

UC Davis

UC Davis Previously Published Works

Title

Effects of magnesium with or without boron on headshaking behavior in horses with trigeminal-mediated headshaking.

Permalink

<https://escholarship.org/uc/item/8193v15q>

Journal

Journal of veterinary internal medicine, 33(3)

ISSN

0891-6640

Authors

Sheldon, Shara A
Aleman, Monica
Costa, Lais RR
[et al.](#)

Publication Date

2019-05-01


DOI

10.1111/jvim.15499

Peer reviewed

STANDARD ARTICLE

Effects of magnesium with or without boron on headshaking behavior in horses with trigeminal-mediated headshaking

Shara A. Sheldon | Monica Aleman  | Lais R. R. Costa | Kalie Weich |
Quinn Howey | John E. Madigan

Department of Medicine and Epidemiology,
School of Veterinary Medicine, University of
California, Davis, California

Correspondence

Monica Aleman, SVM, Department of
Medicine and Epidemiology, 2108 Tupper Hall,
One Shields Avenue, University of California,
Davis, CA 95616.
Email: mraleman@ucdavis.edu

Abstract

Background: Oral administration of magnesium and boron might have a beneficial effect on headshaking behavior in horses.

Objective: Evaluate the effects of oral magnesium alone or in combination with boron on headshaking behavior in affected horses.

Animals: Twelve geldings (6 healthy controls and 6 affected).

Methods: Prospective randomized controlled dietary trial over 42 days in 12 horses (6 horses diagnosed with trigeminal-mediated headshaking and 6 unaffected healthy controls). All horses received a hay diet and were randomized into 3 treatment groups: pelleted feed combination (PF), pelleted feed combination with magnesium (M), and pelleted feed combination with magnesium-boron (MB) with a week washout of hay only between treatments. Headshaking behavior and biochemical blood variables were assessed at baseline (hay only) and then after each week of supplementation.

Results: All 3 diet interventions increased blood ionized and total magnesium. Groups M and MB further increased Mg^{2+} when compared to PF. Horses receiving treatments had a significant reduction in headshaking behavior, as measured by incidence rate ratio (IRR), when compared to unsupplemented hay diet (44% for PF, IRR, 0.558; CI, 0.44, 0.72; $P < .001$; 52% for M, IRR, 0.476; CI, 0.37, 0.62; $P < .001$; and 64% for MB, IRR, 0.358; CI, 0.27, 0.48; $P < .001$).

Conclusions and Clinical Importance: Magnesium in combination with boron had the greatest decrease in headshaking. Oral supplementation with magnesium or magnesium in combination with boron should be considered in horses affected with headshaking.

KEYWORDS

equine, headshakers, magnesium, trigeminal

Abbreviations: AG, anion gap; H, hay diet; IRR, incidence rate ratio; M, magnesium supplement; MB, magnesium-boron supplement; PF, pelleted feed combination; SBE, standard base excess; SID, strong ion difference; tMg, total magnesium.

[Correction added on 10 May 2019, after first online publication: in the first paragraph of section 2.2, the phrase that had read, "as above plus 2 mg/kg BW of boron citrate (Boron Citrate, Platinum Performance, Buellton, California)," now reads, "as above plus 2 mg/kg BW of elemental boron (Boron Citrate = 40 mg/kg, Platinum Performance, Buellton, California)"]

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

1 | INTRODUCTION

Trigeminal-mediated headshaking, formerly known as idiopathic headshaking, is caused by a low threshold of firing of the trigeminal nerve.¹⁻³ Affected horses demonstrate abnormal behavior with headshaking such as sudden jerking of the head with a downward then upward motion, rubbing the nose on their thoracic limbs or objects, lip movements, and excessive snorting, noted mostly during exercise.^{1,4} The clinical signs of headshaking are suspected to result from neuropathic pain, considering the low threshold for firing of the trigeminal nerve in affected horses.² The horse's behavior suggests neuropathic pain that is manifested as an itching, burning, tingling, or electric-like sensation.^{4,5} In most cases, clinical manifestation is seasonal with worsening of signs during spring and summer months, and geldings are overrepresented.^{4,6-9} Various treatments have been attempted to alleviate the signs including physical devices such as face masks with ultraviolet light protection, nose nets, nutritional supplements, drug treatment, including administration of antidepressants, channel blockers, antihistamines or corticosteroids, neuromodulation, and caudal compression of the infraorbital nerve, among others.^{1,9-18} However, the results have been variable. Unsuccessful management of this condition is associated with uncontrollable clinical signs often leading to poor performance, wastage, and, in severe cases, euthanasia, representing an equine welfare issue.

An owner-based survey indicated that magnesium supplementation is perceived to decrease headshaking behavior in 40% of horses affected with trigeminal-mediated headshaking.^{1,19} Owner-based surveys have limitations such as the interpretation of signs and response to treatment, familiarity with the disorder, confusion with other disorders that might cause headshaking, and placebo effects. Some horses affected with trigeminal-mediated headshaking have ionized magnesium concentrations below the reference range (mean of 0.44 ± 0.05 ; reference range 0.47-0.70).²⁰ Administration of intravenous magnesium sulfate infusion given to horses affected with trigeminal-mediated headshaking significantly decreased headshaking behavior by 29%.²¹

Boron increases blood ionized magnesium concentrations.²² Therefore, this study sought to evaluate the effects of dietary supplementation of magnesium and boron on headshaking behavior in horses. We hypothesized that magnesium supplementation with or without boron would decrease headshaking behavior in horses affected with trigeminal-mediated headshaking. We also hypothesized that boron would enhance absorption of magnesium, further increasing blood concentrations of ionized magnesium and further decrease headshaking behavior.

2 | MATERIALS AND METHODS

2.1 | Animals

Twelve male castrated horses, Quarter Horse breeds ($n = 8$) and Thoroughbred ($n = 4$), aged 7 to 16 years, weighing between 465 and 636 kg were enrolled in the study. Six horses were diagnosed with

trigeminal-mediated headshaking and 6 healthy horses matched for age and breed were selected as controls. The inclusion criteria of the affected horses consisted of fulfillment of the diagnosis of trigeminal-mediated headshaking by exclusion of other major causes of headshaking. Furthermore, these horses had been displaying headshaking in a seasonal fashion in previous years and appearing normal in between seasons. The lack of response to the administration of corticosteroids and antihistamines before donation did not support an allergic component to headshaking behavior. All horses had previously received all recommended vaccinations and deworming per University of California Davis, Center for Equine Health protocols. Before entering the study, all horses underwent a thorough physical and neurological examination performed by a board-certified large animal internist and neurologist followed by a detailed diagnostic investigation. Diagnostic investigation included oral, ophthalmic and otoscopic examinations, CBC, serum biochemical profile, skull radiographs, and endoscopy of the upper airway including guttural pouches. None of the horses had apparent cervical or nuchal pain based on palpation, extension, and flexion of the neck that could have contributed to headshaking behavior. Computed tomography of the head to further rule out other causes was not performed. Although not part of this study, these horses were euthanized based on humane grounds at a later time and postmortem macroscopic and microscopic evaluation ruled out other possible causes of headshaking. All affected horses included in this study were displaying headshaking behavior at the time of the experiments. Horses were housed in covered box stalls bedded with wood shavings, having free access to fresh water (automatic waterer), and dietary management as listed below. This study was reviewed and approved by an animal care and use protocol from our institution.

2.2 | Experimental design

The study was a randomized, controlled crossover experimental design, where each horse served as its own control. The study was performed during the spring in April and May. All horses throughout the study received hay from the same batch (analyzed from Dairy One, Ithaca, New York), alfalfa hay in the morning and grass hay in the evening. Hay was weighed out based on body weight from baseline measures so that horses received 1.5% of their body weight (BW) in hay (H) per day. All horses underwent a control period of hay only for 1 week at the beginning of the study before being randomized to treatments. Supplements were weighed and provided to each horse based on their individual BW as listed below. The horses were randomized to 3 dietary treatment groups: (1) pelleted feed combination (PF) which served as control and consisted of 255 g of Purina pelleted feed (Purina Equine Senior, Largo, Florida), $\frac{1}{4}$ cup of canola oil, and 2 tablespoons applesauce; (2) magnesium supplement (M) which consisted of the same pelleted feed, oil and applesauce combination as above plus 24.2 mg/kg BW of magnesium citrate (Magnesium Citrate, Platinum Performance, Buellton, California); and (3) magnesium and boron supplement (MB) which consisted of the same pelleted feed, oil, and applesauce combination and magnesium citrate

(Magnesium Citrate, Platinum Performance, Buellton, California); as above plus 2 mg/kg BW of elemental boron (Boron Citrate = 40 mg/kg, Platinum Performance, Buellton, California). There was a 1-week wash-out period in between treatments where horses received no supplements. The horses did not receive treatment in the same order because treatment order was randomized. Additionally, free choice water was provided with automatic waterers and tested for magnesium and boron levels to get a baseline value. The study period ran from end of April and through the end of May during peak headshaking season for this group of horses.

2.3 | Sample collection

Blood samples (10 mL in evacuated tube containing heparin as anticoagulant and 1.5 mL in heparinized syringes) were collected by venipuncture from the right jugular vein before initiation of dietary supplementation (baseline) and after completion of a week of dietary supplementation. All samples were collected before exercise and samples were placed on ice immediately and analyzed within 2 hours.

2.4 | Blood analysis

Venous blood pH, standard base excess (SBE), HCO_3^- , Na^+ , Cl^- , K^+ , Ca^{2+} , glucose, and lactate concentrations in heparinized blood samples were determined using an ABL815 FLEX (Radiometer America, Inc., Brea, California). Total magnesium (tMg) and ionized magnesium (Mg^{2+}) in heparinized blood samples were determined using NOVA 8 (NOVA Biomedical, Waltham, Massachusetts). The strong ion difference (SID) was calculated using the Stewart equation as follows: $[(\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}) - (\text{Cl}^-)]$.²³ Anion gap (AG) was calculated using the equation as follows: $[(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)]$.²³

2.5 | Behavioral analysis

Horses were placed in an individual round pen, free from any tack or halters, and evaluated by 3 independent experienced evaluators for headshaking behavior while at a walk (1 minute), trot (1-3 minutes), canter (1 minute), and walk again (1 minute) at baseline and then at the end of each week of treatment. Exercise periods were videotaped. Trot time varied because of concurrent lameness caused by navicular disease in 2 horses (1-minute trot). This lameness was ruled out as the cause of headshaking through perineural local anesthesia of the palmar digital nerve. After the exercise period, horses were returned immediately to their adjacent stalls. Evaluators were unaware of which treatment each horse received at any particular day. The headshaking behavior (including headshakes/minute, head-tossing/minute, nose rubbing/minute, dropped head/minute, and snorting/minute) was recorded for each horse during each level of exercise by the 3 evaluators. A median value of headshaking behavior per minute from the 3 evaluators for each horse during each level of exercise was obtained. Agreement between evaluators was determined as described under statistical analysis.

2.6 | Statistical analysis

Data were analyzed using software Stata Statistical Software, Release 14 (StataCorp LP 2015, College Station, Texas). A multilevel mixed-effects Poisson regression model examined the main and interactive fixed effects of treatment groups (H, PF, M, or MB), gait (walk, trot, canter, or walk), and breed (Quarter Horse breeds or Thoroughbreds), with individual horses as the random effect on headshaking behavior. Each gait (first walk, trot, canter, and second walk) was evaluated separately. Each treatment was examined as the baseline value for further effects. Results are presented as incidence rate ratios (IRRs), *P* values, and 95% confidence intervals (CI). The variance among scorer (the 3 trained evaluators) was evaluated statistically, using a Spearman's rank correlation coefficient.

A multilevel mixed effects linear analysis of variance regression model was also used to look at the main and interactive effects of treatment groups (H, PF, M, or MB), status (control or headshaker) with individual horse as random effect, on blood levels (pH, K^+ , Na^+ , Cl^- , SBE, HCO_3^- , Ca^{2+} , glucose, lactate, tMg, and Mg^{2+}), and calculated values of SID and AG.

3 | RESULTS

3.1 | Blood results

3.1.1 | Statistical modeling

There was a significant increase in Mg^{2+} and tMg with all dietary treatments (PF, M, and MB) when compared to baseline diet H for all 12 horses (Figures 1 and 2, $P < .001$). There was an interaction between treatment MB and affected horses that caused a positive effect on tMg ($P = .02$) and on Mg^{2+} ($P = .043$). There was a significant

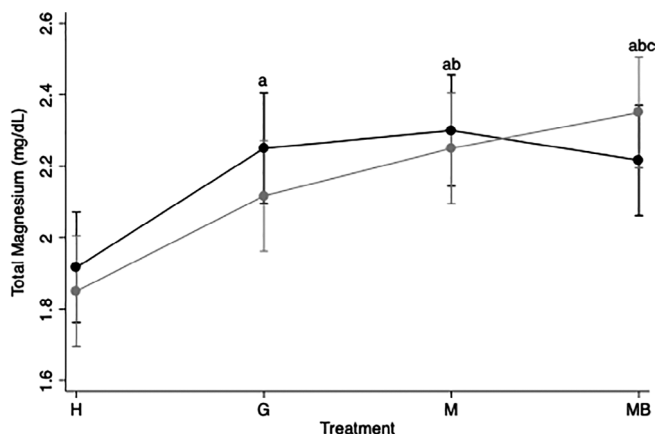


FIGURE 1 Overall effect of treatment on venous tMg. The mean and SEM venous blood tMg for all 6 control and 6 affected horses. Black = control horses, gray = horses affected with headshaking. Treatment H (hay only), treatment PF (pelleted feed combination), treatment M (hay plus magnesium supplement), treatment MB (PF plus magnesium and boron supplements). ^aSignificant differences than treatment H for all horses. ^bSignificant differences than treatment PF for all horses. ^cSignificant differences than treatment M for all horses. $P < .05$ was considered significant

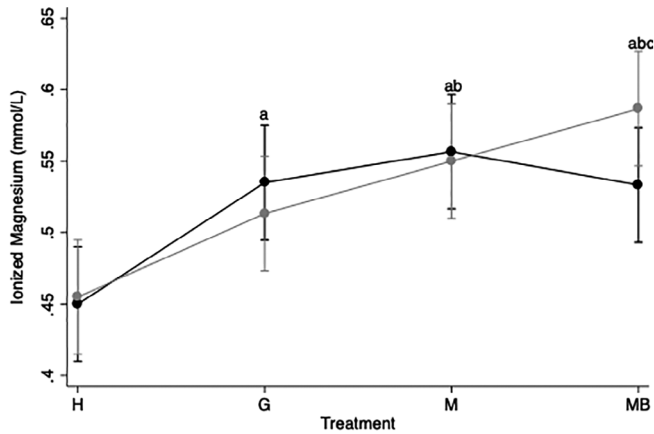


FIGURE 2 Overall effect of treatment on the blood ionized magnesium (Mg^{2+}). The mean and SEM venous blood Mg^{2+} for all 6 control and 6 affected horses. Black = control horses, gray = horses affected with headshaking. Treatment H (hay only), treatment PF (pelleted feed combination), treatment M (PF plus magnesium supplement), treatment MB (PF plus magnesium and boron supplements). ^aSignificant differences than treatment H for all horses. ^bSignificant differences than treatment PF for all horses. ^cSignificant differences than treatment M for all horses. $P < .05$ was considered significant

difference on tMg ($P < .001$) between PF, M, and MB treatments compared to H treatment. There was a significant difference on tMg ($P < .01$) between M and MB treatments compared to PF treatment. There was a significant difference on tMg ($P = .012$) between MB treatment compared to M treatment. There was a significant difference in Mg^{2+} ($P < .001$) between PF, M, and MB treatments compared to H treatment. There was a significant difference in Mg^{2+} ($P < .01$) between M and MB treatments compared to PF treatment. There was a significant difference in Mg^{2+} ($P < .001$) between MB treatment compared to M treatment. Blood variables including pH, SBE, HCO_3^- , Na^+ , Cl^- , K^+ , lactate, SID, AG, and Ca^{2+} had significant changes with all treatments (PF, M, and MB) compared to H treatment ($P < .001$). There was a significant increase in Ca^{2+} with all treatments (PF, M, and MB) when compared to baseline diet H (Figure 3, $P < .001$).

3.1.2 | Effect of baseline hay diet on blood variables

At baseline (H), all blood variables were within reference range except for SBE that were uniformly above reference range (mean, 6.9 ± 0.8 mmol/L for controls; mean, 6.9 ± 1.3 mmol/L for headshakers; Table 1), Mg^{2+} (mean, 0.45 ± 0.03 mmol/L for controls; mean, 0.46 ± 0.03 mmol/L for headshakers; Table 1), and SID (mean, 47.6 ± 1.7 mmol/L for controls; mean, 47.6 ± 2.5 mmol/L for headshakers; Table 2).

3.1.3 | Effect of PF on blood variables

Treatment PF caused a decrease in SBE from baseline (H) but remained above the reference range (mean, 4.3 ± 1.2 mmol/L for controls; mean, 4.1 ± 0.9 mmol/L for headshakers; Table 1). There was a

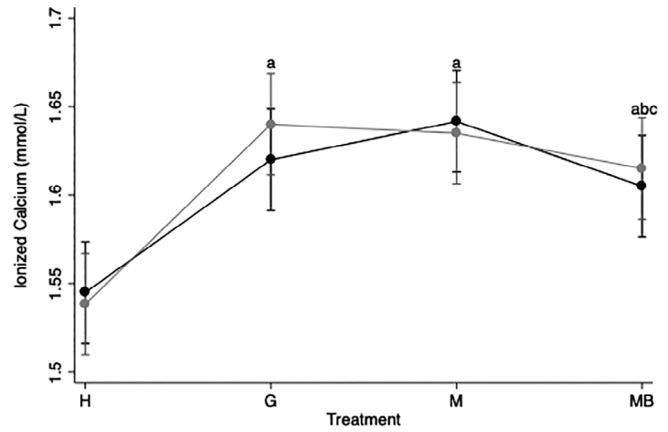


FIGURE 3 Overall effect of treatment on the blood ionized calcium (Ca^{2+}). The mean and SEM venous blood Ca^{2+} for all 6 control and 6 affected horses. Black = control horses, gray = horses affected with headshaking. Treatment H (hay only), treatment PF (pelleted feed combination), treatment M (PF plus magnesium supplement), treatment MB (PF plus magnesium and boron supplements). ^aSignificant differences than treatment H for all horses. ^bSignificant differences than treatment PF for all horses. ^cSignificant differences than treatment M for all horses. $P < .05$ was considered significant

decrease in Na^+ in headshaker horses only (mean, 134 ± 1.1 mmol/L; Table 1). There was an increase in Cl^- to above reference range (mean, 103 ± 1.6 mmol/L for controls; mean, 103 ± 1.0 mmol/L for headshakers; Table 1). There was a decrease in AG to below reference range for all horses (mean, 7.5 ± 1.0 mmol/L for controls; mean, 6.9 ± 0.6 mmol/L for headshakers; Table 2). There was an increase in Ca^{2+} above reference range (mean, 1.62 ± 0.05 for controls; mean, 1.64 ± 0.06 for headshakers; Table 1).

3.1.4 | Effect of magnesium supplement on blood variables

Treatment M caused a decrease in SBE from baseline (H) but was still above reference range (mean, 3.8 ± 1.7 mmol/L for controls; mean, 5.4 ± 1.9 mmol/L for headshakers; Table 1). There was a decrease in Na^+ in headshaker horses only (mean, 134 ± 1.0 mmol/L; Table 1). There was an increase in Cl^- to above reference range in control horses only (mean, 103 ± 1.5 mmol/L for controls; Table 1). There was a decrease in AG to below reference range for all horses (mean, 7.7 ± 0.6 mmol/L for controls; mean, 6.2 ± 1.1 mmol/L for headshakers; Table 2). There was an increase in Ca^{2+} above reference range (mean, 1.64 ± 0.03 for controls; mean, 1.64 ± 0.07 for headshakers; Table 1).

3.1.5 | Effect of magnesium and boron supplement on blood variables

Treatment MB caused a decrease in SBE from baseline (H) but was still above reference range (mean, 4.8 ± 1.2 mmol/L for controls; mean, 4.2 ± 0.3 mmol/L for headshakers; Table 1). There was a

TABLE 1 Changes in blood variables after diet treatments H, PF, M, and MB

Blood variables	Treatments							
	H		PF		M		MB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH (ref 7.35-7.45)								
Control	7.402	±0.009	7.389	±0.012	7.385	±0.019	7.396	±0.019
Headshaker	7.403	±0.009	7.383	±0.017	7.393	±0.017	7.387	±0.017
SBE mmol/L (ref -3.0 to +3.0)								
Control	6.9	±0.8	4.3	±1.2	3.8	±1.7	4.8	±1.2
Headshaker	6.9	±1.3	4.1	±0.9	5.4	±1.9	4.2	±1.7
HCO ₃ ⁻ mmol/L (ref 25-32)								
Control	31.7	±0.9	28.9	±1.2	28.6	±1.6	29.5	±1.0
Headshaker	31.7	±1.3	28.9	±0.9	30.1	±1.8	28.9	±1.7
Na ⁺ mmol/L (ref 135-145)								
Control	139	±0.5	136	±1.4	135	±1.0	136	±1.6
Headshaker	137	±1.0	134	±1.1	134	±1.0	134	±0.8
Cl ⁻ mmol/L (ref 94-102)								
Control	97	±2.3	103	±1.6	103	±1.5	103	±1.9
Headshaker	96	±2.6	103	±1.0	102	±2.3	103	±2.7
K ⁺ mmol/L (ref 3.3-5.0)								
Control	3.8	±0.2	4.1	±0.4	4.3	±0.3	4.1	±0.2
Headshaker	4.0	±0.4	4.5	±0.4	4.4	±0.5	4.4	±0.6
Glucose mg/dL (ref 77-110)								
Control	94	±2	96	±7	100	±4	92	±4
Headshaker	90	±5	90	±9	91	±5	94	±8
Lactate mmol/L (ref <2)								
Control	0.5	±0.1	0.8	±0.1	0.8	±0.1	0.7	±0.1
Headshaker	0.6	±0.1	0.8	±0.1	0.8	±0.1	0.7	±0.1
Ca ²⁺ mmol/L (ref 1.40-1.60)								
Control	1.55	±0.04	1.62	±0.05	1.64	±0.03	1.61	±0.05
Headshaker	1.54	±0.04	1.64	±0.06	1.64	±0.07	1.62	±0.07
tMg mg/dL (ref 1.9-3.0)								
Control	1.9	±0.0	2.3	±0.1	2.3	±0.1	2.2	±0.1
Headshaker	1.9	±0.1	2.1	±0.1	2.3	±0.1	2.4	±0.1
Mg ²⁺ mmol/L (ref 0.47-0.70)								
Control	0.45	±0.03	0.54	±0.04	0.56	±0.03	0.53	±0.05
Headshaker	0.46	±0.03	0.51	±0.07	0.55	±0.09	0.59	±0.09

Abbreviations: H, hay only, no supplement; M, pelleted feed combination, hay, and magnesium supplementation; MB, pelleted feed combination, hay, and magnesium-boron supplementation; PF, pelleted feed combination and hay only.

Numbers in bold for the variables pH, HCO₃⁻, Na⁺, Cl⁻, K⁺, glucose, Ca²⁺, tMg, and Mg²⁺ indicate values above or below the reference range.

Note. For SBE, the baseline values for all horses were above the reference range. For this variable, bold indicates difference from baseline values.

decrease in Na⁺ for headshaker horses only (mean, 134 ± 0.8 mmol/L; Table 1). There was an increase in Cl⁻ to above reference range (mean, 103 ± 1.9 mmol/L for controls; mean, 103 ± 2.7 mmol/L for headshakers; Table 1). There was a decrease in SID for headshaker horses only (mean, 37.3 ± 2.3 mmol/L; Table 2). There was a decrease in AG to below reference range for all horses (mean, 7.5 ± 0.8 mmol/L for controls; mean, 6.2 ± 1.3 mmol/L for headshakers; Table 2). There was an increase in

Ca²⁺ to above reference range (mean, 1.61 ± 0.05 mmol/L for controls; mean, 1.62 ± 0.07 mmol/L for headshakers; Table 1).

3.2 | Behavior results

Control horses did not show signs of headshaking behavior and were not included in the data that follows. In addition to headshaking, other

TABLE 2 Calculated SID and AG. Calculated changes in blood variables after diet treatments H, PF, M, and MB

Blood variables	Treatments							
	H		PF		M		MB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SID mmol/L (ref 38-42)								
Control	47.6	±1.7	38.6	±1.9	38.5	±1.9	39.0	±0.8
Headshaker	47.6	±2.5	37.9	±1.3	38.5	±2.7	37.3	±2.3
AG mmol/L (ref 9-17)								
Control	13.9	±1.1	7.5	±1.0	7.7	±0.6	7.5	±0.8
Headshaker	13.9	±1.3	6.9	±0.6	6.2	±1.1	6.2	±1.3

Abbreviations: H, hay only, no supplement; M, pelleted feed combination, hay, and magnesium supplementation; MB, pelleted feed combination, hay, and magnesium-boron supplementation; PF, pelleted feed combination and hay only.

Numbers in bold indicated values above or below the reference range.

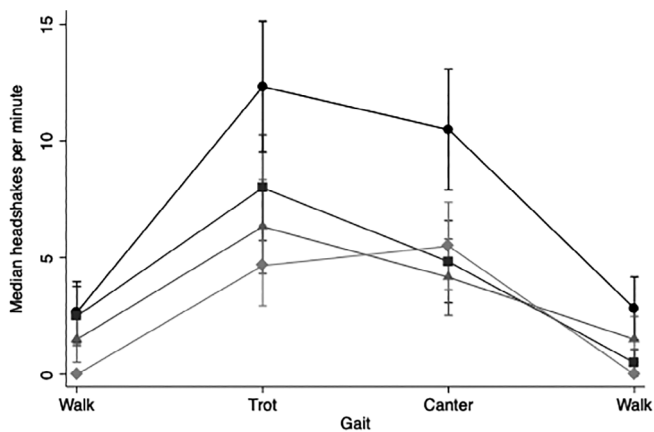


FIGURE 4 Overall effect of treatment on the headshakes per minute at different gaits. The mean and SEM median headshakes per minute for all 6 affected horses. Black circle = treatment H (hay only), dark gray square = treatment PF (pelleted feed combination), gray triangle = treatment M (PF plus magnesium supplement), light gray diamond = treatment MB (PF plus magnesium and boron supplements)

signs displayed by the affected horses in a seasonal manner included head-tossing, nose rubbing, dropped head, and snorting. However, these signs were inconsistent and varied among horses. In order to avoid more variability for the interpretation and quantification of signs, other signs were excluded from further analysis. For the statistical analysis, we focused on the headshakes/minute as this was the most important and consistent behavior of affected horses. Individual headshaking frequency after 1 week of baseline diet H was used for comparison with headshaking frequency after each week of dietary supplementation. Horses receiving treatments PF, M, and MB had a significant reduction in headshaking behavior when compared to unsupplemented hay diet H (Figure 4; $P < .001$). Treatment MB is significantly different than PF (Figure 4; $P = .007$). Treatment M was not significantly different than treatment MB.

A mixed-effects Poisson regression model evaluated the effects of treatment while controlling for breed and gait on median headshakes/minute. There was an effect of PF treatment controlling for

all breeds and gaits (walk, trot, canter, walk) with 0.56 times the rate of headshakes per minute when compared to H, which results in a 44% reduction in median headshakes/minute (IRR, 0.558; CI, 0.44, 0.72; $P < .001$; Table 3). There was an effect of M treatment controlling for all breeds and gaits with 0.48 times the rate of headshaking when compared to H, resulting in a 52% reduction in median headshakes/minute (IRR, 0.476; CI, 0.37, 0.62; $P < .001$; Table 3). There was an effect of MB treatment, controlling for all breeds and gaits, with 0.36 times the rate of headshaking compared to H, resulting in a 64% reduction in median headshakes/minute (IRR, 0.358; CI, 0.27, 0.48; $P < .001$; Table 3). Effect of gait (trot and canter) and diet treatment (PF, M, and MB) on IRR of median headshakes/minute are shown in Table 4. There was an effect of gait when controlling for breed and treatment with the trot and canter compared to the first walk period with trot having almost 4 times increase in headshaking (IRR, 3.750; CI, 2.65, 5.31; $P < .001$) and canter having almost 5 times increase in headshaking (IRR, 4.700; CI, 3.34, 6.61; $P < .001$). For specific information about the number of headshakes per minute per affected horse at the trot and canter, refer to Table S1.

3.2.1 | Treatment PF as control

The model was run with PF treatment as the control value. There was an effect of H treatment with 1.78 times the risk of headshaking as compared to PF treatment, resulting in a 79% increase in median headshakes/minute (IRR, 1.789; CI, 1.39, 2.30; $P < .001$; Table 3). There was an effect of MB treatment with 0.64 times the risk of headshaking compared to PF treatment, resulting in a 36% decrease in median headshakes/minute (IRR, 0.642; CI, 0.47, 0.88; $P = .007$; Table 3). There were no significant differences between M treatment compared to PF treatment.

3.2.2 | Treatment M as control

The model was run with M treatment as the control value. There was an effect of H treatment with 2.09 times the risk of headshaking compared to M treatment, resulting in a 2-fold increase in median

TABLE 3 Effect of diet treatments (H, PF, M, and MB) on incidence rate ratio (IRR) of median headshakes/minute across all breeds and controlled for headshaking horses only

Treatment	Comparisons							
	Compared to H		Compared to PF		Compared to M		Compared to MB	
	IRR (95% CI)	P value	IRR (95% CI)	P value	IRR (95% CI)	P value	IRR (95% CI)	P value
H	-	-	1.789 (1.39, 2.30)	<.001	2.098 (1.61, 2.73)	<.001	2.787 (2.08, 3.73)	<.001
PF	0.558 (0.44, 0.72)	<.001	-	-	1.17 (0.87, 1.58)	NS	1.557 (1.13, 2.15)	.007
M	0.476 (0.37, 0.62)	<.001	0.853 (0.63, 1.15)	NS	-	-	1.328 (0.95, 1.85)	NS
MB	0.358 (0.27, 0.48)	<.001	0.642 (0.47, 0.89)	.007	0.753 (0.54, 1.05)	NS	-	-

Abbreviations: CI, confidence intervals; NS, not significant.

$P < .05$ was considered significant.

TABLE 4 Effect of gait (trot and canter) and diet treatment (PF, M, and MB) on incidence rate ratio (IRR) of median headshakes/minute across all breeds and restricted to headshaking horses only

Treatment	IRR (95% CI)	P value
PF		
Trot	0.658 (0.45, 0.93)	.020
Canter	0.460 (0.30, 0.71)	.001
M		
Trot	0.514 (0.35, 0.76)	.001
Canter	0.396 (0.25, 0.63)	<.001
MB		
Trot	0.378 (0.25, 0.58)	<.001
Canter	0.524 (0.34, 0.80)	.003

Abbreviations: CI, confidence interval; M, pelleted feed combination, hay, and magnesium supplementation; MB, pelleted feed combination, hay, and magnesium-boron supplementation; PF, pelleted feed combination and hay only.

$P < .05$ was considered significant.

headshakes/minute (IRR, 2.098; CI, 1.61, 2.73; $P < .001$; Table 3). There were no significant differences between PF and MB treatments compared to M treatment.

3.2.3 | Treatment MB as control

The model was run with MB treatment as the control value. There was an effect of H treatment with 2.79 times the risk of headshaking compared to MB treatment, resulting in an almost 3-fold increase in median headshakes/minute (IRR, 2.786; CI, 2.08, 3.73; $P < .001$; Table 3). There was an effect of PF treatment with 1.56 times the risk of headshaking compared to MB treatment, resulting in a 56% increase in median headshakes/minute (IRR, 1.557; CI, 1.13, 2.15; $P = .007$; Table 3). There was not a significant difference between M treatment and MB treatment.

3.3 | Agreement between evaluators

Spearman's correlation was run to assess interobserver variability of 3 evaluators. There was strong agreement between observer 1 and

2 ($r_s = 0.95$; $P < .0001$), observer 1 and 3 ($r_s = 0.93$; $P < .0001$), and observer 2 and 3 ($r_s = 0.94$; $P < .0001$).

4 | DISCUSSION

This study revealed a reduction in headshaking behavior with diets providing magnesium supplementation. Horses receiving diet with PF, M, and MB had significant reductions in headshaking behavior. Despite the fact that there was great variability in the severity of headshaking behavior between horses and variability of the same horses on different days, the IRR analysis takes into account these variables and provides a way to look at the effect of supplementation in headshaking behavior. Combined magnesium-boron supplementation induced the highest IRR reduction (64%) in headshaking behavior when compared to hay diet only. Similarly, magnesium-boron supplementation had the greatest IRR reduction (36%) in headshaking behavior when compared to a diet containing pelleted feed. The most severely affected horses had the most benefit to supplementation providing magnesium (data not shown). As previously shown, as exercise intensity increased headshaking behavior also increased.²¹

Diets of PF, M, and MB led to an increase in tMg and Mg^{2+} in all horses. Affected horses had a further increase in tMg and Mg^{2+} when magnesium-boron supplementation was provided. The increased response to boron should be investigated further. In absence of magnesium supplementation, as per baseline, values for Mg^{2+} for all horses in this study (affected and control) were below reference range. It is unclear whether supplementation would have similar effects if horses had ionized magnesium within the normal reference range and further investigation is warranted. Supplementation with all treatments (PF, M, and MB) led to increases in blood levels of Ca^{2+} from baseline (H) despite the fact that the additional amount of calcium was small (approximately 2.55 g of total calcium). This is in contrast with a previous study where Ca^{2+} was transiently decreased after administration of intravenous magnesium.²¹ The effects of Ca^{2+} increase on headshaking behavior are not fully understood but possibly alter the nerve resting membrane potential. Increased Ca^{2+} along with a decreased Mg^{2+} likely bring the nerve resting membrane potential closer to threshold.^{3,24} Furthermore, Mg^{2+} is also involved in the regulation of neuroexcitation and can inhibit Ca^{2+} -dependent presynaptic

excitation-secretion coupling.²⁵ Magnesium also acts on the neuromuscular blockade by inhibiting calcium channels.²⁶

The requirement for magnesium in horses is 13 mg/kg BW and for an average 500 kg horse, the requirement would be 6.5 g of magnesium a day.²⁷ Water would provide 510 mg of magnesium for a horse drinking an average of 7.5 gal per day (standard range is 5-10 gal per day).²⁸ Hay provided an average of 10 g of magnesium and with 45% average bioavailability (ie, approximately 4.5 g of magnesium). The baseline diet was calculated to provide 10 mg/kg magnesium for our group of horses. The pelleted feed selected for this study provided 842 mg of magnesium based on the 255 g serving size per horse. This pelleted feed was selected for palatability and its ability to help mask the flavors of the added supplements and more specifically the boron supplement which had a bitter taste. The addition of this specific pelleted feed in combination with applesauce and canola oil was not expected to greatly increase Mg²⁺ nor affect headshaking behavior; however, it did in this group of horses. Factors such as the canola oil and applesauce might have provided conditions to improve magnesium absorption. These effects were not addressed in this study, but further studies in a larger population of diseased horses could show insight into this intriguing finding. Also, it is unknown if this finding (increase magnesium) would be observed with other types of concentrates or pelleted feed.

The mechanism by which magnesium supplementation in the form of magnesium alone or magnesium in combination with boron resulted in significant reduction in headshaking behavior is not completely understood. Treatment with magnesium in combination with boron induced the greatest reduction (64% decrease in headshakes/minute) in headshaking behavior. This could be because of boron's ability to increase magnesium concentrations in the blood.²² Magnesium can then provide its physiological channel blocking effects on N-methyl-D-aspartate (NMDA) receptors and reduce ion currents.²⁹⁻³² The NMDA receptors affect trigeminal neurons at the trigeminal *subnucleus caudalis* (the orofacial nociceptive processing center) in rats.³³ Boron can also affect neurotransmitters because of its ability to form complexes with sugars.³⁴ When boric acid forms ester complexes with ribose, which is a part of adenosine, boric acid will noncompetitively inhibit adenosine diphosphate ribosyl cyclase (ADP-ribosyl cyclase).³⁵ This slows the production of cyclic ADP-ribose (cADPR) which in turn slows down mobilization of Ca²⁺. Furthermore, calcium might be involved in trigeminal neuropathic pain, but the exact mechanism is unknown.³⁶

The main limitations of the current study included the small number of horses and short-term duration of supplementation. A longer duration of magnesium supplementation would have been useful to investigate the long-term effects in headshaking behavior. The data analyzed in this study included exclusively 1 clinical sign, headshaking. Therefore, the effects of magnesium supplementation in other signs remain unknown. Various types of pelleted diets or concentrates were not evaluated in this study to investigate if the effects on headshaking would have been similar to those found with the PF used here. Our investigation included a short period of exercise and not sustained exercise similar to what a rider would be performing. Seasonality has a potential effect on severity of headshaking however

this study was performed within 6 weeks at the peak of headshaking behavior in our group of horses as an attempt to minimize the effect that seasonality could have played. Nerve conduction velocity of the trigeminal complex before and after dietary supplementation would have been a more objective manner to assess the effects of magnesium supplementation on the threshold for nerve firing. However, this required general anesthesia and was not performed.

Although the overall decrease in headshaking behavior in this group of 6 horses might not reflect the effects on the headshaking population at large, this is a promising therapeutic option that should be investigated in a broader population of horses affected with trigeminal-mediated headshaking. Although magnesium supplementation did not completely alleviate all headshaking behavior, a reduction might improve the horse's quality of life and performance, make a horse rideable or manageable, and avoid euthanasia.

In conclusion, magnesium supplementation (PF, magnesium alone, and magnesium in combination with boron) had effects on reducing headshaking behavior in this small group of horses under the conditions of the study. Magnesium in combination with boron appeared to transiently reduce the severity of headshaking to a greater extent. Magnesium supplementation could be considered, especially if ionized magnesium is low as was found in the horses with headshaking in this study. Supplementation with magnesium could be considered as an adjunct to other treatment attempts for the management of trigeminal-mediated headshaking.

ACKNOWLEDGMENTS

The authors thank Drs. Phil Kass for assistance with statistical analysis.

CONFLICTS OF INTEREST DECLARATION

Platinum Performance provided oral magnesium and boron supplements and partial support for a graduate student.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

University of California Davis approved Animal Care and Use Protocol #19194.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Monica Aleman  <https://orcid.org/0000-0001-5811-9520>

REFERENCES

1. Pickles K, Madigan J, Aleman M. Idiopathic headshaking: is it still idiopathic? *Vet J*. 2014;201:21-30.
2. Aleman M, Rhodes D, Williams DC, Guedes A, Madigan JE. Sensory evoked potentials of the trigeminal nerve for the diagnosis of idiopathic headshaking in a horse. *J Vet Intern Med*. 2014;28:250-253.
3. Aleman M, Williams DC, Brosnan RJ, et al. Sensory nerve conduction and somatosensory evoked potentials of the trigeminal nerve in horses with idiopathic headshaking. *J Vet Intern Med*. 2013;27:1571-1580.
4. Madigan JE, Bell SA. Characterisation of headshaking syndrome—31 cases. *Equine Vet J Suppl*. 1998;30:28-29.
5. Colloca L, Ludman T, Bouhassira D, et al. Neuropathic pain. *Nat Rev Dis Primers*. 2017;3:17002.
6. Bell AJ. Headshaking in a 10-year-old thoroughbred mare. *Can Vet J*. 2004;45:153-155.
7. Madigan JE, Kortz G, Murphy C, et al. Photic headshaking in the horse: 7 cases. *Equine Vet J*. 1995;27:306-311.
8. Madigan JE, Bell SA. Owner survey of headshaking in horses. *J Am Vet Med Assoc*. 2001;219:334-337.
9. Mills DS, Cook S, Jones B. Reported response to treatment among 245 cases of equine headshaking. *Vet Rec*. 2002;150:311-313.
10. Newton SA, Knottenbelt DC, Eldridge PR. Headshaking in horses: possible aetiopathogenesis suggested by the results of diagnostic tests and several treatment regimes used in 20 cases. *Equine Vet J*. 2000;32:208-216.
11. Pickles KJ, Berger J, Davies R, Roser J, Madigan JE. Use of a gonadotrophin-releasing hormone vaccine in headshaking horses. *Vet Rec*. 2011;168:19.
12. Roberts VLH, Perkins JD, SkÄrllina E, et al. Caudal anaesthesia of the infraorbital nerve for diagnosis of idiopathic headshaking and caudal compression of the infraorbital nerve for its treatment, in 58 horses. *Equine Vet J*. 2013;45:107-110.
13. Talbot WA, Pinchbeck GL, Knottenbelt DC, Graham H, Mckane SA. A randomised, blinded, crossover study to assess the efficacy of a feed supplement in alleviating the clinical signs of headshaking in 32 horses. *Equine Vet J*. 2013;45:293-297.
14. Bell C, Hnenny L, Torske K. Internal neurolysis of the maxillary branch of the trigeminal nerve for the treatment of equine trigeminal mediated headshaking syndrome. *Can Vet J*. 2018;59:763-769.
15. Tomlinson JE, Neff P, Boston RC, Aceto H, Nolen-Walston RD. Treatment of idiopathic headshaking in horses with pulsed high-dose dexamethasone. *J Vet Intern Med*. 2013;27:1551-1554.
16. Roberts VL, Patel NK, Tremaine WH. Neuromodulation using percutaneous electrical nerve stimulation for the management of trigeminal-mediated headshaking: a safe procedure resulting in medium-term remission in five of seven horses. *Equine Vet J*. 2016;48:201-204.
17. Roberts VL, McKane SA, Williams A, et al. Caudal compression of the infraorbital nerve: a novel surgical technique for treatment of idiopathic headshaking and assessment of its efficacy in 24 horses. *Equine Vet J*. 2009;41:165-170.
18. Mair TS. Assessment of bilateral infra-orbital nerve blockade and bilateral infra-orbital neurectomy in the investigation and treatment of idiopathic headshaking. *Equine Vet J*. 1999;31:262-264.
19. Pickles K, Aleman M, Adams VJ, et al. Owner-reported response to treatment of 130 headshaking horses. *Proceed Am Assoc Equine Pract*. 2014;60:176-183.
20. Sheldon S, Aleman M, Costa L, Santoyo A, Howey Q, Madigan J. Alterations in metabolic status and headshaking behavior following intravenous administration of hypertonic solutions in horses with trigeminal-mediated headshaking. *Animals*. 2018;8:102.
21. Sheldon S, Aleman M, Costa L, et al. Magnesium sulfate intravenous infusion and its effect on horses with trigeminal-mediated headshaking. *J Vet Intern Med*. 2019;33:923-932.
22. Pizzorno L. Nothing boring about boron. *Integr Med (Encinitas)*. 2015;14:35-48.
23. Stewart PA. Modern quantitative acid-base chemistry. *Can J Physiol Pharmacol*. 1983;61:1444-1461.
24. Bowen JM, McMullan WC. Influence of induced hypermagnesemia and hypocalcemia on neuromuscular blocking property of oxytetracycline in the horse. *Am J Vet Res*. 1975;36:1025-1028.
25. Hoenderop JG, Bindels RJ. Epithelial Ca²⁺ and Mg²⁺ channels in health and disease. *J Am Soc Nephrol*. 2005;16:15-26.
26. Rodriguez-Rubio L, Solis Garcia Del Pozo J, Nava E, Jordán J. Interaction between magnesium sulfate and neuromuscular blockers during the perioperative period. A systematic review and meta-analysis. *J Clin Anesth*. 2016;34:524-534.
27. Stewart AJ. Magnesium disorders in horses. *Vet Clin North Am Equine Pract*. 2011;27:149-163.
28. Stewart AJ. Treatment for hypomagnesemia. In: Fielding CL, Magdesian KG, eds. *Equine Fluid Therapy*. 1st ed. Ames, Iowa: John Wiley & Sons, Inc; 2015:76-87.
29. Cavalcante AL, Siqueira RM, Araujo JC, et al. Role of NMDA receptors in the trigeminal pathway, and the modulatory effect of magnesium in a model of rat temporomandibular joint arthritis. *Euro J Oral Sci*. 2013;121:573-583.
30. Lambuk L, Jafri AJA, Arfuzir NNN, et al. Neuroprotective effect of magnesium acetyltaurate against NMDA-induced excitotoxicity in rat retina. *Neurotox Res*. 2017;31:31-45.
31. Wang LY, MacDonald JF. Modulation by magnesium of the affinity of NMDA receptors for glycine in murine hippocampal neurones. *J Physiol*. 1995;486 (Pt 1):83-95.
32. Zhang L, Rzigalinski BA, Ellis EF, Satin LS. Reduction of voltage-dependent Mg²⁺ blockade of NMDA current in mechanically injured neurons. *Science*. 1996;274:1921-1923.
33. Chen L, Mae Huang L-Y. Protein kinase C reduces Mg²⁺ block of NMDA-receptor channels as a mechanism of modulation. *Nature*. 1992;356:521-523.
34. Nielsen FH. Update on human health effects of boron. *J Trace Elem Med Biol*. 2014;28:383-387.
35. Kim DH, Hee SQ, Norris AJ, Faull KF, Eckhart CD. Boric acid inhibits adenosine diphosphate-ribosyl cyclase non-competitively. *J Chromatogr A*. 2006;1115:246-252.
36. Li KW, Yu YP, Zhou C, et al. Calcium channel alpha2 delta1 proteins mediate trigeminal neuropathic pain states associated with aberrant excitatory synaptogenesis. *J Biol Chem*. 2014;289:7025-7037.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Sheldon SA, Aleman M, Costa LRR, Weich K, Howey Q, Madigan JE. Effects of magnesium with or without boron on headshaking behavior in horses with trigeminal-mediated headshaking. *J Vet Intern Med*. 2019;33:1464-1472. <https://doi.org/10.1111/jvim.15499>