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Effects of pharmacological doses of Vitamin D_3 on mineral balance and profiles of plasma Vitamin D_3 metabolites in horses☆

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Abstract

Metabolism and functions of Vitamin D in horses differ from those in humans, pigs and rats. In horses, calcidiol and calcitriol concentrations in blood plasma are remarkably low (<10 nmol L^{-1}; 20–40 pmol L^{-1}, respectively). When a toxic amount of Vitamin D_3 is administered, the responsiveness of calcium and calcitriol concentrations in blood plasma is much reduced compared to the other domestic animal species but inorganic phosphate (Pi) response is much more marked, leading to an increase of the Ca × Pi product. Also, soft tissue calcifications have been observed to develop in horses during Vitamin D_3 intoxication. It was suggested that the elevation of the Ca × Pi product may play a causative role in this calcification process. To test this assumption, two horses were treated with 40,000 IU kg^{-1} of Vitamin D_3 whilst dietary uptake of Ca and Pi was restricted to prevent or to diminish the increase of the Ca × Pi product. Distribution, number and severity of calcification centres were considerably less in these horses than in the control animals of a previous experiment which had received the same amount of Vitamin D_3 but where Ca and Pi intake was not restricted. It appears from these findings that in horses, the increase of the Ca × Pi product in blood plasma during a Vitamin D intoxication contributes to the soft tissue calcifications. It is further concluded that in the event of a Vitamin D intoxication, it is recommended to restrict the Ca and Pi uptake immediately. The authors believe that this may help to prevent or at least diminish soft tissue calcifications which are often fatal to the horse due to nephrocalcinosis.
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Keywords: Horses; Vitamin-D-intoxication; Calcification; Soft tissue; Calcium × phosphate product

1. Introduction

In horses, metabolism and functions of Vitamin D appear to differ in many respects from those in other domestic animal and mammalian species such as pigs, humans or rats [1]. For example, concentrations of calcidiol (<10 nmol L^{-1}) and calcitriol (20–40 pmol L^{-1}) in blood plasma are remarkably low. If such low concentrations were present in humans or pigs, this would clearly regarded to be in a rachitic range [2,3]. On the other hand, rickets or osteomalacia are very rare in horses.

Another aspect where horses behave differently from many other mammalian species is the metabolic response following an administration of a toxic dose of Vitamin D. If such a high dose is administered parenterally, the responses of some Vitamin D_3 metabolites in blood plasma are much smaller in horses [1] than those usually observed in pigs [4] and sometimes, humans [5]. In particular, this applies to calcitriol.

In a previous study, three ponies received four injections of 0.25 mg Vitamin D_3 per kg BW (10,000 IU kg BW^{-1}) intramuscularly on four consecutive days. These administrations did not result in any increase in the calcitriol concentration in blood plasma of the animals (40 pmol L^{-1}) [2]. Despite the absence of an increase of plasma calcitriol, the toxic amount of Vitamin D_3 led to a lasting (6 weeks) and significant 18% increase of total plasma calcium (from 2.55 to 3.02 mmol L^{-1}) and more notably, to a remarkable 140% increase of plasma inorganic phosphate (Pi) (from 1.38 to 3.33 mmol L^{-1}) [6]. Postmortem examination of the animals 6.5 weeks after the Vitamin D_3 administrations revealed a number of moderate to severe soft tissue calcifications which were mainly located subendocardially at the left ventricle, atrium, in the large arteries and the coronary arteries, the kidneys and the gastric mucosa [7]. However, this study provided no clue concerning the causative factors possibly responsible for the widespread soft tissue calcifications which had formed in the presence of a nearly normal blood calcitriol concentration. One factor which might have con-

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tributed to the precipitation of calcium–phosphate deposits in the soft tissue is the elevation of the calcium·phosphate product in blood plasma which had increased from 3.5 to 10.1.

The aim of the experiment reported here was to examine whether the soft tissue calcifications are related to the increase of the Ca × Pi product and whether they would also develop when the increase of Ca × Pi could be prevented by dietary means. In order to achieve this, feed intake of Ca and Pi was markedly restricted.

2. Materials and methods

2.1. Experimental animals, housing and feeding

Two Standardbred mares (A and B), 11 and 14 years of age with 490 and 525 kg of body weight were used for the experiment. The horses were dewormed during the week before the experiment started. The feeding period for the two horses lasted 49 days, including 7 days before (a.i.) and 42 days after (p.i.) the first Vitamin D_3 injection. Except during the balance trials, the animals were housed individually in 30 m^2 boxes with sand bedding. The horses were fed twice daily at 08:00 and 20:00 h according to the NRC requirements except that the daily intakes of calcium and phosphate were restricted. On fresh weight basis, the feed constituted of wheat straw (53%) and concentrate (47%). The concentrate was composed of 26% saccharose, 18% maize gluten, 16% soya oil and of a mineral/vitamin mix. The latter contained the necessary macro and trace mineral supplements, Vitamin A and D but no calcium or phosphate. An amount of 5.5 kg of this ration was offered daily to the horses. From the 12th day p.i., the horses showed diminished appetite and refused to take up the daily provisions of straw. The composition of the feed was therefore gradually modified in a way that some of the wheat straw and concentrate was replaced by hay. The transitions in feed composition took place from 14th to 42nd days (p.i.). Thirty days p.i., the ration was composed of 34% wheat straw, 34% hay, 16% oat and 16% saccharose. Between the 1st and the 14th days p.i., daily uptakes of calcium and phosphate were restricted to 53 and 34% of their daily requirements, respectively. After 14th days p.i., the daily uptakes of calcium and phosphate were 88 and 34% of their daily requirements or less.

2.2. Balance trials and treatments

Two balance studies for calcium and phosphate each of 5 days duration were carried out with the two horses. One before and one after the Vitamin D_3 administrations. The first (control) balance trial was performed from 7th to 2nd days a.i. and the second balance trial from 7th to 11th days (p.i.). Feed and water intake and faecal and urinary excretions were quantified at 12-h intervals during these balance periods. Starting on day 0 of the feeding period, 0.5 mg (10,000 IU) of Vitamin D_3 per kg BW were injected intramuscularly at 10:00 h on four consecutive days. Until 17th day p.i., heparinized blood samples were collected daily from one of the jugular veins of the horses. From 18th to 42nd days p.i., blood samples were collected every 2–3 days. Both animals were sacrificed 42nd day p.i. and macroscopic and microscopic postmortem examinations were carried out mainly for the presence of soft tissue calcifications.

2.3. Laboratory methods

Ionized calcium (Ca^{2+}), total calcium, inorganic phosphate, alkaline phosphatase activity, calcidiol, 24,25-$(OH)_2D_3$ and calcitriol were measured in blood plasma. Net calcium and phosphate retentions were calculated from the two balance trials. Vitamin D_3 metabolites were measured in plasma samples. For this, the samples were deproteinized followed by solid phase extraction and separation of the metabolites by HPLC. Calcidiol and 1,24-$(OH)_2D_3$ were quantified after HPLC separation by UV absorptiometry. Calcitriol concentrations were determined by use of a calf thymus radioreceptor assay based essentially on the method of Horst et al. [8].

3. Results

3.1. General condition of the animals

After the Vitamin D administrations, feed intake was slightly reduced in both the horses. The reductions of intake were largely reversed by replacing some of the wheat straw with hay. The body temperature (37.1 ± 0.36 °C, mean ± S.D.) remained constant during the experimental period and showed no significant change. The same was true for the heart rate (30 ± 2.5 min^{-1}) and the respiration rate (9 ± 1.2 min^{-1}). The body weight of the horses declined by 7% during the experimental period. Daily uptakes of water were 5.5 ± 1.5 and 6.3 ± 0.5 L (100 kg BW)$^{-1}$ per day for the horses A and B, respectively, and rose after the Vitamin D_3 injections by 4 and 42%, respectively. Renal flow of water was 1.9 ± 0.38 and 3.4 ± 1.1 L (100 kg BW)$^{-1}$ per day and rose by 58 and 72%, respectively.

3.2. Balance studies

Mean values of dietary uptake of calcium and phosphate, their renal and faecal excretion and retention are compiled in Table 1.

Renal excretion of calcium increased by 133% but renal excretion of phosphate rose in the two horses 50- and 15-fold compared to the control period. The single values from the two balance trials are illustrated in more detail in Fig. 1.

After the Vitamin D_3 injections, net losses of calcium and phosphate increased about three-fold in both horses compared to the control period. The detailed values of these

Table 1
Calcium and phosphate balances from two horses before and after administration of a toxic amount of Vitamin D_3 (mmol (kg BW)$^{-1}$ per day, mean ± S.D.) ($n = 5$)

Animal no.	Vitamin D_3 dosage	Uptake	Fecal excretion	Renal excretion	Retention
Calcium					
A	Before	0.54 ± 0.00	0.49 ± 0.05	0.30 ± 0.12	−0.25 ± 0.14
	After	0.43 ± 0.03	0.56 ± 0.14	0.66 ± 0.09	−0.80 ± 0.06
B	Before	0.49 ± 0.03	0.54 ± 0.07	0.20 ± 0.07	−0.25 ± 0.05
	After	0.22 ± 0.09	0.39 ± 0.07	0.49 ± 0.11	−0.66 ± 0.20
Phosphate					
A	Before	0.31 ± 0.00	0.49 ± 0.07	0.01 ± 0.00	0.20 ± 0.07
	After	0.19 ± 0.02	0.11 ± 0.02	0.60 ± 0.10	0.52 ± 0.11
B	Before	0.29 ± 0.00	0.47 ± 0.03	0.04 ± 0.02	0.22 ± 0.03
	After	0.07 ± 0.03	0.06 ± 0.00	0.53 ± 0.17	0.54 ± 0.18

measurements during the two balance studies are presented in Fig. 2.

3.3. Biochemical parameters of blood plasma

The concentration of calcium in blood plasma rose only slightly but significantly after the Vitamin D_3 injections (Fig. 3). The total calcium concentration was elevated by 8.5%, from 2.93 (a.i.) to 3.18 mmol L^{-1} (p.i.). Equally small was the percentage increase in Ca^{2+}. The percentage of Ca^{2+} from the total Ca remained unchanged (Fig. 3). On the other hand, the response of the inorganic phosphate concentration after the Vitamin D injections was much more pronounced resulting in an increase from 1.05 (a.i.) to 2.38 mmol L^{-1} from the third to the 26th day (p.i.). This was about a three-fold increase (Fig. 4).

The activity of the alkaline phosphatase in blood plasma showed no significant change during the first 3 weeks p.i., but began to rise significantly from that time on until the end of the 49 days lasting the feeding period (Fig. 4).

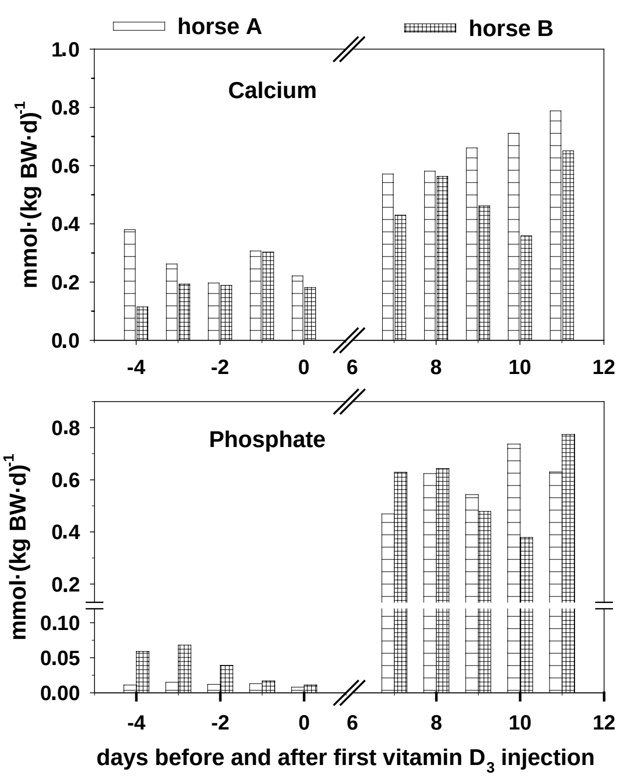

Fig. 1. Renal excretion of calcium and phosphate by two horses before and after the administration of a cumulative dose of 1 mg of Vitamin D_3 per kg BW. The vitamin was intramuscularly injected in four portions on four consecutive days. The horses were on a Ca and Pi deficient diet.

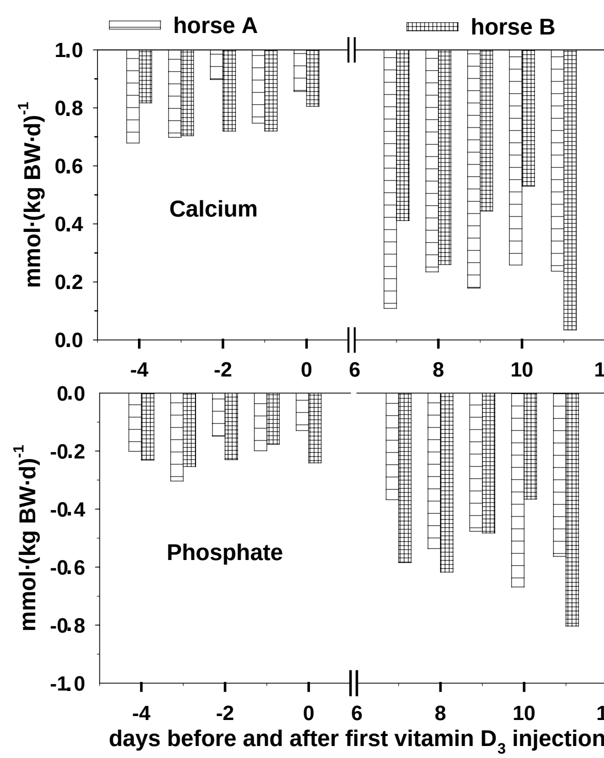

Fig. 2. Calcium and phosphate balance of two horses before and after the administration of a cumulative dose of 1 mg of Vitamin D_3 per kg BW. The vitamin was intramuscularly injected in four portions on four consecutive days. The horses were on a Ca and Pi deficient diet.

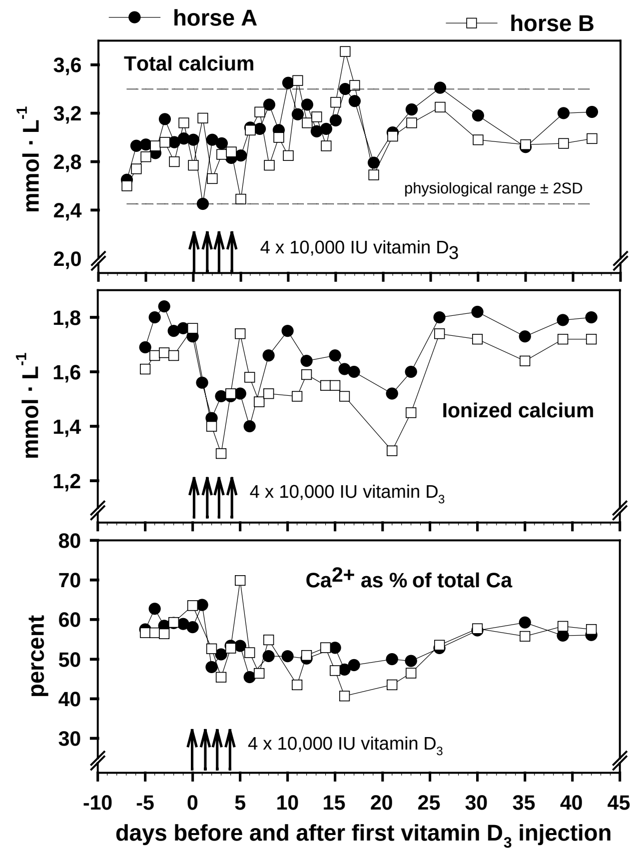

Fig. 3. Concentration of total calcium, ionized calcium (Ca^{2+}) and the percentage ration of Ca^{2+}/total Ca before and after the administration of a cumulative dose of 1 mg of Vitamin D_3 per kg BW. The vitamin was intramuscularly injected in four portions on four consecutive days. The horses were on a Ca and Pi deficient diet.

3.4. *Vitamin D_3 metabolites in blood plasma*

Calcidiol in blood plasma was markedly elevated as a result of the Vitamin D_3 injections. Its concentration rose from about 1.5 to 550 and 1000 nmol L^{-1} in horses A and B, respectively (Fig. 5). The concentration in plasma began to rise immediately after the first Vitamin D_3 injection and reached its maximum at the 16th day (p.i.). The concentration of 24,25-$(OH)_2D_3$ in blood plasma was also elevated p.i. (Fig. 5). Starting from an a.i. concentration of about 6.5 nmol L^{-1}, the time course of its concentration showed some fluctuation in the first part of the experimental period and then remained clearly elevated with levels of 95 and <180 nmol L^{-1}. In contrast to these effects, only minor changes were seen in the calcitriol concentration in blood plasma. As expected, the calcitriol concentration was found to be very low during the control period with 50 pmol L^{-1} with a tendency to increase after the Vitamin D_3 injections. In the second half of the observation period, calcitriol reached concentrations of 78 and 65 pmol L^{-1}, a concentration which is still in the physiological range if present in other mammals (Fig. 5).

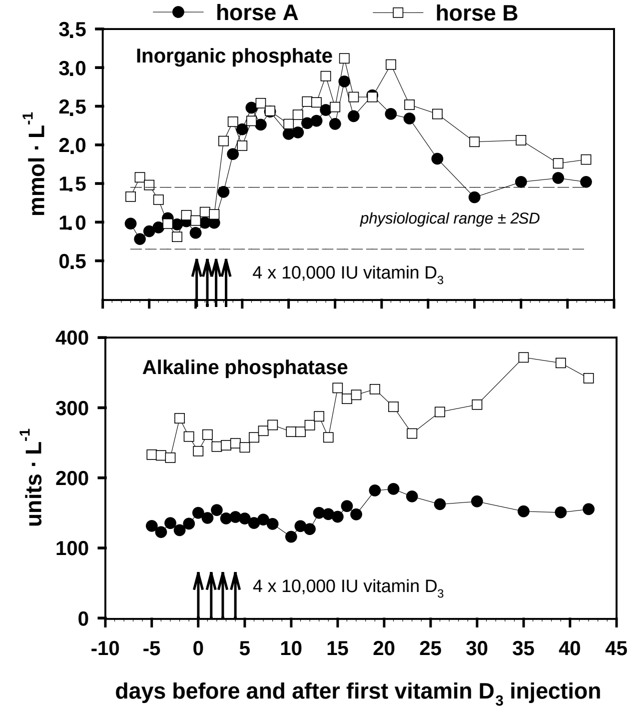

Fig. 4. Concentrations of inorganic phosphate and the activity of alkaline phosphatase in blood plasma before and after the administration of a cumulative dose of 1 mg of Vitamin D_3 per kg BW. The vitamin was intramuscularly injected in four portions on four consecutive days. The horses were on a Ca and Pi deficient diet.

3.5. *Postmortem examinations*

Autopsy of the horses and macroscopic and microscopic examination of soft tissue revealed the presence of some calcification spots. In horse A, these deposits were very rare and were confined to the transition area from the aortic valve to the aorta. In both horses, the walls of the large blood vessels showed however to be somewhat stiff. In horse B, some subendocardial calcifications were seen in the left ventricle in the left atrium and around the atrio ventricular valve. Other multi focal subpleural calcifications were present in the thorax region and some diffuse pinhead like calcifications were observed in the kidneys. The abdominal cavity was affected by a moderate chronic fibrous and filamentous peritonitis. The stomach mucosa showed a moderate ulcerative and erosive gastritis. The small and large intestine was unaffected. Except the places mentioned above, no other calcification spots were detected.

4. Discussion

In the present pilot experiment, the distribution and quantity of calcified areas in the soft tissues were markedly smaller and less severe than in a previous experiment which was carried out with ponies. The amount of Vitamin D_3

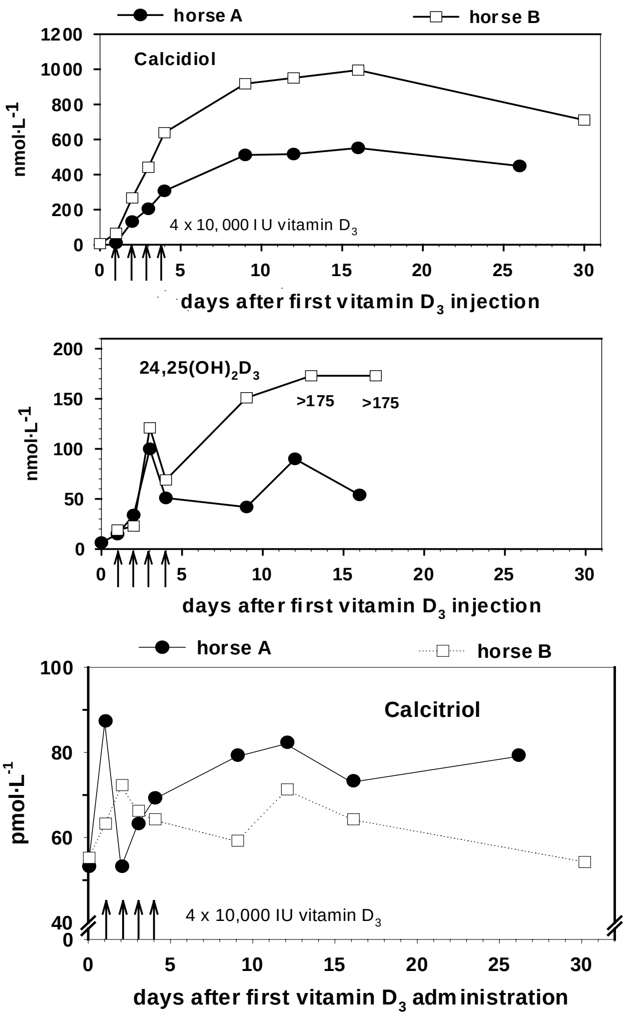

Fig. 5. Concentrations of calcidiol, 24,25-$(OH)_2D_3$ and calcitriol in blood plasma before and after the administration of a cumulative dose of 1 mg of Vitamin D_3 per kg BW. The vitamin was intramuscularly injected in four portions on four consecutive days. The horses were on a Ca and Pi deficient diet.

which was administered per kg BW was the same in both the studies. In both, ponies and horses, the applied dose of Vitamin D_3 resulted into a very high increase in the plasma phosphate concentration but only into a moderate increase in plasma calcium. It resulted further in a marked increase in calcidiol and almost no change in the calcitriol concentration in blood plasma. From these observations, we concluded that ponies and normal horses show comparable responses to toxic doses of Vitamin D_3 and behave similarly in Vitamin D_3 metabolism and calcium and phosphate regulation. On the basis of this assumption, the present study showed that the elevation of the Ca $\times$ Pi product which develops in association with the Vitamin D intoxication plays probably a causative role in the generation of focal soft tissue to calcifications.

Probably as a consequence of the restricted feeding of calcium and phosphate, the Ca $\times$ Pi product in blood plasma rose only from 3.1 to 6.7 as compared to an increase from 3.5. to 11.1 in the previous experiment with ponies where uptake of Ca and Pi was not restricted. The reduced increase of the Ca $\times$ Pi product in this latter experiment was paralleled by a much smaller number and less severe calcification centers in the soft tissue. We conclude from this finding that in order to reduce possible adverse effects of inadvertent Vitamin D intoxications in horses it would be advisable to reduce Ca and Pi intakes as soon as possible.

The absence of a significant increase of the plasma Ca concentration in horses after administration of high doses of Vitamin D_3 has been reported previously by other investigators [9,10]. In some studies with horses [11,12], an elevation of the plasma Ca concentration was also reported after Vitamin D intoxication. Information is lacking in these case reports regarding the amount and nature of the Vitamin D uptake except that it was ingested with feed. It is not known whether different routes of administration of the vitamin, orally or parenterally, lead to different responses.

Significant increases in the plasma Pi concentration in horses after administration of toxic amounts of Vitamin D have also been reported by others [9,10,13]. The moderate or lacking increase of plasma Ca and at the same time the pronounced increase of plasma Pi are probably typical features of a Vitamin D intoxication in horses that differ from those in humans [7], pigs [14] or dogs [15] but may be similar to rabbits [16]. The particularly low calcidiol and calcitriol concentrations in blood plasma of normal horses are two more typical features of the horse Vitamin D system [1]. These peculiarities are accompanied by a lack of any significant increase in blood calcitriol concentration after administration of toxic amounts of Vitamin D, a distinct metabolic feature of horses which differs from that found in many other mammalian species [1]. From a comparative point of view, it would be of interest to further elucidate the peculiar regulatory role of Vitamin D in the equine calcium and phosphate homeostatic system and to find out what is the mechanism of the soft tissue calcifications in horses after Vitamin D intoxication.

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