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Effect of a blend of magnesium oxide on Equine Squamous Gastric Disease in young trotter horses under training

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ABSTRACT

Background: Equine squamous gastric disease (ESGD), as part of the equine gastric ulcer syndrome (EGUS), are common in racing horses. The use of buffering feed supplements to treat and/or prevent gastric ulcers is an option to control this condition.

Objective: The purpose of this study was to evaluate the effect of a 30-day supplementation with a blend of magnesium oxide (MgO) on ESGD scores in trotters under training.

Methods: Forty-two young trotters were submitted to a gastroscopic evaluation to assess their ESGD score and were randomly assigned in a group supplemented with MgO or in a control group. After 30 days, a second evaluation by gastroscopy was performed. The effect of the MgO supplementation was assessed by comparing the evolution of the ESGD score in supplemented and control groups between day 0 and day 30.

Results: The results confirm the high prevalence of EGUS in young Trotters. The supplementation significantly decreased the ESGD scoring in the supplemented group whereas the control group remain unchanged.

Conclusion: The oral MgO supplementation was efficient to control ESGD in the population studied.

Keywords: Horses; gastric ulcers; magnesium; training

INTRODUCTION

Equine squamous gastric disease (ESGD), as part of the equine gastric ulcer syndrome (EGUS), is common in racing and sports horses. Their high prevalence, no specific clinical signs, and negative effect on performance means they represent a significant clinical and economic problem within the horse industry. A variety of management factors have been recognized as contributors to the development of EGUS such as training intensity and/or high starch intakes. These factors increase the exposure of the squamous mucosa to acid content. In racehorses, the prevalence, lesion severity and number of lesion sites within the squamous mucosa are significantly associated with long duration training and increasing intensity. As a consequence, appropriate acid suppressor treatment is indicated in the management of ESGD [1].

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Conceptualization: Leleu C; Funding acquisition: Leleu C; Investigation: Leleu C, Couroucé A; Methodology: Leleu C, Couroucé A; Writing - original draft: Leleu C; Writing - review & editing: Couroucé A.

Conflict of Interest

The authors declare no conflicts of interest.

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The gold standard treatment for ESGD is omeprazole which is an effective treatment [2,3]. However, this molecule is expensive, requires a prescription, can lead to positive antidoping controls and can have potential side effects [4]. Development of a less expensive and natural alternative that could be added to the feed would be of great interest. Recently, there has been increasing interest in the use of buffering feed supplements to treat and prevent gastric ulcers in horses [5-7].

In other species (dairy cows or feedlot cattle), with the aim of controlling rumen pH, a number of studies have evaluated the use of supplemented rations with buffers or alkalinizers (or neutralizing agents) such as magnesium oxide [8-10]. The effectiveness of magnesium oxide sources in raising rumen pH has been reported to differ across sources [11,12]. However, there is no data on the use of magnesium oxide as a gastric buffering agent in horses.

The purpose of this study was to evaluate the effect of a 30-day supplementation with a blend of magnesium oxide (TimRide-up; Timab Magnésium, France) on ESGD scores in young trotters under real conditions of training. We hypothesize that magnesium (Mg) supplemented horses would have less severe ESGD ulcers compared to untreated control horses.

MATERIALS AND METHODS

Ethics statement

As all the procedures were performed on horses for regular diagnostic examinations, ethical review was waived. Formal consent for the use of patient data was provided by the owners and trainers before their inclusion in the study.

Horses

Horses included in the trial were two years-old French trotters ($n = 42$) in active training, during winter (November to December 2021). There were 6 males, 20 geldings and 16 females. Body weight ranged from 380 to 546 kg with a mean body weight of 475 kg (± 40). They were all sound and healthy, with no medical history.

Experimental design

The experiment followed a randomized double-blind placebo-controlled design. The 42 horses under training were recruited and assigned to one of two treatment groups using stratification based on mean ESGD and level of training as level of training is a well-known factor influencing incidence of ESGD and treatment responsiveness [1]. As a result, The mean ESGD score and the level of training were initially similar in Mg treated group ($n = 23$) and control group ($n = 19$).

For 30 days, all horses received either a Mg supplementation or a control. At the beginning (day 0, D0) and end (day 30, D30) of the study, all horses were clinically examined, and went through haemato-biochemical evaluations and gastroscopic examinations. The effect of the Mg supplementation was assessed by comparing the evolution of an ESGD score in supplemented and control groups between D0 and D30.

In both groups, training and nutrition schedules were equivalent during the whole trial and rations fulfilled their nutritional requirements. The experiment was double blinded: neither operators nor trainers knew which of the groups received the supplementation or the

placebo. During the study, the criteria of exclusion were the inobservance of treatment, the interruption of training for any reason, and the occurrence of a medical treatment.

Gastroscopic examination

Gastroscopic examination was performed using a 3-m endoscope (Optomed, France). To improve visualization of the stomach, food and water were withheld for 16 to 18 h prior to gastroscopy. Horses were sedated with detomidine (20 µg/kg/IV) prior to gastroscopic examination. To enable observation of the squamous mucosa and *margo plicatus*, the stomach was insufflated with air. The mucosa was rinsed of adherent food material and mucus with tap water flushed through the endoscope biopsy channel. Each horse's stomach was assigned an ESGD score by two veterinarians in a blinded way. One of the veterinarian was an European College of Equine Internal Medicine (ECEIM) Diplomate used to these examinations. This score was based on the ESGD scoring system where, score 0 = Intact mucosa, score 1 = hyperkeratosis, score 2 = small single or small multifocal ulcers, score 3 = large single or large multifocal ulcers, and score 4 = extensive (often coalescing) ulcers with areas of apparent deep ulceration [1].

Resting haemato-biochemistry and oxidative stress markers

Blood collection by jugular puncture was performed at rest, before the training, between 7.30 and 9.00 am. Blood was collected in 2 ethylenediaminetetraacetic acid (EDTA) tubes (for haematology and erythrocytes superoxide dismutase [SOD] activities) and 2 heparin tubes (biochemistry and lipidic peroxides). Packed cell volume (PCV), haemoglobin concentration (Hb), and red blood cells count (RBC) and basic biochemistry were evaluated on fresh whole blood in EDTA and heparin tubes respectively. Biochemistry analyses included the determination of creatine kinase (CK), aspartate aminotransferase (AST), fibrinogen, alkaline phosphatase (ALP), γ-glutamyltransferase (γGT), bilirubin, urea, and creatinine levels.

One heparin tube was immediately centrifuged, and plasma was stored at -30°C until analysis, as well as one EDTA tube of whole blood.

As oxidative markers, SOD activities and lipidic peroxides plasma concentration were assessed respectively by spectrophotometry using RansodND kits and OxystatsND kits.

Food and Mg supplementation

The ingredient studied, a blend of different sources of Mg oxide (TimRide-up), was included in the concentration of 0.8% in a complete pelletized feed (Racing) by the manufacturer Reverdy, i.e., 20 g of ionized Mg²⁺ per horse per day. The feed used as a control was the same without Mg. The feed of the two groups were not differentiable. The analytical constituents of both concentrates are presented in **Table 1**. Four weeks before experimental phase, all horses received the control feed to homogenize as much as possible the initial feed status (habituation phase). The supplementation with Mg or control started 24 h after the D0 gastroscopy and ended after D30. The palatability was observed and recorded by grooms, who checked the correct observance of treatments. Horses were also fed mixed grass hay (1.5% of body weight) divided into two equal feedings per day. Horses had free access to water at all times.

Statistics

Data were analyzed statistically using a statistical analysis NCSS 2021. A repeated measures analysis of variance using a mixed effects model was used to analyze the data. Fixed effects in

Table 1. Analytical constituents of feedstuff

Composition, %	Control pellets	Mg supplemented pellets
Crude protein	12.4	12.2
Crude fibre	12.2	12
Crude fat	5	5
Crude ash	7.7	8.3
Ca	1.01	0.99
P	0.46	0.46
Na	0.25	0.21
Cl	0.74	0.69
K	0.84	0.84
Mg	0.37	0.66

Ca, calcium; P, phosphorus; Na, sodium; Cl, chloride; K, potassium; Mg, magnesium.

the model included Treatment, Time and the interaction of Treatment*Time. When significant differences were found, *post hoc* pairwise comparisons were conducted with tests of least-squares means for main effects and for interaction effects. Significance was set at $p < 0.05$.

RESULTS

Physical examinations were within normal limits and there was no evidence of clinical gastrointestinal disease in any of the horses prior to enrollment in the study. All hematological and biochemistry data were within normal range with no significant changes between D0 and D30.

During the trial, one Mg-treated horse developed a respiratory infection with nasal discharge and therefore was excluded. One control horse could not be evaluated during the final gastroscopy because of inadequate emptiness of the stomach. The data presented below concerned 22 horses in the control group and 18 horses in the Mg group.

ESGD scores

At D0, considering the 40 horses, the percentage of unaffected horses (score 0 and score 1), of horses with a score 2, with score 3 and with a score 4 were respectively: 27.5%, 30%, 25% and 17.5%. The number of horses of each category at D0 and D30 are available in the **Table 2**.

There was no significant difference in mean scores between treatment groups on day 0, prior to start the supplementation. Means (\pm standard error [SE]) were respectively 2.0 ± 0.27 and 2.1 ± 0.39 in control and Mg groups. On day 30, a significant decrease of mean ESGD scores in Mg group (0.88 ± 0.27) was observed compared to control group (1.81 ± 0.28) (**Fig. 1**).

Table 2. Number of horses on the different stage of ESGD at D0 and D30

Group	Severity of ESGD			
	Unaffected (score 0 and 1)	Light ESGD (score 2)	Severe ESGD (score 3)	Severe ESGD (score 4)
D0				
Control group	5	9	6	2
Mg group	6	3	4	5
D30				
Control group	7	5	10	0
Mg group	11	5	2	0

ESGD, equine squamous gastric disease; D0, day 0; D30, day 30.

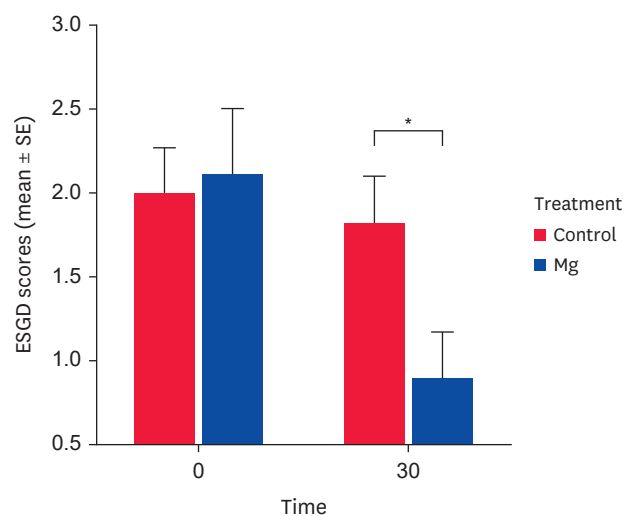


Fig. 1. ESGD scores (mean ± SE) in control group and Mg group. ESGD, equine squamous gastric disease; SE, standard error; Mg, magnesium. *Denotes significant differences ($p < 0.05$) between control and Mg groups.

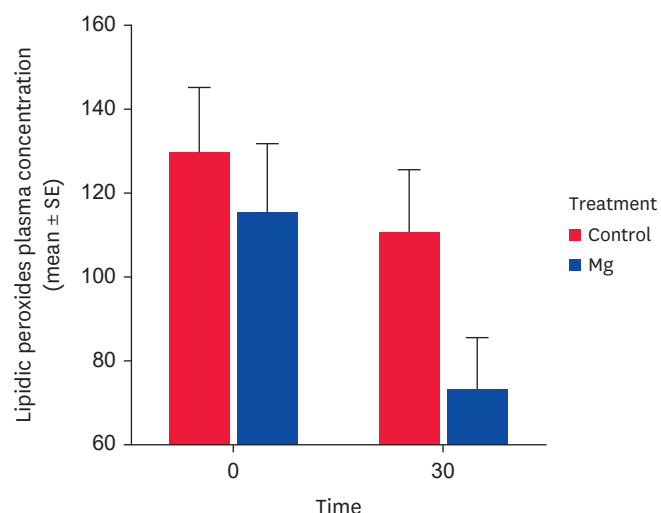


Fig. 2. Lipidic peroxides plasma concentration (mean ± SE) in control group and Mg group. SE, standard error; Mg, magnesium.

Oxidative stress markers

There was no significant difference in mean lipidic peroxides plasma concentration between treatment groups on D0. On D30, a decrease in mean lipidic peroxides plasma concentration in Mg group was observed in comparison to control group ($p = 0.06$) (**Fig. 2**).

Considering the entire population, higher lipidic peroxydes plasmatic concentration in horses presenting higher ESGD scoring was observed ($p = 0.12$). Means ± SE of lipidic peroxydes plasmatic concentrations in unaffected horses (score = 0 and 1), horses scored 2, horses scored 3, and horses scored 4 were respectively 88 ± 12 , 110 ± 12 , 119 ± 17 and 144 ± 25 $\mu\text{mol/L}$.

At D0, a significant difference in mean erythrocytes SOD activity between treatment groups, with significantly higher activities in the control group was observed. On D30, the mean erythrocytes SOD activity was not different between Mg and control groups (**Fig. 3**).

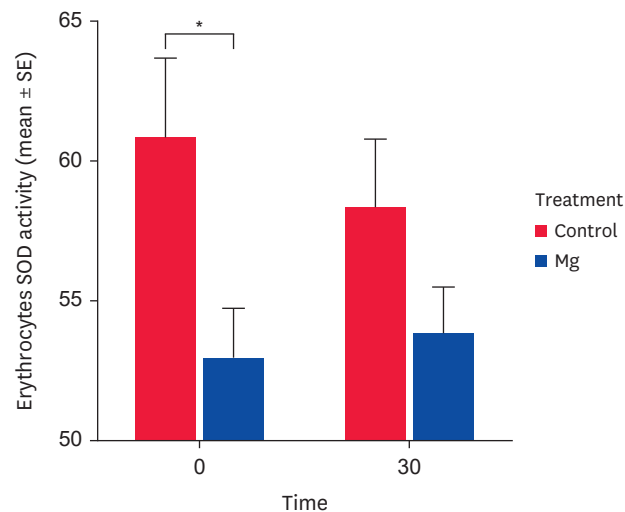


Fig. 3. Erythrocytes SOD activity (mean ± SE) in control group and Mg group. SOD, superoxide dismutase; SE, standard error; Mg, magnesium. *Denotes significant differences ($p < 0.05$) between control and Mg groups.

No correlation was observed between erythrocytes SOD activity and ESGD scoring.

DISCUSSION

In the present study, the percentage of unaffected horses (scores 0 and 1), horses scored 2, horses scored 3, and horses scored 4 were respectively: 27.5%, 30%, 25%, and 17.5%. These data confirmed the high prevalence of ESGD in trotters [13-16]. Dionne et al. [14] and Rabuffo et al. [13] reported respectively a prevalence rates of 63.3% and 86% in Standardbreds. Interestingly, Roy et al. [15] determined the prevalence of this disease by means of repeated examinations and its association with progressive high-intensity training in a population of 48 young Standardbred racehorses in Canada. For a similar level of training, the authors observed comparable percentages of un- and affected horses (14% horses with score 0, 26% horses with score 1–2, 58% horses with score 3–4). Furthermore, they noticed that training intensity increased the odds of observing ESGD and of assigning a higher score.

In the ECEIM consensus statement about EGUS, the pathophysiology of ESGD ulcers is described [1]. Gastric lesions are related to the exposure of the squamous mucosa to acid. *In vitro* experiments clearly show that squamous mucosal cells are susceptible to hydrochloric acid (HCl) and volatile fatty acid (VFA) injury in a pH, dose and time dependent manner. Damage of the outer cell barrier is induced by HCl, later followed by diffusion into the squamous cells of the stratum spinosum ultimately resulting in ulceration. Byproducts of bacterial fermentation of sugars in concentrate diets not only like VFAs and lactic acid, but also bile acids, have been shown to act synergistically with HCl. There is a well-described relationship between exposure of squamous mucosa to acidic content and training. Excessive exposure of the squamous mucosa results from the acidic gastric contents being pushed up by the increased intra-abdominal pressure associated with gaits faster than a walk. Therefore, racehorses have an increased prevalence, lesion severity and number of lesion sites within the squamous mucosa significantly associated with long duration training and increasing intensity.

The most interesting finding of the current study was the significant decrease on mean ESGD scores in Mg group compared to control group. As a particular care was brought to control and maintain similar training and feeding schedules in both groups, this result may be linked to the buffering capacity of the Mg supplementation.

In other species as dairy cows or feedlot cattle, with the aim of controlling rumen pH, a number of studies have evaluated the use of supplementing rations with buffers or alkalizers (or neutralizing agents) such as magnesium oxide [8-10].

The effectiveness of magnesium oxide sources in raising rumen pH and fostering improvements in milk yield and milk fat has been reported to differ across sources [8]. The acid-neutralizing capacity of magnesium oxide depends on several physical and chemical characteristics, which lead to different rates of solubilization in the rumen fluid [12]. Recently, the effects of a magnesium-based product on rumen pH of dairy cattle exposed to an acidogenic diet were evaluated [8]. The authors observed that control cows experienced a marked decrease in rumen pH whereas Mg supplemented cows maintained stable rumen pH during the challenge. Moreover, the Mg supplemented cows spent less time with rumen pH ≤ 5.8 than control cows. Further investigation in horses is needed to confirm such capacity of controlling gastric pH.

Indeed, one critical point in ESGD management with buffers is the duration of intra-day acid suppression required for healing of ESGD and EGGD. In human, maintenance of a pH above 3 and 4 for a minimum of 16 h is required for healing of gastric ulceration and reflux esophagitis, respectively [17]. In the current study, the supplementation in magnesium oxide was included in the concentrate, leading to a 3 times per day administration. As a result, the duration of higher gastric pH might be increased compared to control group and could explain the good evolution of ESGD scoring.

Environmental factors such as food, training/exercise and housing are well known to influence development and healing of gastric ulcers in horses. All trainers were instructed not to perform any differences in feeding or training regimes, it is reasonable to assume that a significant proportion did instigate changes that would very likely influence the development and healing of the ulcers. Due to the blinding of the treatment, these changes have to be assumed equal in the two groups.

Reactive oxygen species (ROS) have been reported to be one of the causative factors for mucosal lesions through oxidative stress. A complete review summarize the pathogenesis of oxidative stress in stomach during the development of various gastric diseases [18]. The radicals promote mucosal damage by causing degradation of the epithelial basement components, complete alteration of the cell metabolism and desoxyribonucleic acid (DNA) damage. The gastric mucosa can be protected from the harmful effects of free radicals through the action of antioxidants that scavenge free radicals. Magnesium ion (Mg^{2+}) plays various roles, such as acting as a cofactor of more than 300 enzymatic reactions, regulating physiological functioning such as neuronal function, energetic metabolism, immunity, etc. [19]. Mg^{2+} by itself and also SOD, antioxidant enzymes with Mg^{2+} as a cofactor, have strong antioxidant properties, which are important in mucosal healing [20].

An interesting finding of the current study was the decrease of lipidic peroxides plasmatic concentration in Mg group compared to control group. Concerning erythrocytes SOD activity, a significant decrease was observed in control group between D0 and D30 whereas

it remained constant in Mg group. In rats undergoing a cold-stress model for inducing gastric ulcers, an increase in gastric mucosal lipid peroxidation and SOD was observed [21]. The authors of that study also reported a positive correlation between free-radical-induced oxidative-stress and gastric ulcers. Using a model of diabetic rats, Ige et al. [22] showed that oral Mg treatment in diabetes reduces serum lipid peroxidation, increases gastric SOD, mucous cell count and reduces the susceptibility of the gastric mucosa to ulceration.

However, in the current study, even if no significant correlation was observed between lipidic peroxides plasmatic concentration and the ESGD scoring, a trend for higher concentrations in horses more severely ulcerated was observed. Thus, further research is needed to investigate if Mg supplementation could attenuate oxidative stress that may initiate and aggravate glandular gastric ulcers in horses.

The results reported here confirm the high prevalence of ESGD in young Trotters in training. Controlling training level and food intake, the 30 days-supplementation with magnesium oxide (TimRide-up) significantly decreased the ESGD scoring in the supplemented group whereas the control group remain unchanged. Further research is needed to better understand physiological mechanisms underlying these findings such as buffering capacity and antioxidant activities.

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