

## Clinical presentation and biochemical profile of horses during induction and treatment of hypocalcemia

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**ABSTRACT.** The aim of this study was to examine the clinical presentation, biochemical profile and response to treatment among horses with experimentally-induced hypocalcemia. Twelve adult, mixed breed mares were used. A 5% ethylenediaminetetraacetic acid disodium (Na<sub>2</sub>EDTA) solution was infused into all the mares until the animals presented clinical signs of hypocalcemia, at which point they were divided into a control group (n = 5) and a treatment group (n = 7). The treated group received an infusion of calcium, phosphorus, magnesium and glucose at a dosage of 1 mL/kg/BW during 30 minutes. The control group received 0.9% saline solution at the same dosage. Clinical examination and blood sample collection were performed at the times T0 (baseline- thirty minutes before beginning the Na<sub>2</sub>EDTA), T1 (at the end of the Na<sub>2</sub>EDTA infusion), T2 (at the end of the treatment) and T3 (24 hours after the end of the experiment). Serum calcium and magnesium concentrations decreased in response to Na<sub>2</sub>EDTA administration. The clinical signs of hypocalcemia in the mares included tachycardia, tachypnea, hypophonesis and cecal atony. These signs disappeared over the course of treatment, while total calcium and magnesium increased. Treatment produced recovery from clinical hypocalcemia within 30 minutes and promoted return of the main biochemical parameters to baseline values.

*Key words:* calcium, phosphorus, treatment, Na<sub>2</sub>EDTA.

**RESUMEN.** El objetivo de este estudio fue evaluar la presentación clínica, perfil bioquímico, y la respuesta al tratamiento en los caballos con hipocalcemia inducida experimentalmente. Doce yeguas se distribuyeron en un grupo control (n = 5) y un grupo tratado (n = 7). Ácido etilendiaminetetraacético disódico (Na<sub>2</sub>EDTA 5%) se infundió a los animales hasta que presentaron signos clínicos de hipocalcemia, momento en el que fueron tratados con una solución comercial con calcio, fósforo, magnesio, y la glucosa en una dosis de 1 mL/kg / PV de 30 minutos. El grupo control recibió 0,9% solución salina en el mismo volumen. Exámenes físicos y colección de muestras sanguíneas se realizaron en cuatro oportunidades: T0 (basal), T1 (al final de la infusión de Na<sub>2</sub>EDTA), T2 (al final del tratamiento), y T3 (24 horas después del fin del experimento). Todas las yeguas presentaron una disminución temporal en las concentraciones de calcio total y de magnesio y se observaron signos clínicos de hipocalcemia. En el curso del tratamiento, la taquicardia, taquípnea, hipofonesis, y la atonía del ciego desaparecieron, así como aumentaron las concentraciones de calcio total magnesio. El tratamiento efectuado posibilitó la recuperación de la hipocalcemia clínica dentro de 30 minutos y retorno de las concentraciones de los principales parámetros bioquímicos a los valores basales.

*Palabras clave:* calcio, fósforo, tratamiento, Na<sub>2</sub>EDTA.

### INTRODUCTION

Calcium is the most abundant mineral in animal tissues, representing 46% of all minerals in the body. It is an essential macro-element for skeleton formation, blood coagulation, cardiovascular regulation, enzyme activation, membrane permeability, muscle contraction, hormone secretion among other important functions (McDowell 1999, Underwood and Suttle 1999).

Calcium in blood is found in a non-ionizable form that is bonded to proteins, mainly albumin, or ionizable forms that represents 50% of the plasma calcium in horses (Kohn 1990, Paulino and Bondan 2006). For survival and for homeostasis of various functional processes, it is vital that the concentration of ionizable calcium in the blood

should be maintained; imbalances may lead to hyper or hypocalcemia (McDowell 1999, Underwood and Suttle 1999).

Although cases of hypercalcemia have been described in the literature, hypocalcemia is a frequent electrolyte abnormality in several species, especially during the postpartum period (Radostits *et al* 2007). Among these species, hypocalcemia is most often seen in dairy cows, but lactating mares or transportations for long period sometimes present with hypocalcemia (Knottenbelt and Pascoe 1998). Hypocalcemia is common in horses with severe gastrointestinal disease, septic foals and under intense physical activity (Toribio 2011). Horses subjected to intense physical activity can develop respiratory alkalosis due to hyperventilation, and lose calcium and chloride in sweat. Alkalosis promotes increased binding of ionized calcium and magnesium to albumin, causing hypocalcemia and hypomagnesemia (Mansmann *et al* 1974).

There have been several studies on experimental induction of hypocalcemia using EDTA in cattle. However, studies on induction of hypocalcemia in horses have mostly been on mild to moderate cases, or even cases secondary to physical exercise or intercurrent diseases (Aguilera *et al*

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1998, Toribio *et al* 2001, Wijnberg *et al* 2002). The reports on severe cases have only been descriptions of isolated spontaneous cases (Richardson *et al* 1991, Scarratt *et al* 1991, Fernandes *et al* 1995, Beyer *et al* 1997, Chiachio *et al* 2005). The few studies on experimental induction of hypocalcemia were done with the objective of evaluating calcium and calcium-related hormone metabolism (Toribio *et al* 2003). In the study by Toribio *et al* (2003), the model used was based on EDTA infusion with subsequent blood calcium determination, but the clinical factors were not fully evaluated. There is therefore a need for studies on experimentally induced equine hypocalcemia: this will enable a more complete description of the clinical signs and laboratory-based characterization of this disease. In addition, validation of the experimental model in horses using clinical factors to determine the endpoint of EDTA infusion is needed.

Thus, the aims of this study were to evaluate the clinical signs and serum biochemistry profile in mares with experimental hypocalcemia, and to assess the efficacy of a therapeutic solution consisting of calcium, phosphorus and magnesium salts treatment for the hypocalcemia.

## MATERIAL AND METHODS

This experiment was carried out in accordance with the ethical standards for animal welfare. This study was pre-approved by the Ethics Committee for Animal Utilization of the School of Veterinary Medicine and Animal Science (FMVZ), University of São Paulo (USP). Conducting this research was a requirement from the Brazilian Agricultural Bureau for approval of the commercialization of the calcium formula used for horses and the minimum number of animals were used.

### ANIMALS AND MANAGEMENT

A total of 12 healthy non-pregnant mixed breed mares were used as subjects. They ranged in age from 6 to 8 years and in weight from 400 to 600 kg. The horses came from a herd belonging to the FMVZ-USP, Pirassununga campus.

One month before the beginning of the experiment, all the animals were bathed with anti-tick shampoo, dewormed, and given multivitamin supplementation (MOV, Vallee, São Paulo, Brazil). The clinical study was performed in the Veterinary Hospital of FMVZ-USP, Pirassununga campus, state of São Paulo. The mares were distributed into three 200 m<sup>2</sup> fenced areas, with four animals in each area. The animals underwent both an adaptation period and a period with an experimental diet, which was composed of 75% dry matter made of coast-cross hay and 25% dry matter made from a commercial concentrate (Vitaly, Qualy Nutrição Animal, Lindóia, Brazil); the food was provided twice daily. The amounts of Ca and P in the hay were 0.4 and 0.1 %, respectively, and the amounts of Ca and P in the concentrate were 1.1 and 0.5%, respectively.

## STUDY DESIGN

Hypocalcemia was induced in all mares as described by Smith and Brown (1963). A 5% solution of ethylenediaminetetraacetic acid disodium (Na<sub>2</sub>EDTA) (Sigma-Aldrich, Germany) with pH adjusted to 7.4, was infused intravenously at a rate of 220 mL/hour through an infusion pump (Digibom, Fundação Adib Jatene). When the animal presented the classic clinical signs of severe hypocalcemia (sternal recumbency with self-auscultation or lateral decubitus), the infusion was stopped and the horses were randomly distributed into a control (n = 5) and a treated group (n = 7). The amount of EDTA needed to induce hypocalcemia ranged from 17.93 to 29.95 g.

The horses in the treatment group received a solution<sup>1</sup> based on an established hypocalcemia treatment, containing 2.44 g of total calcium from three sources (calcium gluconate monohydrate, calcium lactate pentahydrate, and calcium D-saccharate tetrahydrate), 0.185 g of magnesium, 0.472 g of phosphorus (magnesium hypophosphite hexahydrate) and 5 g of anhydrous dextrose per 100 mL at a dose of 1 mL/kg/BW during 30 minutes, thus a horse with 400 kg BW received 9.76 g of Ca, 0.74 g of Mg and 1.89 g of P. The control group received the same dose of a 0.9% saline solution. The infusion rate was calculated individually to ensure that all animals received the volume of the solutions within the 30 minutes period. Clinical examinations were performed and blood samples were taken at the following time points: T0 (baseline, thirty minutes before beginning the Na<sub>2</sub>EDTA infusion), T1 (animal in sternal decubitus with self-auscultation position or lateral decubitus, which was when the Na<sub>2</sub>EDTA infusion was stopped), T2 (30 minutes after the end of the Na<sub>2</sub>EDTA infusion) and T3 (24 hours after the end of the experiment). After the blood sampling and clinical evaluation of T2 had been done, the control group received the same treatment as the treated group so that they would recover from the induced hypocalcemia.

### CLINICAL EVALUATION

The clinical examinations included evaluations of heart rate (HR), respiratory rate (RR), cecal movements (CM), rectal temperature (RT), and capillary refill time (CRT). All other clinical manifestations were addressed with special attention to the horses muscle activity/fasciculation and mental status.

### BLOOD SAMPLING AND BIOCHEMICAL EVALUATION

Blood samples were placed in plain tubes and tubes with anticoagulant (sodium fluoride) for serum and plasma, respectively. Serum biochemical variables analysed included concentrations of total protein (Pt), albumin (Al),

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calcium (Ca), magnesium (Mg), inorganic phosphorus (P) and activity of gamma glutamyl transferase (GGT), aspartate aminotransferase (AST) and creatine kinase (CK). The plasma was analysed for glucose concentration. The biochemical analysis was performed in an automatic biochemical analyser (RX Daytona-Randox Laboratories).

#### STATISTICAL ANALYSES

The statistical analysis was performed using the statistical program SAS 9.3 (2012). Data were evaluated for normality using Kolmogorov-Smirnov and the homogeneity of variances was assessed (Siegel 1975). Data with normal distribution were submitted to analysis of variance (F test) using PROC MIXED (SAS 9.3, 2012) for repeated measures, being studied for each variable the effects of treatment, time and their interaction. The Akaike (AIC) criteria was used for choosing the best covariance structure. A minimum significance level of 5% was adopted.

#### RESULTS

##### CLINICAL FINDINGS

One of the first clinical findings observed in all of the horses after Na<sub>2</sub>EDTA infusion was involuntary continuous movement of the lips and tongue. The first manifestation was a yawn, followed by kinetic movement of the tongue inside the oral cavity and chewing movements. The animals also presented excitation, in the form of incessant locomotion (running around the fence post to which they were tied).

Muscle tremors were observed in most of the horses. Subtle tremors began in the front limbs, in the musculature of the triceps brachii, and immediately proceeded to the back muscles and then to the large muscle groups in the pelvic region. The tremors evolved into pronounced tetany, which was followed by restlessness and stirring. At

this stage, the animals held their forelimbs abducted and stood in a “tripod” position when not moving in order to maintain their balance. After a period of trotting and loss of coordination, 9 of the 12 animals lay down in lateral decubitus, while the others remained in sternal decubitus. During this stage, the appetite of the horses was tested and it was absent.

With continued infusion of the Na<sub>2</sub>EDTA solution, a change was observed in mental status, from excitation to progressive depression marked by somnolence and lack of response to auditory and tactile stimuli. At this stage, all the horses presented marked cecal atony. Other noteworthy signs included tachypnea, dyspnea, temporary apnea, anuria and lack of defecation. The skin temperature in the extremities was also reduced.

After completion of Na<sub>2</sub>EDTA infusion, the animals in the control group were infused with saline solution for 30 minutes and continued to present symptoms of depression, lack of response to tactile and auditory stimuli, and mydriasis. The following symptoms were observed at lower frequency: paddling motions, myoclonus and presence of a purple halo in the perialveolar region of the incisors.

The horses presented significant clinical alterations, including tachycardia (observed at T1), an increase in capillary refill time and a marked decrease in cecal movements. The animals in the control group presented cecal atony for as long as 30 minutes after the end of Na<sub>2</sub>EDTA infusion. Increased respiratory rate was also notable. No significant differences in rectal temperature were observed within or between the groups, and these temperatures remained within the normal range at all times. The results relating to the clinical parameters are presented in table 1.

##### BIOCHEMICAL EVALUATION

The Na<sub>2</sub>EDTA infusion caused a temporary decrease in the total serum calcium concentration (table 2). This

**Table 1.** Mean values and standard deviation of heart rate (HR), respiratory rate (RR), cecal movements (CM) and capillary refill time (CRT) of horses treated and control groups at different times.

Variable	Group	Times				P		
		T0	T1	T2	T3	Trat	Temp	Trat *Temp
HR (beats/min)	Control	39.5±5.9 <sup>b</sup>	58.0±20.9 <sup>a</sup>	49.0±10.3 <sup>ab</sup>	46.0±17.6 <sup>b</sup>	0.8290	0.0094	0.3688
	Treated	40.0±4.5 <sup>b</sup>	70.0±10.9 <sup>a</sup>	46.0±9.0 <sup>b</sup>	40.0±5.6 <sup>b</sup>			
RR (breaths/min)	Control	21.0±11.5 <sup>b</sup>	35.2±13.0 <sup>a</sup>	35.7±19.6 <sup>a</sup>	20.8±1.8 <sup>b</sup>	0.7904	0.0095	0.3259
	Treated	21.1±3.0 <sup>b</sup>	40.6±12.0 <sup>a</sup>	18.9±11.3 <sup>b</sup>	21.4±6.7 <sup>b</sup>			
CM (mov/3')	Control	2.6±0.8 <sup>a</sup>	0.0±0.0 <sup>b</sup>	0.0±0.0 <sup>Bb</sup>	2.8±1.3 <sup>a</sup>	0.0583	<0.0001	0.0172
	Treated	2.5±1.0 <sup>a</sup>	0.0±0.0 <sup>b</sup>	2.0±1.4 <sup>Aa</sup>	2.5±0.6 <sup>a</sup>			
CRT (seg)	Control	2.05±0.0	3.8±1.0 <sup>B</sup>	4.2±1.4	2.5±0.5	0.3610	<0.0001	0.0659
	Treated	2.0±0.0	5.0±0.6 <sup>A</sup>	3.2±0.4	2.7±0.5			

Capital letters in columns indicate significant differences between groups. Small letters in rows indicate significant differences between times.

decrease, which began at T1, was highly significant in relation to the baseline measurement. However, after the end of the treatment (T3) the calcium concentration was higher than the concentration observed at baseline, and returned to the normal concentration at T4.

The concentration of inorganic phosphorus presented a decrease due the infusion of Na<sub>2</sub>EDTA (table 2) with increased values due the P solution infusion in the treated group. The concentration of serum magnesium in the mares decreased significantly over the course of the EDTA infusion, but this concentration became normal again after the animals were treated (table 2).

Even though there was an increase in magnesium concentration between T2 and T3 in the control group, this change was not sufficient to return the serum Mg to the baseline values; at T3, a significant difference remained between the treatment and control groups.

The concentrations of total protein, serum albumin and glucose (table 3) increased during the EDTA infusion in comparison with the baseline values. The GGT activity remained unaltered during the course of the trial, while the AST activity was affected by time only in the treatment group with higher values at T3 when compared with basal.

## DISCUSSION

The horses subjected to experimental induction presented the classic signs of hypocalcemia, similar to other reports of clinical cases in horses (Richardson *et al* 1991). These horses experienced two of the three main phases of clinical hypocalcemia: excitability and muscle tremors (Phase I) and generalised depression, muscular paresis and decubitus (Phase II). Some animals also manifested symptoms of the third phase, with lateral decubitus, intense

**Table 2.** Mean values and standard deviations for total serum calcium (Ca), phosphorus (P) and magnesium in the treated horses and control group at different times.

Variable	Group	Times				P		
		T0	T1	T2	T3	Trat	Temp	Trat* Temp
Ca (mmol/L)	Control	3.3±0.5 <sup>a</sup>	1.6±0.1 <sup>c</sup>	2.0±0.1 <sup>Bb</sup>	3.4±0.1 <sup>a</sup>	<0.001	<0.001	<0.001
	Treated	2.9±0.1 <sup>b</sup>	1.5±0.3 <sup>c</sup>	4.5±0.3 <sup>Aa</sup>	3.3±0.2 <sup>b</sup>			
P (mmol/L)	Control	0.88±0.1 <sup>a</sup>	0.47±0.1 <sup>c</sup>	0.71±0.1 <sup>Bb</sup>	0.87±0.1 <sup>a</sup>	0.1117	<0.0001	0.0112
	Treated	0.90±0.1 <sup>a</sup>	0.52±0.3 <sup>b</sup>	0.98±0.1 <sup>Aa</sup>	0.91±0.1 <sup>a</sup>			
Mg (mmol/L)	Control	1.58±0.4 <sup>ab</sup>	1.92±0.2 <sup>b</sup>	1.80±0.3 <sup>b</sup>	1.25±0.3 <sup>a</sup>	0.3929	<0.0001	0.9282
	Treated	1.83±0.45	2.13±0.5	1.93±0.4	1.37±0.5			

Capital letters in columns indicate significant differences between groups. Small letters in rows indicate significant differences between times.

**Table 3.** Mean concentrations and standard deviations for serum total protein (Pt), albumin (Al), the activities of AST, GGT, CK and plasma glucose in the treated horses and control group during the experiment.

Variable	Group	Times				P		
		T0	T1	T2	T3	Trat	Temp	Trat* Temp
Pt (g/L)	Control	69±4.0	73±5.0	68±5.0	68±4.0	0.978	0.0004	0.6694
	Treated	69±2.0 <sup>b</sup>	74±4.0 <sup>a</sup>	67±3.0 <sup>b</sup>	68±4.0 <sup>b</sup>			
Al (g/L)	Control	32±1.8	34±2.3	32±2.6	32±2.3	0.784	0.0001	0.9257
	Treated	32±1.0 <sup>b</sup>	35±2.0 <sup>a</sup>	32±2.0 <sup>b</sup>	32±1.3 <sup>b</sup>			
AST (U/L)	Control	247±33.8	274±29.0	254±34.3	293±57.5	0.314	0.0003	0.5191
	Treated	270±33.3 <sup>b</sup>	299±31.6 <sup>ab</sup>	260±39.0 <sup>b</sup>	320±44.0 <sup>a</sup>			
GGT (U/L)	Control	12.0±3.1	12.6±3.8	10.5±3.0	12.2±3.1	0.912	0.287	0.7634
	Treated	13.3±6.5	13.8±6.8	12.6±6.5	9.2±3.7			
CK (U/L)	Control	169±25.1	209±96.6	233±98.0	571±544	0.878	0.075	0.9947
	Treated	205±67.8	246±66.5	263±56.4	524±456			
Glucose (mmol/L)	Control	4.9±0.86 <sup>b</sup>	7.2±2.0 <sup>ab</sup>	10.58±2.6 <sup>a</sup>	5.9±1.2 <sup>ab</sup>	0.779	0.001	0.5781
	Treated	5.0±0.45 <sup>b</sup>	8.2±3.0 <sup>ab</sup>	10.5±2.7 <sup>a</sup>	5.2±0.57 <sup>b</sup>			

Small letters in rows indicate significant differences between times.

symptoms of depression, marked tachycardia (> 70 beats per minute) and hypophonesis.

The pathogenesis of these symptoms is closely related to the degree of hypocalcemia present at the time of induction (Herdt 1988, Radostits *et al* 2007). The initial mild hypocalcemia triggers excitation and tetany; consciousness is maintained, and the animal remains standing. With intensification of the hypocalcemia, there is a decrease in the strength of cardiac contractions, which leads to lowered efficiency of cardiac output. The decreased cardiac output triggers compensatory tachycardia, which may be accompanied by hypophonesis. The decrease in blood pressure causes the skin temperature at the extremities to decrease. The anuria is reflective of lower renal perfusion and reduced contractility of the bladder muscle. Inadequate blood perfusion may also contribute towards loss of consciousness.

The decrease in cecal movements and lack of defecation and mydriasis result from decreased contractility of the smooth muscles of the cecum, decreased intestinal motility and decreased contractility of the sphincter muscle of the pupil, respectively. The same occurs with the striated skeletal muscles, which exhibit hypercontractility in phase I and are paretic or even paralysed in phase II, thus causing the animals to remain in lateral or sternal decubitus (Fenwick and Daniel 1990).

Involuntary movements of the tongue and the lips, which were observed in the animals tested, are not found in the classical presentation of natural hypocalcemia in horses (Fernandes *et al* 1995, Wijberg *et al* 2002, Chiachio *et al* 2005).

The signs of hypocalcemia gradually decreased over the course of treatment with the calcium solution. Tachycardia decreased, and HR returned to the baseline within the first 15 minutes of treatment. Over this time period, hypophonesis also disappeared, and the animals appeared more alert. Among the 12 treated horses, two stood up after about 15 minutes of treatment; the others remained in decubitus or stood up immediately after the end of the treatment, with or without stimulus. Concomitantly, cecal movement was reestablished, and the appetite of all of the animals returned. Symptoms of cecal atony and cecal meteorism disappeared, and the animals exhibited increased flatulence. The pupil reflexes also returned.

In healthy horses, nearly half of the serum calcium is present in the ionizable form; the other half remains bonded to the serum proteins. After Na<sub>2</sub>EDTA has been infused into the blood, it gradually exchanges its two cations (Na<sup>+</sup>) for two atoms of ionizable calcium, thus forming a strong bond with the calcium and making it unavailable to the organism (Mellau *et al* 2001). The rate at which ionizable calcium declined in the horses was similar to what was described by Mellau *et al* (2001) in cows, i.e. there was a prolonged and continuous fall until the end of the infusion. In contrast, the treatment with calcium rapidly and significantly increased the total calcium, thus making

calcium available to the horses and reversing the symptoms of hypocalcemia. Unfortunately, in this study, the determination of ionizable calcium was not possible, being the total Ca the variable used to evaluate the variation of this element during hypocalcemia and subsequent treatment.

The increase in the phosphorus concentration may be partially explained by the exaggerated muscle tremors that occurred in some animals. According to Mellau *et al* (2001), this increase in muscle activity leads to increased energy consumption, with the attendant transformation of ATP into ADP and release of phosphate into the circulation. In natural cases of hypocalcemia in dairy cattle, inorganic serum phosphorus concentration tend to fall (Ortolani 2005), unlike what was observed in the present trial.

Cesco *et al* (2004) detected a slight, non-significant decrease in magnesium concentration after Na<sub>2</sub>EDTA infusion in cows. Horses subjected to exhaustive physical activity may have accompanied hypocalcemia and hypomagnesemia, this is due to increased blood pH (respiratory alkalosis) because of hyperventilation developed during physical exercise and loss of electrolytes through sweat (Ca, Mg, Na, Cl). This change in blood pH is responsible for the greater affinity of Ca and Mg by albumin, make these ions unavailable in the ionized form (Schryver, Hintz and Lowe 1978, Kerr and Snow 1983, Toribio 2011).

Na<sub>2</sub>EDTA is not known to have any immediate influence over protein synthesis or over the degree of proteolysis. The observed increase may have been due to the excitation observed during phase I of the hypocalcemia, this excitation may have caused splenic contraction, thus leading to hemoconcentration, which would increase the quantity of elements found in the blood and decrease the percentage of fluids in this medium, and would indirectly cause increases in the concentrations of protein and albumin. This excitability, can also cause hyperventilation and consequent change in blood pH, contributing to reduction of Ca and Mg, and this hypothesis is strengthened by the increase of total protein concentration.

Na<sub>2</sub>EDTA does not appear to interfere in glucose metabolism, but the stress of manipulation and excitation can led to cortisol release, which stimulates gluconeogenesis. The treatment with the calcium solution, which contained glucose, maintained hyperglycemia and normal levels were reestablished 24 hours after hypocalcemia had been induced.

The maintenance of normal GGT and AST values indicated that no hepatic lesions resulted from the induction or the treatment of hypocalcemia. The AST values alone increased at the end of the 24 hours in relation to the other time points, a pattern that was seen in both groups. This elevation in AST activity is closely linked to muscle changes that probably resulted from small lesions that developed during the pathological decubitus or were caused by the muscle tremors. Increases in CK, just like increases in AST, are indicative of muscle injury.

Use of Na<sub>2</sub>EDTA for experimentally inducing hypocalcemia was effective in reducing the concentration of

ionized and total calcium, thus validating this experimental model for horses, which uses clinical information as the Na<sub>2</sub>EDTA infusion endpoint. The experimental induction of hypocalcemia using Na<sub>2</sub>EDTA promoted significant drops in the serum concentration of total calcium and magnesium and also led to an increase in serum phosphorus concentration. Treatment with a calcium-rich solution temporarily increased the calcium values above the baseline concentration. Calcium concentration returned to baseline 24 hours after treatment, thus proving that the solution tested was effective in promoting recovery from hypocalcemia.

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