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


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 represents 50% of the plasma calcium in horses that is bonded to proteins, mainly albumin, or ionizable calcium, 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).

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...
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 m² 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.

Blood sampling and clinical evaluation.

Hypocalcemia was induced in all mares as described by Smith and Brown (1963). A 5% solution of ethylenediaminetetraacetic acid disodium (Na₂EDTA) (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 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 Na₂EDTA infusion), T1 (animal in sternal decubitus with self-auscultation position or lateral decubitus, which was when the Na₂EDTA infusion was stopped), T2 (30 minutes after the end of the Na₂EDTA 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),

1 Valleé Calcio, Vallecè S.A.
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 Na₂EDTA 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 Na₂EDTA 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 Na₂EDTA 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 Na₂EDTA 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 Na₂EDTA infusion caused a temporary decrease in the total serum calcium concentration (table 2). This decrease was followed by an increase in inorganic phosphorus concentration and a decrease in the activity of gamma glutamyl transferase (GGT). The aspartate aminotransferase (AST) and creatine kinase (CK) activities remained unchanged.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Times</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T0</td>
<td>T1</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>Control</td>
<td>39.5±5.9b</td>
<td>40.0±4.5b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>39.5±5.9b</td>
<td>40.0±4.5b</td>
</tr>
<tr>
<td>RR (breaths/min)</td>
<td>Control</td>
<td>21.0±11.5b</td>
<td>35.2±13.0a</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>21.1±3.0b</td>
<td>40.6±12.0a</td>
</tr>
<tr>
<td>CM (mov/3')</td>
<td>Control</td>
<td>2.6±0.8a</td>
<td>0.0±0.0b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.5±1.0a</td>
<td>0.0±0.0b</td>
</tr>
<tr>
<td>CRT (seg)</td>
<td>Control</td>
<td>2.0±0.0</td>
<td>3.8±1.0b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.0±0.0</td>
<td>5.0±0.6a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Times</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T0</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>Control</td>
<td>3.3±0.5a</td>
<td>1.6±0.1c</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>2.9±0.1b</td>
<td>1.5±0.3c</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>Control</td>
<td>0.88±0.1a</td>
<td>0.47±0.1c</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0.90±0.1a</td>
<td>0.52±0.3b</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>Control</td>
<td>1.58±0.4ab</td>
<td>1.92±0.2b</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>1.83±0.45</td>
<td>2.13±0.5</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Times</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T0</td>
</tr>
<tr>
<td>Pt (g/L)</td>
<td>Control</td>
<td>69±4.0</td>
<td>73±5.0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>69±2.0b</td>
<td>74±4.0b</td>
</tr>
<tr>
<td>Al (g/L)</td>
<td>Control</td>
<td>32±1.8</td>
<td>34±2.3</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>32±1.0b</td>
<td>35±2.0a</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>Control</td>
<td>247±33.8</td>
<td>274±29.0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>270±33.3b</td>
<td>299±31.6ab</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>Control</td>
<td>12.0±3.1</td>
<td>12.6±3.8</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>13.3±6.5</td>
<td>13.8±6.8</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>Control</td>
<td>169±25.1</td>
<td>209±96.6</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>205±67.8</td>
<td>246±66.5</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>Control</td>
<td>4.9±0.86b</td>
<td>7.2±2.0ab</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>5.0±0.45b</td>
<td>8.2±3.0ab</td>
</tr>
</tbody>
</table>

Small letters in rows indicate significant differences between times.
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 me-}


diphonia also disappeared, and the pupils 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 Na₂EDTA has been infused into the blood, it gradually exchanges its two cations (Na⁺) 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 Na₂EDTA 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, making these ions unavailable in the ionized form (Schryver, Hintz and Lowe 1978, Kerr and Snow 1983, Toribio 2011).

Na₂EDTA 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.

Na₂EDTA does not appear to interfere in glucose metabolism, but the stress of manipulation and excitation can lead 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 Na₂EDTA 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 Na₂EDTA infusion endpoint. The experimental induction of hypocalcemia using Na₂EDTA 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.

ACKNOWLEDGEMENTS

The authors thank Valleé S.A. for funding this experiment.

REFERENCES


