Dose-dependent effects of magnesium supplementation on serum and intracellular magnesium concentrations in healthy horses

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Summary: For magnesium supplementation in horses, dosages for MgO, $MgCO_3$, and $MgSO_4$ were described previously. However, for magnesium as magnesiumaspartate-hydrochloride (MAH) dose-dependent effects on the magnesium levels have not yet been determined in horses. Therefore, the magnesium concentrations in blood lymphocytes, serum, urine and the fractional magnesium excretion in the urine were measured in five healthy adult horses before and after oral supplementation with 15, 30 and 60 mg/kg bodyweight (BW) magnesium as MAH respectively for seven days. There was no wash-out period in between experimental periods. In erythrocytes, the intracellular baseline magnesium concentration and the intracellular magnesium concentration after one week of supplementation with 60 mg/kg BW magnesium were determined. All horses were exclusively fed with hay of which the magnesium concentration was measured each week. All horses were clinically examined daily, including signs of colic and soft feces. There were no significant changes in the magnesium concentration in the diet. Signs of colic, soft feces or other side effects were not observed. The serum magnesium concentration after supplementation with 60 mg/kg BW magnesium as MAH was significantly higher than the baseline and exceeded the reference range in four of five horses. After 15 and 30 mg/kg BW magnesium as MAH, the serum magnesium concentration showed a significant increase over time. Neither a significant increase of the intracellular magnesium concentration nor significant changes in urine magnesium concentration or fractional excretion were observed. In erythrocytes, a trend for higher magnesium concentrations was found after supplementation with 60 mg/kg BW magnesium as MAH. For short-term supplementation in cases of increased magnesium requirements, these dosages seem effective and the dosages of 15 mg/kg BW and 30 mg/kg BW safe to use. For supplementation with 60 mg/kg magnesium as MAH, veterinary supervision and monitoring of the serum magnesium concentration is recommended.

Keywords: magnesium, intracellular magnesium concentration, magnesiumaspartate-hydrochloride, serum magnesium concentration

Introduction

Magnesium is the second most abundant intracellular cation in the body (*Altura* and *Altura* 1996) and its extracellular concentrations depend on gastrointestinal absorption, renal excretion and bone exchange (*Quamme* and *de Rouffignac* 2000, *Schweigel* and *Martens* 2000). In the horse, about 25 % of the magnesium are absorbed in the proximal small intestine, 30–35 % in the distal small intestine and 5–10 % in the large colon (*Hintz* and *Schryver* 1972). For most adult horses, a magnesium intake of 13–15 mg/kg seems to be sufficient (*Hintz* and *Schryver* 1973). However, in several physiologic states, such as lactation, growth, and intense exercise, a higher daily magnesium supply (15–30 mg/kg and day) may be necessary (*Council* 2007). To replace unavoidable magnesium losses, a horse has to absorb 5 mg/kg BW per day (*National Research Council* 2007). A state of hypomagnesaemia

can be induced with a magnesium intakes of 5–6 mg/kg BW magnesium per day (*Meyer* and *Ahlswede* 1977).

For oral magnesium supplementation, dosages for MgO (30–50 mg/kg BW and day), $MgCO_3$ (60–80 mg/kg BW and day), and $MgSO_4$ (80–100 mg/kgBW and day) have been described as safe to use in horses (*Toribio* 2010). For magnesiumaspartate-hydrochloride (MAH), dose-effect relations have not been determined in horses, yet. However, when compared to inorganic magnesium salts, organic magnesium salts such as magnesiumaspartate were better absorbed in Wistar rats (*Coudray* et al. 2005). In humans (n = 24), MAH was significantly better absorbed than magnesiumoxide. Additionally, MAH has a high degree of safety: single dose toxicity in rats, mice, and dogs was 6.8, 6.9, and 4.5 g/kg BW, respectively (*Classen* 2002). Decreased serum magnesium concentration is a reliable indicator for a magnesium deficit (*Vormann*

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2003). However, as serum magnesium concentration can be stabilized by emptying the body's functional reserves, it is also a rather insensitive marker for magnesium deficiency (*Chaudhary* et al. 2010)

Recently, reference values for free intracellular magnesium concentrations in equine lymphocytes have been published (0.16–0.42 mmol/l) (*Winter* et al. 2018). To the authors' knowledge, reference ranges for intracellular magnesium concentration in erythrocytes have not been determined yet, probably because alterations in the intracellular magnesium concentration in erythrocytes caused by erythrocyte age (*Elin* and *Hosseini* 1985, *Deuster* et al. 1987, *Flatman* 1988) and the HLA phenotype (*Millart* et al. 1995, *Nadler* and *Rude* 1995) cannot be excluded.

The purpose of this study was to examine the effect of different dosages of magnesium as MAH on serum, intra-lymphocyte, and intra-erythrocyte concentrations in five healthy horses.

Material and Methods

Five clinic-owned horses (two Standardbreds, one Arabian Horse, one Arab-Cross and one Icelandic Horse) aged seven to 27 years (19.0 ± 8.0 years) were enrolled in the study. None of the horses was in a magnesium-deficient state at the beginning of the study. For 1 week respectively, they received increasing oral doses of 15, 30, and 60 mg/kg BW magnesium as MAH (Nupafeed®, Verla-Pharm Arzneimittel GmBH, Tutzing, Germany). The supplement was provided once daily (between 8:00 and 10:00 a.m.). It was directly given into the horses' mouth with a syringe. The horses were maintained on a high quality hay diet exclusively for 3 months prior to the start of the study, of which the magnesium content was determined weekly to prove a steady magnesium content. The horses were kept either in a paddock with shelter for 24 hours a day (3 horses), or on a paddock during daytime and stabled on wood shavings at night. They had no access to pasture. Hay was provided on an ad libitum basis in the paddocks. The horses that were stabled at night were fed 1 kg hay/kg bodyweight overnight and hay ad libitum during the day. During the study period, all horses were examined daily and especially checked for signs of colic or soft faeces. The horses were weighed at the beginning and the end of the study as well as before feeding a different dosage of magnesium as MAH. Additionally, the body condition score (*Henneke* et al. 1983) was determined. On a weekly basis, i.e. before receiving magnesium as MAH and after receiving each dosage for a week, blood was obtained by antiseptic jugular venipuncture between 9:00 and 10:00 a.m.. Two lithium-heparin tubes, one EDTA tube, and one serum tube (all from Sarstedt, Nuembrecht, Germany) were collected. Urine was collected as a spot urine sample. Complete blood count (CBC, Abaxis VetScan® HM5, Viernheim/Germany), creatinine (in blood and urine), blood urea nitrogen (BUN), aspartate transaminase (AST) and gamma-glutamyltransferase (GGT) were determined by reflection measurement (Reflotron® plus, Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany). The total magnesium content in serum and urine was determined by an external laboratory using photometry (Laboklin, Bad Kissingen, Germany) and the fractional excretion of mag-

nesium was calculated. For measurements performed at the Equine Clinic's laboratory, the urine did not require dilution for the measurements.

Additionally, the magnesium content of blood lymphocytes was determined upon completion of each 1 wk dosing step. In erythrocytes, the basal magnesium concentration and the magnesium concentration after receiving 60 mg/kg BW orally for 1 wk was determined in four of the five horses.

Determination of the free intracellular magnesium concentration in lymphocytes

For lymphocyte isolation, 10 ml heparinized blood were diluted with 8 ml of phosphate buffered saline (PBS; Lonza, Basel, Switzerland) supplemented with 0.8 mmol/l magnesium chloride mixed by inverting. The diluted blood was layered carefully onto Histopaque 1077 (Sigma-Aldrich, Munich, Germany). Immediately thereafter, the sample was centrifuged at 700 g at 21 °C for 30 min. After centrifugation, the layer in which lymphocytes were suspended was removed. The lymphocyte suspension was centrifuged at 300 g and 21 °C for 15 min. The supernatant was discarded and the cells resuspended in 10 ml Hank's balanced salt solution (HBSS; Biochrom, Berlin, Germany) supplemented with 0.8 mmol/l magnesium as magnesium chloride and centrifuged again at 300 g and 21 °C for 15 min. Magnesium was added to the solution to imitate a physiological cell environment. This protocol has already been successfully used to study intralymphocytic free magnesium concentrations in humans (*Delva* et al. 1996, *Delva* et al. 2006). After another washing step with HBSS + 0.8 mmol/l magnesium chloride, the supernatant was discarded and the cells were resuspended in 1 ml HBSS with 0.8 mmmol/l $MgCl_2$. Using a TC20 automated cell counter (Bio-Rad, Munich, Germany) cell number and cell viability were determined, averaging at 6.3×10^6 cells/ml and 89.5 %, respectively. The suspended cells were loaded with mag-fura 2 AM (8.7 μmol/L) (Thermo Fischer Scientific, Dreieich, Germany) and incubated on a shaking plate at 37 °C for 30 min. After centrifugation (400 g, 5 min), removal of the supernatant and resuspension in HBSS + 0.8 mmol/l $MgCl_2$, the cells were incubated for another 20 min at 37 °C in HBSS + 0.8 mmol/l $MgCl_2$ to allow the complete de-esterification of the dye. Cells were subsequently pelleted by centrifugation (400 g, 5 min) and resuspended in completely HBSS + 0.8 mmol/l $MgCl_2$.

Measurements were performed at 37 °C in 3 ml cuvettes containing 2 ml cell suspension under stirring. The ratio of emissions at 508 nm was determined during alternating excitation at 340/380 nm using a spectrofluorometer LS55 (PerkinElmer, Rodgau, Germany) equipped with a fast-filter accessory. According to the protocol published by *Delva* et al. (*Delva* et al. 1998), baseline emission was measured first over at least 1 min, followed by the addition of EDTA and EGTA (both from Sigma-Aldrich, Taufkirchen, Germany) to a final concentration of 5 mmol/l each. By adding EDTA/EGTA to the measurement buffer, extracellular Mg^{2+} and Ca^{2+} were complexed, resulting in an immediate drop in the fluorescence intensities. The fluorescence ratio obtained directly after this drop (< 10 s) represents the intracellular Mg^{2+} concentration. Next,

Triton X-100 was added to a final concentration of 0.1% to lyse the cells, thereby yielding the minimum fluorescence ratio. The maximum fluorescence ratio was determined by addition of $MgCl_2$ to a final concentration of 100 mM. Mg^{2+} concentrations were calculated using the FL WinLab software provided by the LS 55 based on the method by *Grynkiewicz* (*Grynkiewicz* et al. 1985).

Determination of intracellular magnesium in erythrocytes

Of 10 ml heparinized blood, 1 ml was removed for photometric (546 nm) hemoglobin determination (Spectrophotometer LP300S, Dr. Lange, Düsseldorf, Germany and haematocrit determination (Centrifuge 5702, Eppendorf AG, Hamburg, Germany). The remaining blood was placed on ice until centrifugation at 1500 g and 4°C for 10 min. The plasma was removed and frozen in liquid nitrogen until magnesium determination with atomic absorption spectrometry. The erythrocytes were resuspended in 3 ml cold magnesium-free PBS. For magnesium determination with atomic absorption spectrometry 1 ml of this suspension was frozen in liquid nitrogen to destroy the cells. Another milliliter was used to determine the haemoglobin concentration and the haematocrit.

The remaining erythrocytes were centrifuged again at 1400 g at 4°C for 10 min. The supernatant was discarded and the cells again resuspended in 3 ml cold magnesium-free PBS. Again, 1 ml was frozen in liquid nitrogen to produce erythrocyte lysate and 1 ml was used for haemoglobin and haematocrit determination. The intracellular magnesium was calculated from the magnesium concentration measured in the erythrocyte lysate divided by the haematocrit ($[Mg]/Hct = [Mg]_i$).

Statistical analysis

The statistical analysis was conducted with IBM SPSS Statistics 23 (IBM, Armonk, New York United States). The Friedmann test was applied to analyse the changes of the magnesium concentration in serum, lymphocytes, erythrocytes, and urine. The Friedmann test is similar to the parametric repeated measures ANOVA and therefore used to detect differences in treatments across multiple test attempts. As a post-hoc test, the Wilcoxon signed-rank test was used. Normal distribution was not tested.

Data are displayed as mean ± standard deviation if not mentioned otherwise. The significance level was set at p = 0.05.

Results

Throughout the study, all horses remained clinically healthy. Signs of colic and/or soft faeces were not observed. One horse developed a mild leukopenia and a mild elevation of the serum creatinine, which did not require further treatment.

The horses weighed 349–589 kg (447.0 ± 79.7 kg) and weight did not change significantly during the study. There was no change in body condition score in any of the horses during

the time of magnesium supplementation with magnesium as MAH (Table 1).

There were no significant changes of the magnesium concentration in the hay (1.7 ± 0.2 g/kg dry matter, Table 2).

The serum magnesium concentration increased significantly over time during the study period (P = 0.008). The serum magnesium concentration after supplementation with 60 mg/kg BW magnesium as MAH (1.00 ± 0.07 mmol/) was significantly higher than baseline (0.76 ± 0.05 mmol/l; P = 0.039) and exceeded the reference range (0.5–0.9 mmol/l) in four out of five horses. After supplementation of 15 and 30 mg/kg BW magnesium as MAH, the serum magnesium concentration showed a significant increase over time (after 15 mg/kg BW magnesium as MAH: 0.82 ± 0.13 mmol/l; after 30 mg/kg BW magnesium as MAH: 0.86 ± 0.09 mmol/l; P = 0.05, Figure 1). Neither a significant increase of the intra lymphocyte magnesium concentration nor significant changes in the urinary concentration (Table 3) and fractional excretion of magnesium were observed.

The magnesium concentration in erythrocytes tended to be higher after one week of supplementation with 60 mg/kg BW magnesium as MAH, though this increase was statistically not significant (P = 0.144, Figure 2).

Discussion

Several studies have investigated the effect of magnesium supplementation on plasma and intra-erythrocyte magnesium

Table 1   Bodyweight and body condition score (BCS) of the horses during the study | *Körpergewicht und Body Condition Score (BCS) der Pferde während der Studie*

Horse no.	Day 0		Day 7		Day 14		Day 21	
	BW (kg)	BCS	BW (kg)	BCS	BW (kg)	BCS	BW (kg)	BCS
1	579	7	589	7	588	7	590	7
2	449	6	448	6	446	6	452	6
3	432	6	436	6	438	6	435	6
4	349	5	350	5	350	5	351	5
5	418	6	417	6	415	6	421	6

Table 2   Mineral content of the hay provided during the study | *Mineralstoffgehalt des während der Studie gefütterten Heus*

	Mineral				
	Mg^{2+}	Ca^{2+}	K^+	Na^+	PO_4^{3-}
Prior to study	1.6	3.6	15.4	0.6	1.9
Day 0	1.8	4.5	11.9	5.5	1.8
Day 7	1.9	4.2	13.7	0.6	2.4
Day 14	1.6	3.5	16.6	0.3	1.8
Day 21	1.5	3.5	11.9	0.6	1.7

Mineral content given in g/kg dry matter

concentration in humans. A significant increase in the plasma magnesium concentration compared to baseline was found in high-performance athletes after two month supplementation with 100 mg magnesium as magnesium oxide. A significant change in the intracellular magnesium concentration, however, was not present after two months magnesium supplementation compared to two months without (*Molina-Lopez* et al. 2012) which is comparable to our study, although the horses were supplemented for a shorter time period than the athletes. Another study reported a statistically significant increase in the plasma magnesium concentration and a trend for an increase in magnesium concentration in erythrocytes after 4 weeks of magnesium supplementation with magnesium citrate malate in 20 women (*Basso* et al. 2000). Similarly to the latter
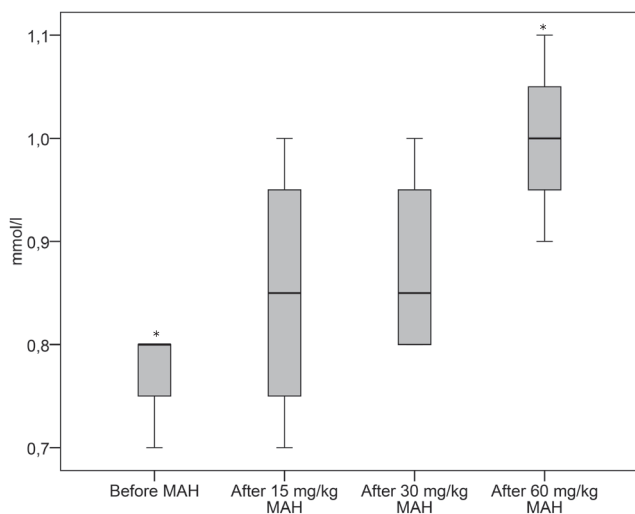

Fig. 1   Serum magnesium concentration in 5 healthy horses before and after supplementation with increasing dosages of magnesium as MAH. * Values marked with a star differ significantly | *Serummagnesiumkonzentration bei 5 gesunden Pferden vor und nach Supplementation mit ansteigenden Dosierungen von Magnesium als MAH * Mit einem Stern markierte Werte unterscheiden sich signifikant*
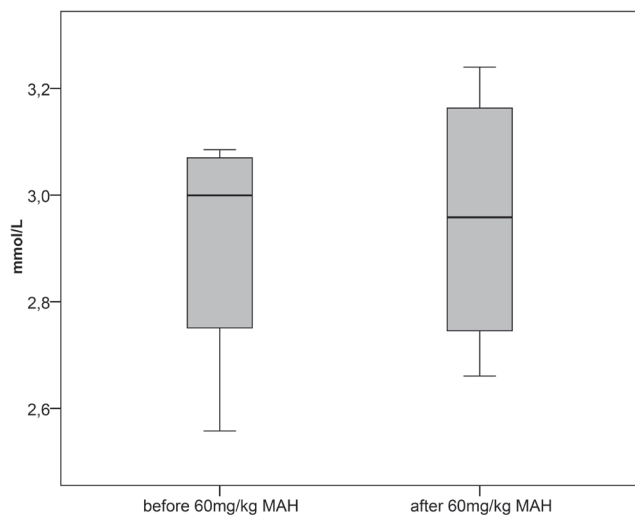

Fig. 2   Intracellular magnesium in erythrocytes of 4 healthy horses before and after supplementation with 60 mg/kg magnesium as MAH *Intrazelluläre Magnesiumkonzentration in Erythrozyten bei 4 gesunden Pferden vor und nach Supplementation mit 60 mg/kg Magnesium als MAH*

study in women, in which magnesium was supplemented at increasing dosages (125 mg/d magnesium for one week followed by 250 mg/d magnesium for three weeks; (*Basso* et al. 2000), we also supplemented magnesium (as MAH) at increasing dosages. Despite the fact that we could not identify an increase in the free cytosolic magnesium in leukocytes, we observed a trend for an increase of total intra-erythrocyte magnesium concentration after 1 wk supplementation of the highest magnesium dose (60 mg/kg BW), which is in agreement with the findings of *Basso* and colleagues (*Basso* et al. 2000). The latter finding was not statistically significant, which may be attributed to the small sample size of only four horses in our study. Nonetheless, *Molina-López* et al. did also not report a significant change in intra-erythrocyte magnesium concentration after 2 months of magnesium supplementation (100 mg Mg^{2+} as MgO) in 14 professional handball-players (*Molina-Lopez* et al. 2012). It may thus appear that the intracellular magnesium concentration is little affected by changes in extracellular magnesium availability in healthy subjects. Similarly, a significant change of intra-erythrocyte magnesium concentration was neither found after a single nor after multiple intra-peritoneal injections of magnesium chloride (50 mg/kg q 24 h) for seven days in male Wistar rats when compared to placebo (*Wlaz* et al. 2016).

As the magnesium concentration in erythrocytes can be influenced either by age (*Elin* and *Hosseini* 1985, *Deuster* et al. 1987, *Flatman* 1988), or the HLA phenotype (*Millart* et al. 1995, *Nadler* and *Rude* 1995), or the activity of the erythrocyte sodium-magnesium exchanger (*Widmer* et al. 1995), erythrocytes are probably not the best choice for determining the effect of short time magnesium supplementation, but rather long time supplementation. On the other hand, *Witkowski* et al. concluded after reviewing 27 studies in 21 publications, that beside serum/plasma and urinary magnesium concentration, erythrocyte magnesium concentration, responds well to dietary manipulation (*Witkowski* et al. 2011). Additionally, the protocol we used for intra-erythrocyte magnesium measurement delivers reliable results and is also quick and easy to apply. In horses, especially in horses with a suspected long-term magnesium deficiency, this protocol might be applicable in routine diagnostics. Of course, further research to determine reference ranges is required.

In healthy subjects, there should be no intracellular magnesium deficiency in the peripheral tissues. In a magnesium-deficient state, the situation may be different though. After inducing a magnesium-deficient state in inbred male rats by feeding a magnesium-deficient diet for 6 weeks, the plasma magnesium concentration decreased significantly during the

Table 3   Urinary magnesium concentration (mean ± standard deviation) | *Magnesiumgehalt des Urins (Mittelwert ± Standardabweichung)*

	Urinary magnesium concentration (mmol/l)
Day 0	3.58 ± 0.10
Day 7	3.62 ± 0.04
Day 14	3.55 ± 0.05
Day 21	3.54 ± 0.12

first week and remained significantly lower for the rest of the test period (*Elin* et al. 1980). Similarly, the erythrocyte magnesium content was significantly reduced in 16 humans after 3 weeks on a magnesium-free liquid diet. Such reduced intra-erythrocyte magnesium concentration was restored to normal with oral magnesium supplementation for eight weeks (400 mg/day) without significant changes of the serum magnesium concentration (*Nadler, Malayan* et al. 1992).

Looking at diseased subjects, the insulin resistance index values were directly correlated to intra-lymphocyte free magnesium. Patients with the lowest intra-lymphocyte magnesium content showed the greatest peripheral insulin resistance (*Delva* et al. 1998). Magnesium supplementation with 365 mg/d magnesium as MAH for six months in 25 humans with decreased insulin sensitivity resulted in a significant increase of the ionized magnesium in whole blood when compared to patients receiving placebo (n = 22). After the supplementation, there was a trend for higher serum magnesium concentrations in the verum group while there was no significant change in the total intra-erythrocyte magnesium concentration (*Mooren* et al. 2011). Furthermore, *Thomas* et al. found out that migraine patients (n = 29) had significantly lower (15 %) intracellular magnesium concentrations in lymphocytes than controls (n = 19). After magnesium supplementation with mineral water containing 4.5 mmol/l magnesium, the magnesium content in lymphocytes was increased by 16 % in the migraine patients without an effect on the plasma magnesium level (*Thomas* et al. 2000). In contrast to this, our horses showed a significant increase of serum magnesium concentrations after supplementation of magnesium as MAH whereas an increase of the free magnesium concentration in lymphocytes was not observed. However, as our five horses were healthy, an intracellular magnesium deficiency was not expected. Since the tissue electrolyte concentrations in healthy individuals should be constant, it is possible that less magnesium was taken up into the cell in our healthy horses than would have been the case in a magnesium deficient state. However, about 99 % of the total body's magnesium is located in bone, muscles, and non-muscular soft tissues which means that only 1 % of the body's magnesium can be found in the blood (*Elin* 2010). The examination of for example bone or muscle biopsies might yield more accurate results, but these methods seem to be too invasive for repeated examinations. Moreover, the determination of intracellular free magnesium concentration in equine blood lymphocytes has only just recently been established (*Winter* et al. 2018). Additionally, the reliable and exact determination of intracellular Mg^{2+} might have been hampered by the experimental setup although due care was taken to avoid intracellular Mg^{2+} depletion during the experiments; i.e., lymphocytes were kept in media with a magnesium concentration of 0.8 mmol/l throughout the isolation procedure and the mag-fura 2 loading and activation steps. The magnesium was added to the medium in order to reduce magnesium loss of the cells due to osmosis. However, the several washing and incubation steps that are necessary to measure free cytosolic magnesium in lymphocytes may have an effect on the intracellular free magnesium concentration of lymphocytes, leading to a partial harmonization of the measured concentrations among the different dosage steps. Due to different concentration gradients, the speed and the extent of the magnesium uptake may have been different with

different intra-lymphocyte magnesium concentrations. It is therefore possible that a potential increase in the intracellular magnesium concentration caused by the supplementation with MAH was masked by the treatment conditions prior to the measurement. Erythrocytes on the other hand were processed within 20–30 min after blood collection and not incubated in media containing magnesium. Uptake of extracellular magnesium prior to atomic absorption spectrometry was hence not possible and the measured results should reliably reflect the intracellular magnesium concentration at the time of sampling.

Since ionized magnesium (Mg^{2+}) represents the biologically active form of the ion, its exact determination is of high medical interest. However, determination of total magnesium concentrations in erythrocytes is a significantly faster method and might also represent a more reliable method in particular for routine diagnostic purposes.

The resorption of magnesium can be affected by the composition of the feed. The digestibility (praecaecal and overall) of magnesium was shown to be better in horses on a roughage than on a mixed feed diet (*Stadermann* et al. 1992). The horses in this study were kept on a hay diet exclusively, so that the magnesium absorption should have been optimal.

Assuming that the horses consumed 2 kg/hay per 100 kg BW, the horses would have taken in 31.6 mg magnesium/kg BW (calculation per kg feed, not dry matter) with which even the daily magnesium requirements of physiologic states of increased magnesium requirements were met. With the supplementation, the horses received approximately 45 mg/kg BW, 60 mg/kg BW, and 90 mg/kg BW magnesium, respectively, which exceeds the daily requirements by far. However, magnesium toxicity is rarely reported in horses and mostly iatrogenic associated with renal failure or animals with extensive cellular damage such as cancer, myonecrosis, haemolysis, and severe sepsis (*Toribio* 2010). When used as cathartics, dosages for magnesiumsulfate of up to 1 g/kg BW have been reported (*White* 2005) which exceeds the recommended use for magnesium supplementation given by *Toribio* (80–100 mg/kg BW and day) by one hundred-fold. 100 mg $MgSO_4$ will provide 9.7 mg Mg^{2+} (*Plumb* 2018), which means that 1 g of $MgSO_4$ will provide 97 mg Mg^{2+}. With an increased magnesium intake of approximately 90 mg/kg magnesium as MAH, our horses were therefore not at risk to develop magnesium toxicity, but rather at risk to develop diarrhea due to the cathartic effect of the magnesium. However, no changes were observed in the consistency of the horses' faeces.

The urine excretion of magnesium and its fractional excretion did not change during the present study even though a greater change in either magnesium concentration or magnesium excretion was expected. It is known that the intestinal absorption of magnesium is reduced if the magnesium supply is above requirements (*Toribio* 2010). The faecal magnesium excretion was not determined in this study. However, it is likely that the faecal magnesium excretion increased due to reduced intestinal absorption. During short-term supplementation, the dosages of 15 mg/kg BE, 30 mg/kg BW, and 60 mg/ kg BW did not lead to any side effects. However, the reference range for serum magnesium concentration was exceeded in

4/5 horses after supplementation with 60 mg/kg BW magnesium as MAH, suggesting that hypermagnesamia may be induced by a longer supplementation. Effects of a long-term supplementation of magnesium as MAH in different dosages thus remains to be examined.

Conclusion

During the study period, none of the horses showed any side effects caused by the supplementation of magnesium as MAH. For short-term supplementation in cases of increased magnesium requirements, these dosages seem effective and the dosages of 15 mg/kg BW and 30 mg/kg BW appear safe to use. For supplementation with 60 mg/kg magnesium as MAH, veterinary supervision and monitoring of the serum magnesium concentration is recommended.

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Animal welfare statement

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References

Altura B. M., Altura B. T. (1996) Role of magnesium in patho-physiological processes and the clinical utility of magnesium ion selective electrodes. Scand. J. Clin. Lab. Invest. Suppl. 224, 211–234

Basso L. E., Ubbink J. B., Delport R. (2000) Erythrocyte magnesium concentration as an index of magnesium status: a perspective from a magnesium supplementation study. Clin. Chim. Acta 291, 1–8; DOI 10.1016/s0009-8981(99)00168-0

Chaudhary D. P., Sharma R., Bansal D. D. (2010) Implications of magnesium deficiency in type 2 diabetes: a review. Biological trace element research 134, 119–129

Classen H. (2002) Magnesium-L-aspartate hydrochloride: experimental and clinical data. J. Clin. Basic Cardiol. 5, 43–47

Coudray C., Rambeau M., Feillet-Coudray C., Gueux E., Tressol J. C., Mazur A. Rayssiguier Y. (2005) Study of magnesium bioavailability from ten organic and inorganic Mg salts in Mg-depleted rats using a stable isotope approach. Magnes. Res. 18, 215–223

Delva P., Degan M., Trettene M., Lechi A. (2006) Insulin and glucose mediate opposite intracellular ionized magnesium variations in human lymphocytes. J. Endocrinol. 190, 711–718

Delva P., Pastori C., Montesi G., Degan M., Micciolo R., Paluani F., Lechi A. (1998) Intralymphocyte free magnesium and calcium and insulin tolerance test in a group of essential hypertensive patients. Life Sci. 63, 1405–1415

Delva P. T., Pastori C., Degan M., Montesi G. D., Lechi A. (1996) Intralymphocyte free magnesium in a group of subjects with essential hypertension. Hypertension 28, 433–439

Deuster P. A., Trostmann U., Bernier L. L., Dolev E. (1987) Indirect vs direct measurement of magnesium and zinc in erythrocytes. Clin. Chem. 33, 529–532

Elin R. J. (2010) Assessment of magnesium status for diagnosis and therapy. Magnes. Res. 23, S194–198; DOI 10.1684/mrh.2010.0213

Elin R. J., Hosseini J. M. (1985) Magnesium content of mononuclear blood cells. Clin. Chem. 31, 377–380

Elin R. J., Utter A., Tan H. K., Corash L. (1980) Effect of magnesium deficiency on erythrocyte aging in rats. Am. J. Pathol. 100, 765–778

Flatman P. (1988) The control of red cell magnesium. Magnes. Res. 1, 5–11

Henneke D. R., Potter G. D., Kreider J. L., Yeates B. F. (1983) Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15, 371–372

Hintz H. F., Schryver H. F. (1972) Magnesium metabolism in the horse. J. Anim. Sci. 35, 755–759

Hintz H. F., Schryver H. F. (1973) Magnesium, calcium and phosphorus metabolism in ponies fed varying levels of magnesium. J. Anim. Sci. 37, 927–930

Meyer H., Ahlswede L. (1977) Magnesium metabolism in the horse. Zentralbl Veterinarmed A 24, 128–139

Millart H., Durlach V., Durlach J. (1995) Red blood cell magnesium concentrations: analytical problems and significance. Magn. Res. 8, 65–76

Molina-Lopez J., Molina J. M., Chirosa L. J., Florea D., Saez L., Millan E., Planells E. (2012) Association between erythrocyte concentrations of magnesium and zinc in high-performance handball players after dietary magnesium supplementation. Magnes. Res. 25, 79–88; DOI 10.1684/mrh.2012.0311

Mooren F. C., Kruger K., Volker K., Golf S. W., Wadepuhl M., Kraus A. (2011) Oral magnesium supplementation reduces insulin resistance in non-diabetic subjects – a double-blind, placebo-controlled, randomized trial. Diabetes Obes. Metab. 13, 281–284; DOI 10.1111/j.1463-1326.2010.01332.x

Nadler J. L., Malayan S., Luong H., Shaw S., Natarajan R. D., Rude R. K. (1992) Intracellular free magnesium deficiency plays a key role in increased platelet reactivity in type II diabetes mellitus. Diabetes Care 15, 835–841

Nadler J. L., Rude R. K. (1995) Disorders of magnesium metabolism. Endocrinol. Metabol. Clinics of North America 24, 623–641

Plumb D. C. (2018) Plumb's Veterinary Drug Handbook. Stockholm, Wisconsin, Pharma Vet Inc.

National Research Council (2007) Nutrient Requirements of Horses: Sixth Revised Edition. Washington, DC, The National Academies Press.

Quamme G. A., de Rouffignac C. (2000) Epithelial magnesium transport and regulation by the kidney. Front. Biosci. 5, D694–711

Schweigel M., Martens H. (2000) Magnesium transport in the gastrointestinal tract. Front. Biosci. 5, D666–677

Stadermann B., Nehring T., Meyer H. (1992) Calcium and Magnesium absorption with roughage or mixed feed. Pferdeheilkunde 1, 77–80

Thomas J., Millot J.-M., Sebille S., Delabroise A.-M., Thomas E., Manfait M., Arnaud M. J. (2000) Free and total magnesium in lymphocytes of migraine patients – effect of magnesium-rich mineral water intake. Clinica Chimica Acta 295, 63–75; DOI 10.1016/s0009-8981(00)00186-8

Toribio R. E. (2010). Magnesium and Equine Disease. In: Equine Internal Medicine. Reed S. M., Bayly, W. M., Sellon, D. C. (eds) St. Louis Saunders 1293–1295

Vormann J. (2003) Magnesium: nutrition and metabolism. Mol. Aspects Med. 24, 27–37

White N. (2005) Intestinal diseases. Western Veterinary Conference: Proceedings

Widmer J., Feray J. C., Bovier P., Hilleret H., Raffin Y., Chollet D., Gaillard J. M., Garay R. (1995) Sodium-magnesium exchange in erythrocyte membranes from patients with affective disorders. Neuropsychobiology 32, 13–18

Winter J. C., Sponder G., Merle R., Aschenbach J. R., Gehlen H. (2018) Intracellular free magnesium concentration in healthy horses. J. Anim. Physiol. Anim. Nutr. (Berl); DOI 10.1111/jpn.12921

Witkowski M., Hubert J. Mazur A. (2011) Methods of assessment of magnesium status in humans: a systematic review. Magnes. Res. 24, 163–180; DOI 10.1684/mrh.2011.0292:.

Wlaz P., Serefko A., Szopa A. Poleszak E. (2016) The effect of an acute and 7-day administration of magnesium chloride on magnesium concentration in the serum, erythrocytes, and brain of rats. Pharmacol. Rep. 68, 289–291; DOI 10.1016/j.pharep.2015.09.006