

# Plasma Calcium, Inorganic Phosphate and Magnesium During Hypocalcaemia Induced by a Standardized EDTA Infusion in Cows

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**Mellau LSB, Jørgensen RJ, Enemark JMD: Plasma calcium, inorganic phosphate and magnesium during hypocalcaemia induced by a standardized EDTA infusion in cows. Acta vet. scand. 2001, 42, 251-260.** – The intravenous Na<sub>2</sub>EDTA infusion technique allows effective specific chelation of circulating Ca<sup>2+</sup> leading to a progressive hypocalcaemia. Methods previously used were not described in detail and results obtained by monitoring total and free ionic calcium were not comparable due to differences in sampling and analysis. This paper describes a standardized EDTA infusion technique that allowed comparison of the response of calcium, phosphorus and magnesium between 2 groups of experimental cows. The concentration of the Na<sub>2</sub>EDTA solution was 0.134 mol/l and the flow rate was standardized at 1.2 ml/kg per hour. Involuntary recumbency occurred when ionised calcium dropped to 0.39 - 0.52 mmol/l due to chelation. An initial fast drop of ionized calcium was observed during the first 20 min of infusion followed by a fluctuation leading to a further drop until recumbency. Pre-infusion [Ca<sup>2+</sup>] between tests does not correlate with the amount of EDTA required to induce involuntary recumbence. Total calcium concentration measured by atomic absorption remained almost constant during the first 100 min of infusion but declined gradually when the infusion was prolonged. The concentration of inorganic phosphate declined gradually in a fluctuating manner until recumbency. Magnesium concentration remained constant during infusion. Such electrolyte responses during infusion were comparable to those in spontaneous milk fever. The standardized infusion technique might be useful in future experimental studies.

*Na<sub>2</sub>EDTA; induce.*

## Introduction

Induction of hypocalcaemia by means of infusion with EDTA has been performed in experimental veterinary medicine and physiology for over 36 years (*Smith & Brown 1963*) primarily as a model for spontaneous cases of milk fever and subclinical hypocalcaemia in dairy cows. The intravenous Na<sub>2</sub>EDTA infusion allows effective specific chelation of circulating Ca<sup>2+</sup> leading to a progressive hypocalcaemia (*Desmecht et al. 1995*). A review of the Na<sub>2</sub>EDTA induced hypocalcaemia by *Jørgensen et al.*

(1999) indicated that the regulation of infusion among animals has been variable between researchers. Furthermore, the descriptions of the methods in published investigations were not detailed and results obtained by monitoring total plasma calcium and free ionic calcium are often not comparable due to differences in sampling and analysis. *Desmecht et al.* (1995) regulated the infusion speed by a continuous on-line monitoring of systemic arterial pressure (SAP) to estimate the range of Ca<sup>2+</sup> decay.

Payne (1964) used mathematical formula to calculate the exchangeable calcium pool and an immediately available calcium reserve to indirectly monitor the rate of calcium decay. Contreras et al. (1982) used Paynes' formula but the results could not be reproduced. Reproducibility failure was associated with the variability in the length of infusion period and hence the flow rate on the excretion of the so formed Ca-EDTA complexes (Contreras et al. 1982). The assumptions during calculations that the trend is linear (Payne 1964) or curvilinear (Contreras et al. 1982) had a remarkable effect on calculating the mobilizable calcium pool. A biphasic pattern of  $\text{Ca}^{2+}$  was reported by Riond et al. (1997) whereas Schröter & Seidel (1976) infused the total amount of  $\text{Na}_2\text{EDTA}$  over a 20-min period and found the drop in plasma total calcium approached a linear curve. Finally, Berger & Gerber (1977), Desmecht et al. (1995) and van de Braak et al. (1997) all reported a triphasic pattern of calcium decay with an initial fast drop followed by a plateau, and then a relatively fast drop again. Factors such as cow's response to the gradually developing hypocalcaemia, the dietary calcium and its solubility might influence  $\text{Ca}^{2+}$  decay during EDTA infusions. However, a disagreement between blood [ $\text{Ca}^{2+}$ ] and clinical signs at an infusion speed above 2 mg/kg per minute has been recorded by the authors (unpublished) by cow side monitoring of  $\text{Ca}^{2+}$ . This has probably resulted from differences between vascular and tissue  $\text{Ca}^{2+}$  concentrations during the fast infusions (Mellau et al. 1999). For these reasons standardization of the method would greatly improve the comparability of such studies (van de Braak et al. 1997).

The present study was aimed at standardizing the infusion flow rate, to stop infusion at involuntary recumbency in order to establish the pattern of ionized calcium decay. It was also meant to monitor clinical parameters during infusion

as well as the response of plasma total calcium, inorganic phosphate and magnesium in cows.

## Materials and methods

### Animals

Six dry and non-lactating cows (3 Holstein and 3 Red Danish Dairy) that had calved at least 3 times were used. The cows had no history of parturient paresis previously. Eight weeks before the start of the experiment, cows were surgically installed with rumen cannulas. After recovery, cows were randomly assigned to 2 treatment sequences of diets intended to influence calcium homeostasis (see below). Each diet was offered for 10 days and on day 11 cows were challenged until involuntary recumbency with an intravenous EDTA infusion.

### Diets

Cows were first offered a control ration consisting of wrap grass silage (BR). The second diet during the experiment was the same control ration that in addition, was supplemented with ammonium chloride and ammonium sulphate at the rate of 0.23 g/kg BW of each salt per cow per day as described by Wang & Beede (1992). The addition of these anionic salts was intended to induce metabolic acidosis. Calculated amount of salts were first dissolved in 1 liter of water administered via the rumen fistula. Daily intake of the feed was adjusted to an amount of 14 kg DM/ cow per day.

### EDTA solution

The high quality  $\text{Na}_2\text{EDTA}$  salt (Merck nr.8418 pro analysi, E. Merck, D-6100 Darmstadt), was used. A 5%  $\text{Na}_2\text{EDTA}$  solution was prepared by dissolving 50 g of the salt in 1 litre of sterile distilled water. This is equivalent to a concentration of 0.134 mol/l.

### EDTA infusion

Two cows at a time were inserted with central

vein indwelling catheters (Secalon® Seldy Ohmeda, Faraday Road, Swindon, London) the day before the start of the experiment. To insert the catheters, cows were pre-medicated by intramuscular injection with a mixture of 2 ml butorphenol (1% Torbugesic Vet®, SCANVET, DK-3480) and 1 ml Detomidine hydrochloride (1% Domosedan, Orion Animal Health DK-3490). Catheters were kept patent by flushing with physiological saline containing 0.2 ml of heparin/100 ml, after collection of each blood sample. The right catheter was used for EDTA infusion and the left for collection of blood samples during the EDTA test.

#### *Flow rate*

During intravenous infusion with EDTA solution to challenge calcium homeostatic mechanisms in cows, the dosage rate of 60 mg/kg per hour equivalent to the flow rate of 1.2 ml/kg per hour, was adjusted using an electronic infusion pump (Masterflex® model No 7523-37, Barnant Co. Barrington, IL 60010 USA). Intravenous EDTA infusion was stopped when the cows showed clinical signs of circulatory collapse manifested by cold extremities, increased pulse rate to over 120 beats/min, generalized paresis and involuntarily recumbency. Thereafter, cows were allowed to recover spontaneously from EDTA-induced hypocalcaemia.

#### *Blood sampling*

From each cow 1 blood sample was collected before the start of infusion into evacuated heparin tubes (Venoject®, Terumo Europe N.V. 3001 Leuven, Belgium). During intravenous EDTA infusion, blood samples were collected every 20 min until the cow went involuntarily recumbent. The first 10 ml of blood were always discarded because it might contain heparin that was routinely used to flush the catheter after each collection of blood sample. At involuntary recumbency one blood sample was taken

and thereafter, blood samples were taken on hourly intervals until  $[Ca^{2+}]$  level of 1.00 mmol/l was regained.

#### *Calcium regaining time (CRT)*

The time spent by cows from involuntary recumbency until  $Ca^{2+}$  level of 1.00 mmol/l was regained during recovery from hypocalcaemia was calculated by subtraction. This was defined as calcium regaining time (CRT).

#### *Analytical procedures*

Plasma total calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer 5000, Perkin-Elmer Corp. Analytic Instruments Norwalk, CT 06856 USA). Plasma inorganic phosphate was determined by means of a spectrophotometric analysis (Unimate-kit (Roche) catalogue No. Roche 0736775, Switzerland) applied to Cobas Fara Roche automated centrifugal analyser. The ionised calcium fraction was determined cow side using a transportable acid-base analyzer (IRMA® SL Blood Analysis System (Diametrics Medical Inc., St, Paul, MI 55113, USA).

#### *Statistics*

Linear regression was used to compare the ionised calcium, total calcium, inorganic phosphate and magnesium decaying trends during intravenous EDTA infusion among the 2 groups of cows. The statistical model for simple linear regression is the line with addition of errors;  $Y_i = \beta_0 + \beta_1 x_i + \epsilon_i$ , where  $i = 1, \dots, n$ ,  $\beta_0 = y$  intercept  $\beta_1 =$  the slope of the line  $\epsilon_i =$  the unobservable error variation which is independent and  $N(0, \delta^2)$ .

## **Results**

#### *Clinical parameters*

All cows continued to eat normally as the intravenous EDTA infusion continued until a time

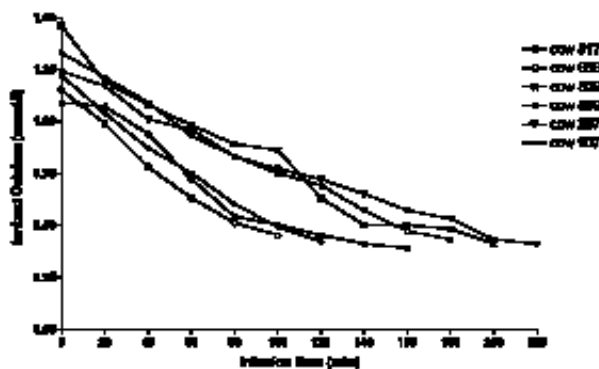


Figure 1a: Plasma ionized calcium in cows fed basic ration then infused intravenously with EDTA from time zero onwards. The last blood sample was taken at recumbency.

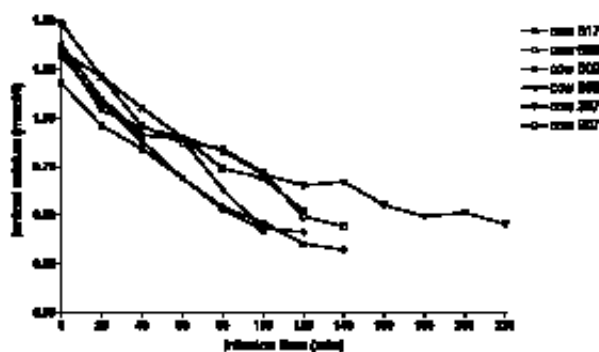


Figure 1b: Plasma ionized calcium in cows supplemented with anions then infused intravenously with EDTA from time zero onwards. The last blood sample was taken at recumbency.

was reached when chewing activity and the rumen contraction force started to decline. At this period cows appeared dull but were still eating though sluggishly and the blood ionized calcium dropped to around 0.8 mmol/l as a result of chelation with EDTA. As the intravenous EDTA infusion continued and therefore more free calcium became chelated the muzzle became progressively dry, ocular mucous membranes became congested and the respiration became dyspnoeic. A state of hallucination manifested by bellowing was observed at this stage. When ionized calcium concentration fell to around 0.60 mmol/l the rumen contractions became muffled and the chewing activity disap-

peared. The ears, tail and the caudal part of the limbs became cold probably due to circulatory collapse and the cow became uneasy shifting weight from one hind leg to another, and sometimes crossing the forelegs. Other signs included frequent urination, starry coat and muscle twitching. On the later stages the cows started to sway on their hind limbs and attempted to support themselves to the feed trough or even to the personnel before they went involuntarily recumbent.

#### *Ionized calcium*

An initial fast drop was observed during the first 20 min of infusion followed by a constant

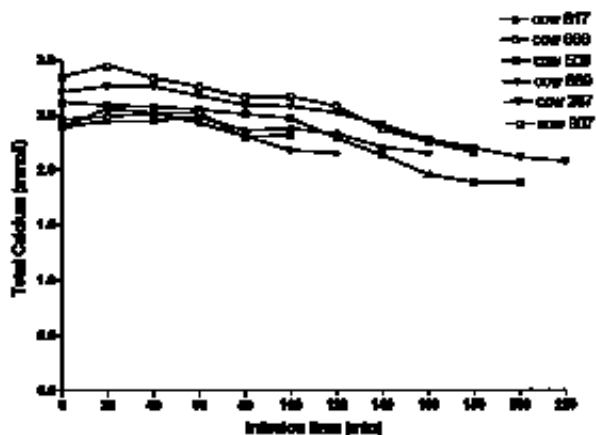


Figure 2a: Plasma total calcium in cows infused intravenously with EDTA from time zero onwards. The last blood sample was taken at recumbency. Cows were supplemented with anions in their ration for 10 days before EDTA infusion.

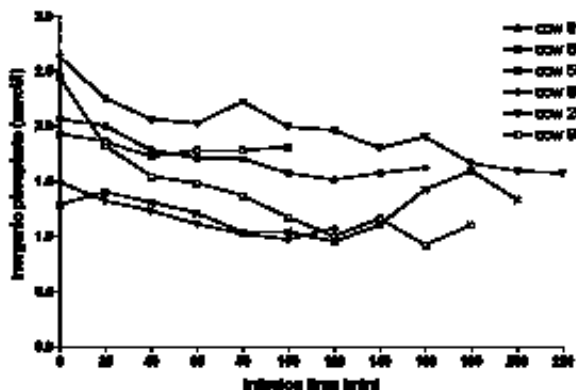


Figure 2b: Plasma inorganic phosphate in cows infused intravenously with EDTA from time zero onwards. The last blood sample was taken at recumbency. Cows were supplemented with anions in their ration for 10 days before EDTA infusion.

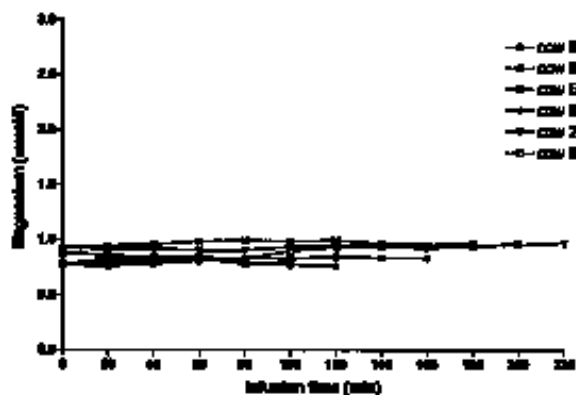


Figure 2c: Plasma magnesium in cows infused intravenously with EDTA from time zero onwards. The last blood sample was taken at recumbency. Cows were supplemented with anions in their ration for 10 days before EDTA infusion.

drop until recumbency in the control cows as well as the anion supplemented cows (Figs. 1a and 1b). The length of infusion period until recumbency varied between cows and there was no correlation between the pre-infusion concentration of calcium and the total amount of EDTA infused until recumbency ( $r^2 = 0.024$ ).

#### *Total calcium, inorganic phosphate and magnesium*

The experimental diets in this study did not influence the trend of plasma mineral response to the intravenous EDTA infusion. The declining pattern of plasma total calcium, inorganic phosphate and magnesium was almost the same during the standardized EDTA infusion following the 10-day period on wrap grass silage. The declining trends were also the same following 10 day of anion salt supplementation. Figs. 2a, 2b and 2c concentration trends for total calcium, inorganic phosphate and magnesium during a standardized intravenous EDTA infusion following a 10 day of anion salt supplementation to cows. Plasma total calcium concentration remained almost constant during the first 100 min of infusion. It started to decline gradually in cows that resisted EDTA induced hypocalcaemia and hence the infusion period was prolonged. Plasma inorganic phosphate concentration declined gradually although a fast drop was observed during the first 20 min of infusion. A further drop was observed until recumbency in some cows but was fluctuating in others. Plasma magnesium concentration remained constant during infusion.

#### **Discussion**

The clinical signs observed in this study were comparable to those in spontaneous milk fever. Reduced appetite was the first sign observed during infusion and was most likely due to reduced rumen contraction force (Daniel 1983). Jørgensen et al. (1998) observed a clear de-

pression in the frequency and amplitude of rumen contractions at ionized calcium concentration of 0.8 mmol/l and later tympanitis at 0.56 mmol/l indicating paresis of the rumen. In earlier studies complete paresis of the rumen was observed when plasma ionized calcium dropped to between 0.45-0.48 mmol/l (Fenwick & Daniel 1990). In our study complete off feed occurred at ionized calcium of 0.6 mmol/l which was within the range of  $0.48 \pm 11$  mmol/l observed by Desmecht et al. (1996). Other clinical signs observed in our study have been documented elsewhere (Daniel & Moodie 1978, Fenwick & Daniel 1990, Desmecht et al. 1996), but increased salivation and raising of the tail was not observed in this study.

In our study plasma ionized calcium declined fast during the first 20 min of infusion followed by a fluctuating tendency until recumbency. Others observed a triphasic regression pattern following an accelerated infusion from 1.65 to 2 ml/kg per min in cows that resisted induced hypocalcaemia (Desmecht et al. 1995). The flow rate was standardized in our procedure so we were not expecting a pattern other than a straight line. As previously mentioned we have observed a disagreement between blood  $[Ca^{2+}]$  and clinical signs at an infusion speed above 2 mg/kg per minute where cows may stand and eat at blood  $[Ca^{2+}]$  of  $\leq 0.4$  mmol/l. Probably, this might have resulted from differences between vascular and tissue  $Ca^{2+}$  concentration during fast infusions because, at least in our standardized procedure, concentrations of plasma  $Ca^{2+}$  of  $\leq 0.40$  mmol/l were associated with paresis and recumbency. Though fluctuating, the persistent decline (Figs. 1a and 1b) of ionized calcium that was observed in our trial could be explained by the fact that, the constant infusion rate of the homogeneous EDTA solution chelated calcium at a rate exceeding the amount replaced through mobilization.

In our experiment total calcium concentration,

which included chelated calcium still present intravascularly remained almost constant during the initial 100 min of infusion. Plasma total calcium concentration started to decline slowly when the infusion period was prolonged in cows that resisted the induced hypocalcaemia for more than 2 hours. The later decline in total calcium might be due to the excretion of EDTA bound calcium by the kidney. *Desmecht et al.* (1995) infused EDTA for 3-4 h and observed an increase in plasma total calcium measured by the same technique used in this study (atomic absorption spectrophotometry). They associated the elevation of total calcium with a mild intoxication of the renal cells by EDTA preventing a rapid clearance of the so formed calcium EDTA complexes.

The concentration of inorganic phosphate declined gradually during our standardized infusion tests and the longer the infusion period the lower the inorganic phosphate concentration attained. A reduction in plasma inorganic phosphate has been shown in spontaneous milk fever (*Littlelike et al.* 1969) and in experimental hypocalcaemia (*Daniel & Moodie* 1979) where the decrease may be marginal. In our study an increased concentration of inorganic phosphate was observed only in one out of 6 cows after 120 min of infusion and the cow was actually struggling. It was hypothesized that such an increase in plasma inorganic phosphate during infusion occurs in struggling cows in which increased muscular activity releases energy from ATP. This reaction might have released inorganic phosphate into the extracellular fluid. *Ramberg et al.* (1967) did not observe any changes in the inorganic phosphate levels in cows simultaneously infused with EDTA and calcium chloride. In hypocalcaemic cows treated with calcium borogluconate the plasma inorganic phosphate rises significantly within 5 min of the intravenous infusion (*Daniel & Moodie* 1979). *Blum et al.* (1974) associated

this elevation to PTH effect on renal clearance of inorganic phosphate.

It has also been observed in the present study that the concentration of total magnesium remained constant, and could be related to the selective affinity of  $\text{Na}_2\text{EDTA}$  to calcium ions (*Jørgensen et al.* 1999). In spontaneous milk fever plasma magnesium increases particularly in paretic cows (*Olson et al.* 1971). In other studies the ionized and total plasma magnesium concentration remained constant throughout the infusion process suggesting that  $\text{Na}_2\text{EDTA}$  administration does not influence  $\text{Mg}^{2+}$  bioavailability (*Desmecht et al.* 1995). *Payne* (1964) and *Berger & Gerber* (1977) observed that the plasma levels of magnesium remained unchanged during  $\text{Na}_2\text{EDTA}$  infusion. In contrast *Belyea et al.* (1976) found a mean rise in plasma magnesium following infusion. *Van Mosel et al.* (1993) in studies with 2 groups of cows fed either a negative or positive dietary cation-anion difference (DCAD) observed constant plasma magnesium concentration in EDTA induced hypocalcaemia and no significant differences were observed in plasma inorganic phosphate concentration due to the dietary treatments. The dietary DCAD is normally calculated as the sum total of  $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$  of the daily ration (*Oetzel* 1988). A negative DCAD prevents milk fever whereas the positive DCAD does not and the preventive effect is due to enhanced effect of parathyroid hormone and  $1, 25(\text{OH})_2\text{D}_3$  on target organs responsible for calcium homeostasis (*Goff et al.* 1991).

In our study the average  $\text{Ca}^{2+}$  concentration at recumbency was 0.43 mmol/l range 0.39 - 0.52 mmol/l. This did not deviate much from previous results in which  $\text{Ca}^{2+}$  concentration at recumbency were  $0.65 \pm 0.12$  mmol/l (*Berger & Gerber* 1977); 0.53-0.61 mmol/l (*Wang & Beede* 1990; 1992), 0.45-0.48 mmol/l (*Jørgensen et al.* 1998) and  $0.48 \pm 0.11$  mmol/l

(Desmecht et al. 1995). This indicates that paresis occurs within a range of 0.39-0.65 mmol/l of ionised calcium.

In our study the observed time range of 90-220 min from the start of infusion until to recumbency was also quite wide among cows. This suggests a behavioural variability of cows to a gradually developing hypocalcaemic state (Desmecht et al. 1995) and whether the cow was feeding during infusion. In our opinion cows that continue to eat during EDTA infusion might be able to resist hypocalcaemia slightly longer due to absorption of dietary calcium. The absorbed calcium probably replaces EDTA-chelated fraction although this effect might be temporary. The lack of correlation between pre infusion calcium concentrations and the total EDTA used to induce recumbency might be explained by the redistribution of ionized calcium between blood and tissues. On the other hand the efficiency and the rapidity with which calcium homeostatic mechanisms could respond can determine resistance to hypocalcaemia during EDTA infusion until recumbency.

Although figures are not shown in this text the calcium regaining time (CRT) expressed as time in minutes spent by cows to regain ionised calcium level of 1.00 mmol/l after EDTA-induced hypocalcaemia, was faster in cows supplemented with anions compared to cows fed wrap grass silage only. This observation suggests further that metabolic acidosis induced by anion salt supplementation improves the ability of the cows to mobilize calcium when demands for calcium were suddenly increased as a result of EDTA induced hypocalcaemia.

In conclusion, our standardized flow rate of 1.2 ml/kg per hour of the 5% Na<sub>2</sub>EDTA solution until recumbency resulted into responses for plasma ionized calcium, total calcium, inorganic phosphate and magnesium comparable to spontaneous milk fever. This infusion tech-

nique might be useful in future experimental studies of hypocalcaemia that require comparison of methods involving monitoring of calcium homeostatic mechanisms. Ionized calcium not total calcium monitoring may serve as a tool in monitoring the level of induced hypocalcaemic state in cows. The pre infusion concentration of plasma ionized calcium should be judged carefully as a predictor of time to recumbency during infusion. The slope of ionized calcium regression lines during EDTA infusion as well as those during recovery from hypocalcaemia could be used to compare calcium homeostatic responses. Calcium regaining time could be another useful tool for monitoring the ability of the cows to mobilize calcium reserves following a sudden increase in calcium demands. Plasma ionized calcium concentration of 0.4 mmol/l would require immediate restitution of calcium infusion when milk fever prone cows are used in experiments.

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### Sammendrag

*Plasma calcium, uorganisk fosfat og magnesium ved hypocalcæmi induceret med standard EDTA infusion i køer.*

Den intravenøse Na<sub>2</sub>EDTA infusionsteknik tillader en specifik og effektiv binding af cirkulerende calciumioner førende til tiltagende grad af hypocalcæmi. De metoder, der sædvanligvis anvendes til monitorering af blodets totale og frie calciumpulje, er ikke beskrevet i detaljer, og de derved opnåede resultater

er ikke sammenlignelige på grund af forskelle i prøveudtagning og analyse. Nærværende artikel beskriver en standardiseret EDTA infusionsteknik, som gør det muligt at sammenligne indvirkningen på blodets calcium-, fosfor- og magnesiumkoncentration mellem to grupper af forsøgskøer. Koncentrationen af den anvendte EDTA-opløsning var 0.134 mol/l. Infusionshastigheden blev standardiseret til 1.2 ml/kg legemsvægt per time. Parese indtraf i området 0.39-0.52 mmol/l ioniseret calcium. I de første 20 min sås et hurtigt fald i ioniseret calcium, efterfulgt af en periode med fluktuerende koncentrationer, igen efterfulgt af et fald førende til parese. Koncentrationen af ioniseret calcium før infusion af EDTA

havde kun ringe korrelation til det volumen af EDTA, der var nødvendig for at fremkalde parese. Koncentrationen af total calcium, målt ved atomabsorption, var næsten konstant igennem de første 100 min af infusionen. Ved fortsat infusion faldt koncentrationen gradvist. Koncentrationen af uorganisk fosfor faldt gradvis og i et fluktuerende mønster indtil paresestadiet. Koncentrationen af magnesium forblev konstant under hele infusionen. Det observerede respons er sammenligneligt med det, der ses ved spontane tilfælde af mælkefeber, hvorfor den her beskrevne standardiserede infusionsteknik kan være værdifuld i fremtidige eksperimentelle undersøgelser.

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