the same amount of salt was dissolved in a larger volume of water, spillage was 10% (Grünberg et al., 2013). A possible explanation is that in a more concentrated solution, spillage of a certain volume has more impact on salt losses than in a more diluted solution. Same principal goes for residual volume in the drench gun.

Conclusion

All three salts tested in this study were fit to increase plasma [Pi]. 302 g NaH_2PO_4 dihydrate and 263 g KH_2PO_4 both elevated plasma [Pi] from 90 to at least 1440 min after treatment, which proves KH_2PO_4 to be equally suitable for treatment of hypophosphatemia as the current best established treatment with NaH_2PO_4. 244 g $Ca(H_2PO_4)_2$ monohydrate was found to be less effective for increasing plasma [Pi] than NaH_2PO_4 and KH_2PO_4, but when a combination of Ca and Pi is desirable it is an acceptable alternative. Also, it is a much more suited solution than the more researched $CaHPO_4$.

No indication was found that the reticular groove reflex could be activated by oral administration of a 3.2 M NaH_2PO_4 solution.

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