

Effects of a supplement containing hyaluronan with TBG-136™ schizophyllan β -glucan on gastric ulcer scores in horses

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Summary

Background: Polysaccharides, hyaluronan and TBG-136™ Schizophyllan (S β -glucan) have not been thoroughly evaluated in horses with equine squamous gastric disease (ESGD) or equine glandular gastric disease (EGGD).

Objectives: To evaluate the effect of hyaluronan with S β -glucan ([RELGI] Relyne^{GI}®, Hagyard Pharmacy, Lexington, KY) on ESGD and EGGD in stall-confined horses.

Study Design: Single period non-crossover.

Methods: Healthy adult thoroughbreds ($n=12$) were divided into RELGI ([$N=6$], 30 mL gel) or control ([$N=6$], 30 mL gel) groups and fed 3 $\times$ daily for 35 days. The horses were stratified by ESGD ulcer score on Day -1, and gastroscopy was repeated on Days 14, 21, 28 and 35. ESGD number and severity (NGN and NGS) and EGGD number and severity (GN and GS) scores were assigned at scoping by a masked clinician. Gastric fluid pH and bodyweight were measured.

Results: The median ESGD scores were lower on Day 28 in the RELGI group (median: 1.0, IQR: 1.0–1.0), compared to the control group (median: 1.5, IQR: 1.0–2.25; $p=0.037$). The median NGN and NGS scores were lower on Days 28 and 35 in the RELGI group (median: 0; IQR: 0–0) than in controls. On Day 28, NGN had a median of 1.0 (IQR: 0–2.5) and NGS had a median of 1.0 (IQR: 0–2.25). On Day 35, NGN had a median of 0.5 (IQR: 0–2.25) and NGS had a median of 1.0 (IQR: 0–2.5). ESGD ulcers healed in the treatment group on the same days. EGGD was low, so statistical analysis was not reported. Gastric fluid pH was low and horses gained weight.

Main Limitation: Thoroughbred horses had very mild EGGD lesions.

Conclusions: The supplement Relyne^{GI}® was palatable and safe. Relyne^{GI}® administration resulted in improvement in stomach health without altering stomach pH and might be an adjunct to pharmacologic treatment for ESGD.

KEYWORDS

horse, equine gastric ulcer syndrome (EGUS), hyaluronan, stomach, supplements, β -glucan

Abbreviations: ANOVA, analysis of variance; CBC, complete blood count; EGGD, equine glandular gastric disease; EGUS, equine gastric ulcer syndrome; ESGD, equine squamous gastric disease; GN, glandular ulcer number score; GS, glandular ulcer severity score; NGN, non-glandular ulcer number score; NGS, non-glandular ulcer severity score; PSG, polysaccharide gel; RELGI, Relyne^{GI}®, S β -glucan, TBG-136™ Schizophyllan.

INTRODUCTION

Equine gastric ulcer syndrome (EGUS) is an umbrella term used to describe lesions and erosive diseases in the non-glandular squamous (equine squamous gastric disease [ESGD]) and glandular (equine glandular gastric disease [EGGD]) regions of the horse stomach (Sykes et al., 2015). Because of the difference in the appearance and pathophysiology of lesions, the regions are considered separately. ESGD is an ulcerative condition and is likely caused by exposure of the stomach mucosa to hydrochloric (HCL) and other stomach acids (volatile fatty acids, bile acids), a decreased stomach pH (pH < 4), and compromise to the squamous mucosal barrier (Nadeau et al., 2003; Sykes et al., 2015). EGGD can lead to ulcers, but for the most part, it is a non-ulcerative condition affecting certain breeds of horses, and lesions are caused by damage to the mucous-bicarbonate protective layer, alterations in prostaglandin concentrations, and the erosive effect of stomach acid (Banse & Andrews, 2019). Common risk factors for ESGD and EGGD include breed, exercise intensity and number of days per week in work, stall confinement, and intermittent feeding regimes (Banse et al., 2018; Banse & Andrews, 2019; Murray, 1994; Paul & Banse, 2024; Sykes et al., 2015). Most equine competitions are designated as "Clean Sport" and forbid the use of substances with the potential to affect equine performance, health or welfare and/or with a high potential for misuse. This is contrary to the integrity of equestrian sport and the welfare of horses (<https://inside.fei.org/fei/cleansport/horses>). Therefore, the use of clean drug-free supplements is preferable and might improve long-term stomach health after pharmaceutical treatment has been discontinued or intermittently administered while actively competing.

A previous study showed oat-derived β -glucan (without hyaluronan) in a feed supplement improved stomach health after 35 days of administration; however, the effect of β -glucan alone could not be determined as the feed supplement contained multiple ingredients (dried apple pectin pulp, lecithin, sodium bicarbonate, calcium carbonate, polar lipids, and natural antioxidants) in alfalfa meal (Woodward et al., 2014). Recently, a polysaccharide gel (PSG), containing hyaluronan and β -glucan, reduced ulceration in horses in active training and racing that were nonresponsive to traditional ulcer treatments (Slovits, 2017). However, in that study, all horses were treated, which was a limitation. In that study, 90% of the horses showed complete resolution and/or improvement for ulcer grades 2 and 3+ in both ESGD and EGGD, based on gastroscopic examination before and after administration of the polysaccharide blend. The author speculated that the high molecular weight hyaluronan and β -glucan gel conferred protection to the mucosa by coating and supplying epidermal growth factors, basic fibroblast growth factors and anti-inflammatory properties to ulcerated tissues.

High molecular weight hyaluronan, a biologically active surface polysaccharide, has been shown to stimulate tissue regeneration, morphogenesis, angiogenesis and provide anti-inflammatory and antimicrobial properties (Reitinger & Lepperdinger, 2012; West & Kumar, 2007). It also provides hydration and forms a protective barrier on the stomach mucosa, decreases gastric acid secretion and

prevents diffusion of corrosive acids that might damage the gastric mucosa (Al-Bayat et al., 2011). Hyaluronan is found throughout the gastrointestinal tract and deficiencies in hyaluronan might lead to intestinal inflammation and disease (Fraser et al., 1997; Kvietys & Granger, 2010; Slovis, 2017).

β -glucan (β -1,3 β -glucan with β -1,6 branching) is present in the cell wall of the common wood fungus, *Schizophyllum commune*, and imparts its surface bioactivity by forming linear, branched and cyclic macroparticles that coat tissues. Dissolved β -glucan absorbs large amounts of water to form high-viscosity gums on the surface of intestinal and other tissues (Du et al., 2019; Gudej et al., 2021). They do not undergo enzymatic degradation in the stomach, which allows the creation of a mucus layer, protecting against acid irritation and lessening inflammation (Tanaka et al., 2011). In addition, β -glucan might stimulate the immune system by activating macrophages to synthesise anti-inflammatory cytokines (Pan et al., 2020; Zheng et al., 2016). The β -glucan has also been shown to have antimicrobial activity and might reduce bacterial contamination of the ulcer bed, which may delay gastric ulcer healing (Laroche & Michaud, 2007; Paul et al., 2023; Yuki et al., 2000). Finally, β -glucan has been shown to provide antioxidative and anti-inflammatory effects in people with chronic gastritis (Gudej et al., 2021).

The purpose of the study was to evaluate the anti-ulcerogenic properties of Relyne^{GI}, a polysaccharide gel containing hyaluronan with β -Glucans, in stall-confined horses with naturally occurring non-glandular (ESGD) and glandular (EGGD) gastric diseases. We hypothesise that the Relyne^{GI} supplement mixed in the grain portion of the feed will decrease ESGD and EGGD scores in stall-confined horses.

MATERIALS AND METHODS

Animals

All procedures performed on horses during the study were approved by the Louisiana State University (LSU) Institutional Animal Care and Use Committee (IACUCAM-23-022). Horses (N=12) used for the study were Thoroughbreds of average size, bodyweight (median 491 kg, range 443–556 kg) and competition age (median 8 years, range 4–18 years) randomly selected from the resident herd at the LSU School of Veterinary Medicine, Equine Health and Sports Performance (EHSP) and were not enrolled in concurrent studies. A complete physical examination was performed on all horses to exclude the presence of clinical signs of disease. Venous blood samples were obtained from either jugular vein of the horses on Days -1 and 35, to determine normality and the effect of the supplements on blood parameters (Figure 1). A complete blood count (Siemens Advia 120 Haematology System) and whole blood gas and biochemical panel (EPOC[®] Blood Analysis System) were performed on Day -1, prior to administering the supplements, and Day 35, prior to the final gastroscopy examination. In addition, bodyweight was measured weekly using a calibrated digital livestock scale.

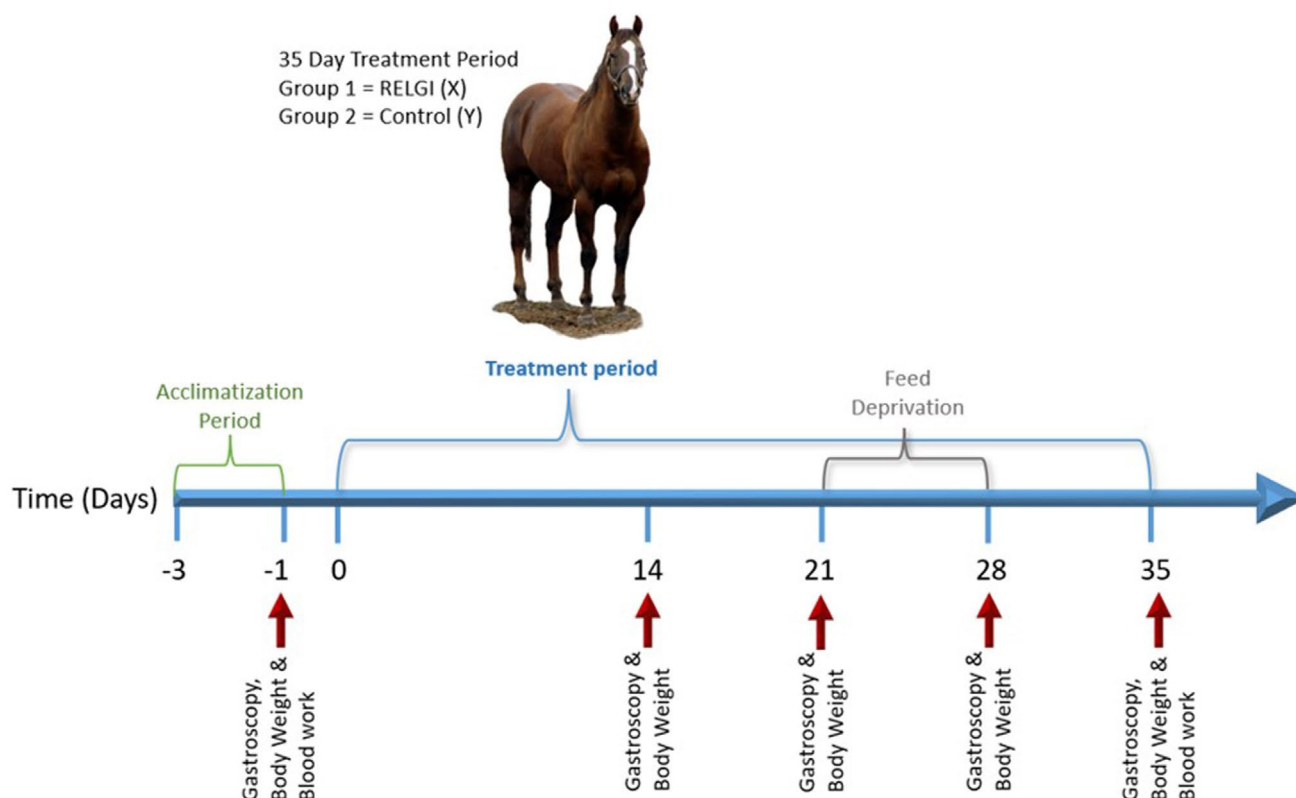


FIGURE 1 Study timeline.

Experimental design

The study was performed as a single-period non-crossover design, 38 days in duration (Figure 1). On Day -3, horses ($N=16$) were brought in from nearby pastures and placed in 3- $\times$ 3-m box stalls for the 2-day acclimation period. Diet consisted of locally derived mixed grass square-bale hay fed at 1.5% body weight and a commercial concentrate feed (2.2 kg, daily, Purina® Impact® All Stages 14%, Purina Animal Nutrition). Horses were fed twice daily on a consistent schedule. On Day -1, gastroscopy was performed on the 16 horses to arrive at the 12 horses who had ESGD scores of ≥ 1 (Andrews, Bernard, et al., 1999) and did not have clinical signs of disease. After the gastroscopy, horses were stratified by ESGD score and allocated to the treatment group ($N=6$, RELGI; Relyne^{GI}®, Hageyard Pharmacy, Lexington, KY) or control group ($N=6$, Relyne^{GI}® supplement without the active ingredients, hyaluronan and β -glucan) using a random number calculator. Treatment and control supplements (30 mL; 2 pumps) were mixed in the grain portion of the feed and fed three times daily for 35 days. Prior to shipping the containers of RELGI, the β -Glucans fraction was assayed (Beta Bio, Corp., Gyonggi-Do, Korea) and found to be within the specifications (58% pure) for the product. The RELGI and the control supplements were shipped overnight in gallon containers labelled X or Y, so that all personnel at the study site were masked to treatment groups. The treatment groups were unmasked after all animal work was completed and data was tabulated and analysed.

Gastroscopy was repeated on all horses on Days 14, 21, 28 and 35 of the study period (Figure 1). To improve visualisation of the stomach, food was withheld beginning 16–18 h prior to examination, and water was not withheld. A muzzle was placed on each horse at the time when food was withheld to prevent ingestion of shavings or other environmental material. Horses were moved to stocks in an environmentally controlled room and sedated with xylazine (XylaMed™ VETONE®, 0.4 mg/kg bwt, IV once). Gastroscopic examinations were performed on an empty stomach using a 3.25-m video endoscope (Karl Storz Endoscopy). The stomach was insufflated with air using an electric air pump (Airhead 120V Hi-Pressure Air Pump, Aqua Leisure Recreation, LLC) until the rugae of the stomach were no longer visible. Mucosal surfaces of the stomach were cleansed of mucus and debris with tap water flushed through the biopsy channel of the endoscope. Lesions in the non-glandular mucosa were scored by size using the ESGD (EGUS) scoring system (Andrews, Bernard, et al., 1999; Andrews, Sifferman, et al., 1999; Sykes et al., 2015) and ESGD number (NGN) and severity (NGS) scoring system and EGGD number (GN) and severity (GS) scoring, respectively (MacAllister et al., 1997). Scores were assigned by a single masked investigator (FMA) during the gastroscopy examination. Gastric fluid was aspirated from the ventral fundic region of the stomach upon entry and prior to adding water. The aspirated gastric fluid was collected in plastic cups and was immediately capped with lids. The pH was measured within 1 h using a bench-top pH metre (Thermo Orion pH Meter Model 410A). A complete examination of each empty stomach was performed, and, in some cases, fluid was aspirated from

the stomach to improve the view. Suction was also used to remove insufflated air from the stomach at the completion of the examination. Horses were allowed to recover from sedation, approximately 20 min, prior to refeeding.

All horses remained stall-confined for the duration of the study, but were able to see other horses in stalls and in the nearby pastures. Furthermore, from Days 21 to 28, all horses underwent an alternating feeding model, where they were muzzled and deprived of feed for 24 h, then fed their normal ration for 24 h, until a total of 96 h of cumulative feed deprivation was achieved (Murray, 1994). During the feed deprivation period, horses in the treatment groups continued to receive the daily dose of the RELGI or control supplement mixed with a small amount of the grain portion of the feed (about 10% of the regular ration) three times daily. From Days 28 to 35, horses were returned to their normal diet and allowed to recover from the intermittent feeding period. Horses were monitored daily for clinical signs or adverse events throughout the study.

Statistical analysis

Statistical analyses were performed using JMP Pro 17.0.0 (JMP Statistical Discovery LLC). For blood work, pH and weight, a repeated measures analysis of variance (ANOVA) with a mixed effects model was used. Treatments, time points and their interactions were entered as the fixed effects, and each animal was entered as the random effect. When a fixed effect was detected, a post hoc Tukey comparison was used against the baseline. Normality of residuals was assessed and confirmed by examining standardised residuals and quantile plots. Data are presented as mean $\pm$ SEM. Ulcer scores were evaluated via the Friedman test with pairwise Dunn's post hoc comparisons within each treatment from Day -1 and via Mann-Whitney test within each time point between the two treatments. Data are presented as median (minimum – maximum), with interquartile ranges (IQR) shown in the figures. Significance was set at $p < 0.05$.

RESULTS

Physical examinations completed on Days -1 and 35 were within normal limits and there was no evidence of adverse events or adverse clinical signs in any of the horses prior to enrolment or at the end of the study. All horses readily consumed the supplements mixed in the grain portion of the feed and all 12 horses completed the study.

ESGD score, ESGD number (NGN) and ESGD severity (NGS) scores

Although the ESGD (EGUS) scores were slightly higher in the control group on Day -1, there was no significant difference in ESGD scores between treatment groups (Figure 2). The ESGD score

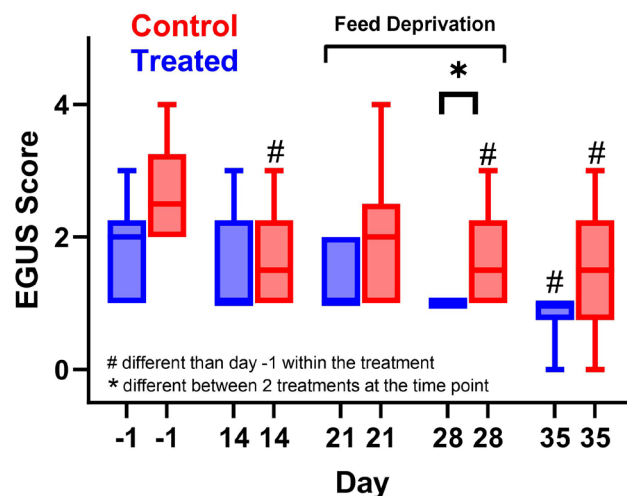


FIGURE 2 Equine squamous gastric ulcer disease (ESGD) non-glandular ulcer scores in horses fed a supplement (treated) containing hyaluronan and β -glucan (RELGI, Relyne^{GI}, Hagyard Pharmacy, LLC, Lexington, KY, USA) or control supplement (without the active ingredients) for 35 days. Box plots represent median ESGD scores with interquartile ranges; whiskers indicate minimum and maximum values. Days indicate gastroscopy times, and the EGUS non-glandular scoring system was adapted from Andrews, Bernard, et al. (1999). Feed deprivation occurred between Days 21 and 28, as indicated by the bracket. #Significant difference from Day -1 within the treatment group ($p < 0.05$). *Significant difference between the two treatment groups at the same time point ($p < 0.05$).

decreased significantly ($p < 0.037$) in the control group after 14 days of treatment (median 1.5; IQR, 1.0–2.25), when compared to the ESGD score on Day -1 (median, 2.5; IQR, 2.0–3.25), but there was no difference in scores when compared to the RELGI group on Day 14. On Day 28 of treatment, following the feed deprivation model, there was no significant increase in ESGD score in either treatment group due to the intermittent feeding protocol. However, the ESGD score decreased ($p = 0.037$) in the RELGI-treated group (median, 1; IQR, 1–1), when compared to controls (median, 1.5; IQR, 1.0–2.5). In addition, the ESGD number score (NGN) and ESGD severity score (NGS) were significantly lower in the RELGI-treated horses on Days 28 and 35, compared to controls (Figure 3a,b). On Day 28, the treated group had a median score of 0 (IQR, 0–0) versus a control median of 1.0 (IQR, 0–2.5 for NGN; 0–2.5 for NGS; $p = 0.037$). On Day 35, the treated group maintained a median score of 0 (IQR, 0–0) compared to the control median of 0.5 and 1 (IQR, 0–2.25 for NGN; 0–2.5 for NGS; $p = 0.037$). Also, non-glandular ulcers were healed (except for mild hyperkeratosis) in all the RELGI-treated horses on the same days (Figure 4a,b).

Equine glandular gastric ulcer scores (GN and GS)

EGGD lesions were observed in only 3/12 (25%) horses at the beginning of the study. The EGGD ulcer number (GN) and severity

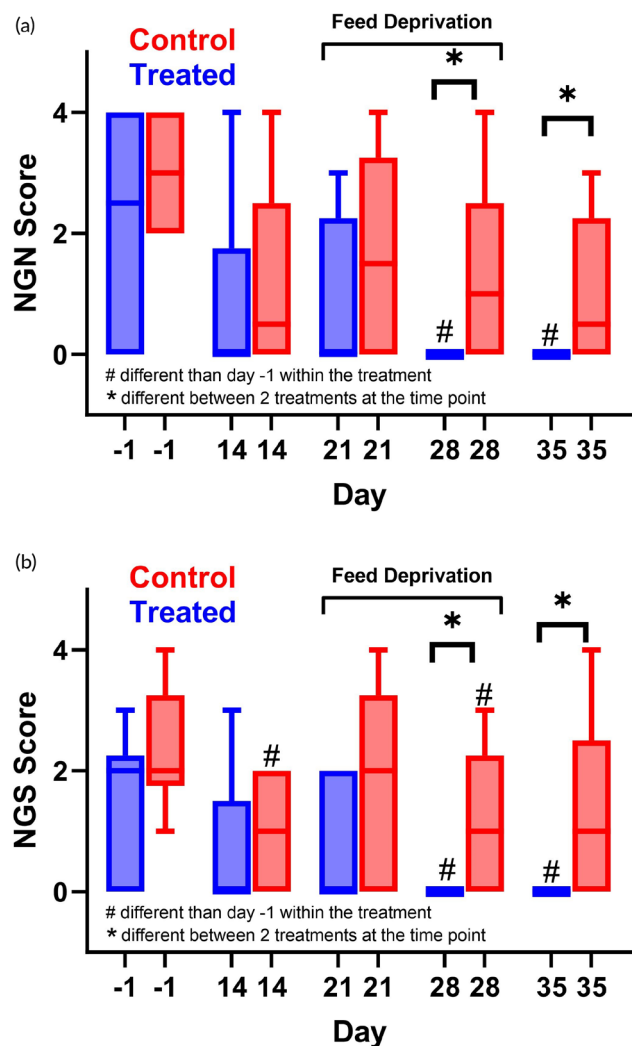


FIGURE 3 (a and b) ESGD number scores (a) and ESGD severity scores (b) in horses fed a supplement (Treated) containing hyaluronan and β -glucan (Relyne^{GI}) or control supplement (without the active ingredients) for 35 days. Days indicate gastroscopy times, and the ESGD number and scoring system were adapted from MacAllister et al. (1997). Feed deprivation occurred between Days 21 and 28, as indicated by the bracket. #Significant difference from Day -1 within the treatment group ($p < 0.05$). *Significant difference between the two treatment groups at the same time point ($p < 0.05$).

scores (GS) were mild and variable in both groups throughout the treatment period. Due to the small number of horses with EGGD in this study, statistical analysis was considered underpowered and was not reported.

Gastric fluid pH and bodyweight

Gastric fluid pH was low and variable, with a mean range of 1.82 to 2.85 and 1.92 to 2.85 in the RELGI and control groups, respectively. There was a mild increase ($p = 0.0362$) in mean gastric fluid pH on Day 35 in the RELGI horses compared to Day -1, but no treatment by

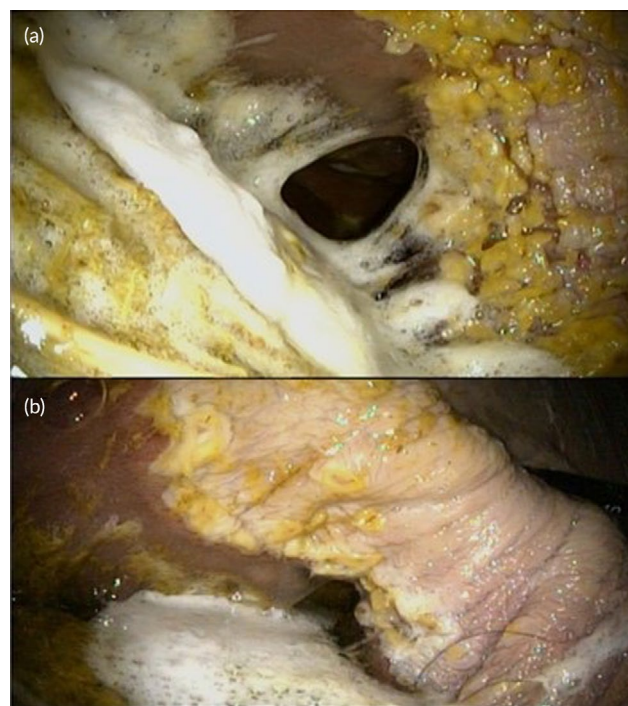


FIGURE 4 (a and b) Non-glandular ulcers on the lesser curvature of a horse before (Day -1, a) and after (Day 35, b) treatment with Relyne^{GI} containing hyaluronan and β -glucan. Ulcers were healed except for mild hyperkeratosis remaining.

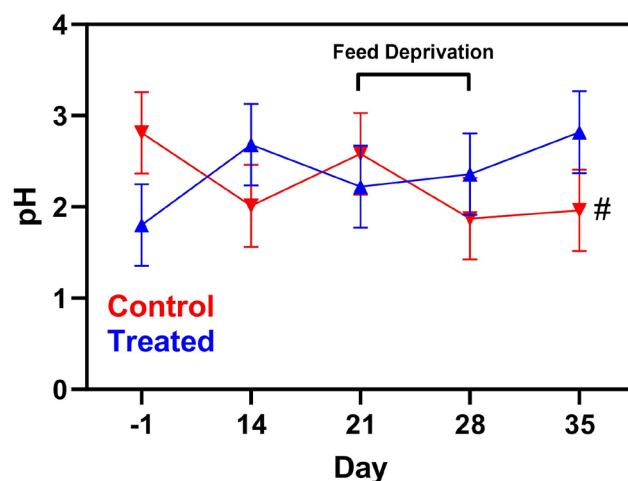


FIGURE 5 Mean $\pm$ SEM gastric fluid pH in horses fed a supplement (treated) containing hyaluronan and β -glucan (Relyne^{GI}) or control (supplement without the active ingredients) for 35 days. Days indicate gastroscopy times when gastric fluid was suctioned via the biopsy chamber of the endoscope. #Significant difference in gastric fluid pH on Day 35 compared to Day -1 in the treated group only. There was no treatment by day effect on day 35.

day effect was observed (1.96 ± 0.45 , Day 35 vs. 2.81 ± 0.45 , Day -1; Figure 5). The variability in gastric fluid pH might have been associated with a dilution effect, as horses were not held off water before endoscopic collection.

There was also a significant increase in mean bodyweight in both groups of horses on Day 35 when compared to Day -1 in both treatment groups (control: 493.4 ± 16.3 kg vs. 479.7 ± 16.3 kg; treated: 518.3 ± 16.3 kg vs. 504.1 ± 16.3 kg; Figure 6). Otherwise, there was no significant treatment or treatment-by-day effect on bodyweight on any of the study days.

Blood values

Mean blood values were within reference range on Days -1 and 35 for the horses. Except on Day 35, the mean plasma sodium concentration was significantly ($p=0.0343$) higher in the control horses (139.8 mg/dL ± 0.5) compared to the RELGI horses (137.7 mg/dL ± 0.5). However, despite the treatment by day difference, the sodium values remained within the reference range (128 – 142 mg/dL) in both groups.

DISCUSSION

The supplement, Relyne^{GI}®, with hyaluronan and β -glucan, when mixed in the grain portion of the feed, was readily consumed by the horses, and no adverse events or clinical signs were observed. The supplement, Relyne^{GI}®, resulted in significant improvement in non-glandular stomach health by Days 28 and 35. Gastric fluid pH was low and variable throughout the study, but higher in the RELGI-treated horses on Day 35, and did not differ from the control horses. In addition, bodyweight increased in both treatment groups by Day 35.

The intermittent feed-deprivation model used in this study was not successful in worsening ESGD gastric ulcer scores, as shown in previous studies (Murray, 1994; Woodward et al., 2014). However, on Day

28, ESGD scores in the control group remained the same compared to Day 21, whereas the median ESGD score in the treatment group was significantly lower, denoting a treatment-by-day effect on Day 28 after feed deprivation. This contributed to the evidence that the Relyne^{GI}® supplement used in this study resulted in a positive effect on ESGD lesions in horses undergoing stall confinement and feeding stress.

In the study reported here, there was a decrease in the median ESGD score and severity (NGS) gastric ulcer scores in the control group of horses after 14 days of administration, compared to Day 0. However, the median ESGD and NGS scores in the control group were not significantly different compared to the RELGI group on Days -1 and 14. Previous studies have shown that pastured horses typically have less severe non-glandular ulcers than stall-confined horses (Feige et al., 2002). However, dry and marginal pastures with dominant horses might lead to stressful events and increase the prevalence of ESGD, whereas horses fed in individual stalls might be less stressed and have fewer ulcers. Furthermore, in one study in managed non-pregnant broodmares, the prevalence of non-glandular ulcers was 71%, and some were severe (le Jeune et al., 2009). The exact reason for the decrease in gastric ulcer severity in the control group of horses in the study reported here is unknown, but it might have been due to changes in management for the study period. Typically, pasture grass and round bales fed to the EHSP herd have poor nutritional quality during the late fall and early winter (study dates, 24 October to 28 November), and horses are fed in pastured groups. When horses were moved to individual stalls, they were fed individually, reducing the stress of group feeding, lack of competition for feed, and increased nutrition in the square bales fed. In a previous study, moving horses from pastures to stalls resulted in decreased ESGD ulcer scores (Woodward et al., 2014). Also, diet and stress have been identified as risk factors for ESGD. It should be noted that a similar decrease was not noted in the RELGI group, but ulcer scores were slightly, but not statistically different from control horses on Days -1 and 14. Unfortunately, a feed analysis was not performed during the study period, although bodyweights increased in the horses in both groups by Day 14, when compared to Day -1, but this was not significant.

Non-glandular ESGD, NGN and NGS gastric ulcer scores in this study were significantly lower in the RELGI-treated horses on Days 28 and 35 of treatment, when compared to controls. In the study reported here, five of six horses (83%) showed complete resolution in the non-glandular ulcerated areas, while one horse in that same group did not develop ulcers during the study. Mild hyperkeratosis persisted in five of six RELGI-treated horses after 35 days of treatment. It should also be noted that 3 of 6 (50%) control horses also showed improvement in ESGD scores. The results of the study presented here were similar to a previous study where horses were administered a similar polysaccharide gel containing hyaluronan and schizophyllan for 30 days (Slovits, 2017). In that study, 90% (9 of 10) of the horses showed complete resolution and/or improvement in ulcerative areas in both non-glandular and glandular regions and showed increased appetite, weight gain and positive behavioural changes. In addition, a positive response was noted in 87% of the horses. The improvement in ESGD in the control horses the study

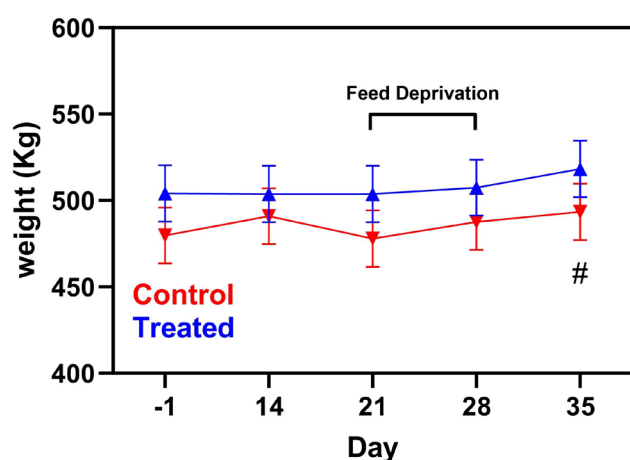


FIGURE 6 Mean $\pm$ SEM bodyweight (weight [kg]) in horses fed a supplement, treated containing hyaluronan and β -glucan (Relyne^{GI}®) or control (supplement without the active ingredients) for 35 days. Day indicates gastroscopy times. Horses were weighed the morning of the gastroscopy examination. #Significant difference in bodyweight in the treatment and control groups combined compared to Day -1.

reported here was likely to be due to reduction in stress, improved management and nutrition.

The reason for the improvement in the ESGD scores in the study reported here was due to the polysaccharides, hyaluronan, and TBG 136™ β -glucans in the supplement, as the control group received the same supplement without the active ingredients, hyaluronan and S β -glucan. Hyaluronan and S β -glucan are surface-acting polysaccharides that provide mucosal protection against the corrosive and oxidative effects of prolonged exposure to stomach acids (hydrochloric acid, volatile fatty acids, and bile acids), which likely cause non-glandular ulcers in horses (Andrews et al., 2006; Murray & Eichorn, 1996; Nadeau et al., 2000). The ESGD in horses has been likened to gastroesophageal reflux disease (GERD) in people, where prolonged exposure to stomach acids can breach the defensive barrier and lead to inflammation and loss of barrier integrity, resulting in ulceration (Herdiana, 2023). Although inflammatory cells are not typically found in the histopathological examination of ulcerated ESGD, a recent study showed that inflammatory biomarkers, adenosine deaminase (ADA) and the protein S100A12, were significantly higher in the saliva and serum of horses with ESGD compared to horses without ESGD (Contreras-Aguilar et al., 2022).

Horses in the study reported here had very few mild glandular lesions. The low prevalence of EGGD in these horses was likely due to breed differences, as Thoroughbred horses have been reported to have a lower prevalence of EGGD compared to sport horses and Warmblood show jumping horses (Banse & Paul, 2024).

Hyaluronan is a naturally occurring polysaccharide and a common constituent of the extracellular matrix of synovial fluid, connective tissue, and stomach mucosa (Goa & Benfield, 1994). Hyaluronan, the RELGI supplement administered in this study, was high molecular weight (2,000,000 Daltons), which has been shown to regulate the inflammatory response and reduce the deleterious effects of oxygen-free radicals and enzymes. Inflammation likely occurs in horses with EGUS, and a recent study showed that adenosine deaminase (ADA) and the protein, S100A12, inflammatory markers, were significantly increased in saliva samples in horses with endoscopically confirmed EGUS (Contreras-Aguilar et al., 2020, 2022; Yan et al., 2008), and these two salivary markers were strongly associated as markers of inflammation (Gutiérrez et al., 2024). In addition, two previous reports identified inflammatory infiltrates histologically in non-glandular (Yuki et al., 2000) and glandular (Banse et al., 2023) mucosa. Although neither salivary markers nor mucosal samples were evaluated in the study reported here, inflammation is likely to occur once the mucosa is damaged, and the properties of high molecular weight hyaluronan have been shown to downregulate inflammation. The exact mechanism is unknown, but hyaluronan receptors in other species are present on leukocytes, fibroblasts, endothelial and epithelial cells, and high molecular weight hyaluronan might have recruited these cells and stimulated them to produce inhibitory cytokines, leading to mucosal cell repair and the reduction of inflammation (Wolny et al., 2010).

In addition, high molecular hyaluronan has been shown to have antibacterial, antifungal, and antiviral properties (Ardizzoni

et al., 2011; Cermelli et al., 2011). Previously, a study showed that ESGD, once formed, were colonised by resident bacteria (gram-positive cocci), resulting in delayed ulcer healing (Yuki et al., 2000). In addition, a recent study showed that there were modest differences in the mucosal microbiome associated with EGGD (Paul et al., 2021, 2023; Voss et al., 2022), which suggested a bacterial component might contribute to the formation and/or persistence of these lesions.

Finally, hyaluronan is a hygroscopic macromolecule that is highly osmotic. Hyaluronan has been shown to form scaffolds by binding to sulphur proteoglycans on the gastric mucosa. This scaffolding might cover a large surface and be able to trap large quantities of water and ions, providing tissue hydration and turgidity to resist acid damage. Tissue hydration during the inflammatory process might facilitate the response to tissue injury and lead to rapid healing, and in some situations, prevent further ulcer formation. In addition, high molecular weight hyaluronan scaffolding prevents diffusion of other substances from the mucous membranes, which supports cell protection and renewal (Volpi et al., 2009). A horse in the study reported here did not develop either ESGD or EGGD during the trial. Although in this horse, one might conclude that hyaluronan protected the stomach from the formation of ulcers.

S β -glucan (TBG-136™ Schizophyllan) used in this study was a schizophyllan produced from the patented strain of common wood fungus *Schizophyllum commune* and was crossbred to produce heightened immune activity. The S β -glucan is found as an integral cell constituent in the fungus. Studies in other species have shown that S β -glucan activates differentiation and proliferation of immune cells, including dendritic cells, macrophages, natural killer cells, and B and T lymphocytes via the plasma membrane bound receptor Dectin1 (Brown et al., 2003; Herre et al., 2004). These are biological defence modulators that nutritionally potentiate the immune response and have probiotic properties, including antioxidant and antibacterial actions. The S β -glucan and high molecular weight hyaluronan potentially have a synergistic effect to protect the stomach mucosa and enhance barrier function. Hyaluronan might strengthen the physical barrier of the stomach lining, while S β -glucan indirectly supports healing and a healthy gut environment that can further contribute to reducing inflammation and protecting the stomach. In addition, high molecular weight hyaluronan provides a structural framework for the newly formed tissue, helping organise collagen and other components (Al-Bayaty et al., 2011). This contributes to the strength and integrity of the healed wound. High molecular weight hyaluronan, combined with S β -glucan's immune stimulation, might promote faster healing of gastric lesions. In addition, S β -glucan has been shown to accelerate wound healing by stimulating cell growth through increased production of fibroblasts, which are essential for creating new tissue and collagen in the wound bed. S β -glucan promotes angiogenesis with the formation of new blood vessels, crucial for delivering nutrients and oxygen to the injured areas and enhances immune response by stimulating the activity of macrophages (immune cells might clear debris and fight infection at the wound site). Overall, hyaluronan and S β -glucan might act synergistically

to impart a positive effect on stomach health in horses when given orally.

There were several limitations in this study, including a small sample size, a short treatment period (35 days), the prevalence of EGGD was low, and lesions seen were not severe; horses were individually stalled and were not exercised or in training, and the Thoroughbred breed. We did find significant differences in ESGD scores in the study, even though the sample size was small. The treatment period (35 days) was short, and a longer treatment period might have shown different results, including the long-term effects of the RELGI supplement or recurrence of ulcers. In addition, ESGD was not very severe in these horses and ESGD ulcer scores decreased in control horses after moving into stalls from pastures, which was somewhat unexpected. However, significant treatment effects were found regarding ESGD scores. Although there was a treatment effect seen in horses in this study, a supplement should not be used to treat ESGD; instead, it should be used during or after appropriate pharmaceutical treatment.

Furthermore, horses were not exercised or in training, and exercise has been shown to be a risk factor for ESGD in horses. The RELGI supplement studied might not have such a robust effect on horses that are exercising. Although in a previous study, horses treated with a similar supplement containing hyaluronan and Schizophyllan showed marked improvement (87%) in ulceration scores and clinical signs (Slovits, 2017).

CONCLUSIONS

Overall, the supplement RELGI was readily consumed, and no adverse events were observed in this study. The RELGI (1 oz., orally, 3x daily) improved non-glandular stomach health in this population of Thoroughbred horses undergoing stall confinement and feeding stress, while maintaining the physiological gastric pH. Horses also increased bodyweight, and blood values remained in the normal reference ranges. The RELGI supplement administered to horses in this study reported here should not be used to treat ESGD, but as an adjunct or continued therapy after appropriate pharmacologic treatment. Comprehensive management of horses is recommended, including diet, housing, exercise and stress management to maximise treatment and prevention of ESGD. Future studies with larger cohorts would be valuable to further validate these findings, particularly in breeds with a higher prevalence of EGGD, where individual variation may be greater.

CLINICAL RELEVANCE

- The supplement containing hyaluronan (hyaluronic acid) and Schizophyllan (beta-glucan) (Relyne^{GI}, 1 ounce, orally, Q8h) was readily consumed and no adverse events were observed.
- Administration resulted in improvement in non-glandular and glandular stomach health in this population of stall-confined

horses undergoing feed stress. Gastric juice pH and body weight did not change throughout the study period.

- This supplement improved stomach health and should be used as an adjunct along with appropriate pharmacologic treatment and management of gastric ulcers.

AUTHOR CONTRIBUTIONS

F.M. Andrews: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; visualization; writing – review and editing; supervision; project administration; resources. **S. Mordoh:** Investigation; methodology; visualization; writing – review and editing; project administration; resources. **M. L. Keowen:** Investigation; methodology; validation; visualization; writing – review and editing; project administration; supervision. **F. Garza Jr:** Investigation; methodology; validation; visualization; writing – review and editing; project administration; supervision. **C. -C. Liu:** Methodology; validation; writing – review and editing; formal analysis.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

ETHICS STATEMENT

The procedures performed on the horses during study were approved by the Louisiana State University Institutional Animal Care and Use Committee (IACUCAM-23-022).

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