Effectiveness of a complementary feed containing extract of *Griffonia Simplicifolia and Olea Europaea* on horses affected by equine gastric ulcer syndrome

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SUMMARY

Equine gastric ulcer syndrome (EGUS) is a worldwide disease, found especially in racehorses, although it can affect different categories of animals with difference in prevalence. Recently, two different diseases have been recognized, the first affecting the squamous mucosa (Equine Squamous Gastric Disease, ESGD) and the other the glandular mucosa (Equine Glandular Gastric Disease, EGGD), with different pathophysiology, risk factors and management. To date, the main strategy for the treatment involves acid-suppressive therapy with omeprazole, often associated with sucralfate; yet the total safety and absence of side effects of this treatment have recently been questioned. Recently, an increasing number of studies have been investigating the use of complementary feeds to manage of these health issues. The purpose of this study is to evaluate the effectiveness of a complementary feed administered for 28 days for EGUS control. A gastroscopy (T0) was used to select sixteen horses with lesions on the squamous mucosa, graded between 0/4 and 4/4. A second gastroscopy was performed at the end of the supplementation (T1) to assess any changes in the lesions score. The data obtained did not reveal statistically significant differences that might enable us to objectively establish whether the product is effective in the case of these gastric pathologies. However, gastroscopic images showed a clear improvement in the lesions and in the appearance of the mucosa even in the absence of score variations, suggesting that 28 days of administration may not be sufficient for complete healing. During the trial the treated subjects displayed an improvement in behavior and appeared to be more relaxed and inclined to work. The results allow us to affirm that, despite not being statistically relevant, the feed supplement improved not only the appearance of the gastric mucosa, especially of the squamous mucosa, but also the well-being of the horses, making them more willing to work. Further studies are needed, with an increased number of horses and a longer length of supplementation period.

KEY WORDS

Griffonia simplicifolia; *Olea europaea*; ESGD; EGGD; gastric ulcers.

INTRODUCTION

Equine Gastric Ulcer Syndrome (EGUS) is a multifactorial pathology characterized by the presence of erosive and ulcerative lesions in the gastric mucosa. Recently [1-5], EGUS has been divided into two different diseases: Equine Squamous Gastric Disease (ESGD) and Equine Glandular Gastric Disease (EGGD) in relation to the location of ulcers in the squamous or glandular portion of the stomach, respectively. Prevalence varies with breed and training level and different incidence between ESGD and EGGD has been found [1-13]. All ages and breeds are susceptible to EGUS, yet factors such as diet and management play a pivotal role in the ulcer formation and maintenance [1,2,5,14]. The highest prevalence is found in sport horses, with percentages ranging from 66-91% in thoroughbred horses and with increases of up to 80% -100% when in full activity [1-5,7,15]. A prevalence of 40% has been reported in Quarter Horses [1,2,5,16,17]. In addition to intense exercise and competitions, the risk factors include nutrition and stress [1-5,12,18,19]. The reported clinical signs appear to be varied and nonspecific and include not only anorexia or disorexia, a poor body condition score and weight loss, chronic diarrhea, a dull, shaggy coat, bruxism, behavioral changes (including aggression and nervousness) and recurrent or acute colic but also reduced performance [1-5,14,17,20]. The treatment is based on the maintenance of a gastric pH above 4 to allow the mucosa to initiate the reparative processes. Proton pump inhibitors and H2 receptor antagonists are the most commonly used drug classes in horses. In the first class, omeprazole is the most widely studied [1-5,17,20]. However, these treatments entail several disadvantages: they are expensive and require a veterinary prescription, if discontinued they may lead to relapses, and, if administered for prolonged periods of time they may increase the

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gastric pH excessively, leading thus to inadequate digestion [17,21,22]. In two recent papers, Sykes sought to assess whether the use of omeprazole was correctly deemed as safe and devoid of both short-term and long-term adverse effects. In the author's view there may be a correlation between the use of omeprazole and gastric hyperacidity due to a rebound effect, a decrease in calcium absorption and the destruction of normal balance and activity in the large intestine [21,22]. Furthermore, omeprazole-based pharmaceutical products are not permitted in some competitions [17]. For this reason, there has been a growing interest in evaluating valid alternatives based on the use of complementary feeds [14,17,23-27].

Griffonia simplicifolia is an African plant, the seeds of which are rich in 5-hydroxy-l-tryptophan (5-HTP), a precursor of serotonin (5-HT) [28]. This is a monoamine that has a role as a neurotransmitter in the central nervous system, including a role in the control of mood and memory. Its depletion is responsible for anxiety, depression, schizophrenia, etc., but the administration of 5-HTP orally increases the concentrations of 5-HP in the central nervous system. In human medicine, this plant is used successfully to treat migraine, depression, anxiety, weight loss and insomnia [29-32].

Olea europaea is a fruit tree used mainly for nutritional purposes. In phytotherapy, it is used to prevent diabetes, cardiovascular diseases, cancer and malaria. Fruits and leaves, rich in polyphenoles, especially oleuropein and hydroxytyrosol, have various effects, e.g. antioxidant, hypoglycaemic, anticancer, hypotensive, etc. [33-36]. On gastric diseases, *Olea europaea* extracts, from both fruits and leaves, have demonstrated a positive effect, with a reduction in the acidity of the stomach content and the prevention of the development of gastritis and gastric ulcers in rats [33,34,37]. Furthermore, the leaf extracts have the ability to block calcium channels, reducing the secretion of histamine and the production of gastric acid [38].

Considering the properties of the extracts of *G. simplicifolia* and *O. europaea*, and the need for effective nutraceutical products for the treatment and prevention of gastric ulcers in horses, aim of this study was to investigate the use of a novel commercial feed supplement (Gastro Horse Relax, NBF lines, Milan, Italy) to manage of gastric ulcers in Quarter Horses.

MATERIALS AND METHODS

Animals

The study was carried out in collaboration with the University of Teramo and the University of Perugia and authorized by the Ethical Committee of the University of Teramo (CEISA) under protocol No. 15/2019. American-riding trained adult horses were included, with no restriction of breed or sex. Horses were excluded if they presented signs of systemic diseases or were treated with any kind of medication, especially those of the stomach and the gastrointestinal system. Animals could be excluded at any time at the owners' request.

Sixteen horses, were enrolled, aged between 3 and 16 (mean 9, s.d 5). All were used for Reining or Performance training. Most were Quarter Horses (9/16, 56%), 2/16 (13%) were Paint, 2/16 (13%) were mix breed, 1/16 (6%) was Thorougbred, 1/16 (6%) Appaloosa and 1/16 (6%) Italian Saddlebred. Based on the sex, 6/16 (38%) were geldings, 1/16 (6%) were males and 9/16 (56%) were females (Table 1).

Table 1 - List of horses enrolled in the study, QH: quarter horse, SCN: mixed breed, SI: Italian saddlebred, G: gelding, M: male, F: female.

	Breed	Gender	Age
1	QH	G	3
2	QH	G	13
3	QH	M	16
4	QH	F	8
5	QH	G	4
6	Paint	F	3
7	QH	F	4
8	Appaloosa mix	F	14
9	PSI	G	15
10	QH	F	10
11	QH	G	4
12	Appaloosa	G	10
13	Paint	F	6
14	SCN	F	14
15	SI	F	10
16	QH	F	6

Before the start of the study, a questionnaire was given to the owners, with questions about the management and feeding regimen of the horses (e.g., type of housing, the access to a paddock, type of food and diet, the potential use of additional complementary feeds, the level of exercise carried out daily/weekly, whether the horse had been moved in the months prior the study and the presence of any previous or current systemic pathologies that may be associated with therapeutic treatments). The owners were interviewed to inquire whether they had noticed symptoms or behaviors related to EGUS (weight loss, recurrent colic, reduced performance, behavioral changes, etc.[1-5]). According to the questionnaires, management was similar for all horses: all the animals were housed in boxes with access to a sand paddock a few times a week and were trained for one hour every day. The diet involved the administration of 8-10 kg of mixed hay (with alfalfa) and an average of 4 kg of concentrate per day. Only 1/16 (Table 2 n.5) had been moved to a different stable the month before the study began and consequently both food and work management had changed. According to the owners, none of the horses showed any sign of gastric ulcers,

Gastroscopic examination

Before each gastroscopy, a physical examination was carried out and the weight of the animal was measured with a metric tape [39].

Endoscopy was performed according to bibliography [2,3], the animals were fasted for 16-18 hours and were sedated with alpha-2-agonist drugs (xylazine, 0.6-1 mg / kg IV). A portable processor (Tele Pack Vet X Led, Karl Storz, Germany) and an endoscope 3m long and 10.4mm in diameter (60130PKS; Karl Storz, Germany) were used. The scope entered through the lower nasal meatus, the nasopharynx and the esophagus to reach the stomach. The organ was distended with air to allow a clear view and the mucosa was rinsed with water if it was covered

Table 2 - Composition of the feed supplement (Gastro Horse Relax, NBF Lanes Milano) used in the study.

Ingredients	Percentage
Soya shell powder	25,52
Maltodextrin	24,75
Dextrose	10,75
Spray dried malt powder	8
Lecithin powder	4
Guar gum	4
Kaolinitic clay	4
Pectin powder	4
Alfalfa leaf fine powder	4
Carrots fine powder	4
Magnesium hydroxide	2
Olea europaea leaf extract	2
Griffonia simplicifolia (98%)	1
Precipitated silicic acid	1
Magnesium oxide	0,80
Apple flavoring powder	0,10
Saccharomyces cerevisiae	0,08

with foam and/or residual food material. The feed supplement was added to the standard diet of the horses for 28 days. At the end of this period (T1), a complete physical examination, the recording of the weight with a metric tape and a control endoscopy were performed. At the same time, the owners were asked whether they had noticed any improvement in the horse's clinical situation, including its willingness to work.

At the end of the trial, gastroscopy recordings were reviewed and the grading score was assigned by a veterinarian who was unaware of the time (T0 or T1) when the investigations had been carried out. Ulcers in the squamous portion were graded using the scoring system by the ECEIM Consensus Statement. As bibliography lacks a standard scoring system to classify the severity of lesions for EGGD, the horse was considered positive or negative only on the basis of the presence or absence of lesions, regardless of their severity [1,2].

Feed supplement

The feed supplement (Gastro Horse Relax, NBF Lanes Milano) introduced into the diet is a product containing *Griffonia simplicifolia* and *Olea europaea* extracts associated with other nutraceutical components (Table 2). It was administered orally in quantities of 30 g twice daily for 28 days, mixed with the feed. During this period the daily management of the animal remained unchanged.

Statistical Analysis

The data was collected in a Microsoft Excel for the subsequent descriptive statistical evaluation. The difference between the grade for ESGD, the presence of EGGD and weight estimation before and after treatment was assessed using paired sample sign test with significance set to $p < 0.05$. Statistical analysis was performed using R commander package (R Core Team, 2020).

RESULTS

Before supplementation, 14/16 (88%) animals had ESGD with a score between ¼ and 4/4, whereas 5/16 (31%) were positive for EGGD, as shown in Table 3 and pictures 1 and 2.

Table 3 - Presence of ESGD (grade 0-4/4) and EGGD (0: negative, 1: positive) and weight estimation before and after 28 days of administration of the feed supplement (Gastro Horse Relax, NBF Lanes Milano) [2,39].

Horse	T0 ESGD	T1 ESGD	T0 EGGD	T1 EGGD	T0 Weight	T1 Weight
1	1	1	0	0	477	477
2	1	0	0	1	477	484
3	2	2	1	0	514	530
4	2	1	0	0	522	545
5	2	4	0	0	484	484
6	2	2	1	1	393	407
7	1	2	0	0	407	448
8	2	1	1	0	463	463
9	0	1	0	0	470	507
10	4	4	0	1	470	484
11	4	4	0	0	430	477
12	4	1	0	0	448	470
13	1	1	0	0	470	463
14	0	0	0	0	593	585
15	1	1	1	0	570	600
16	4	2	1	0	455	470

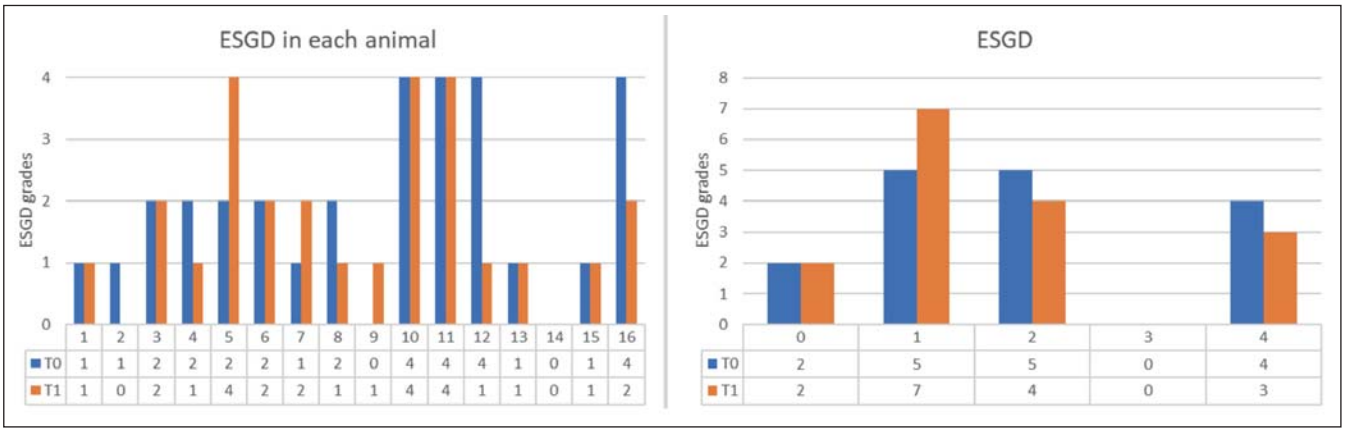

Figure 1 - Distribution of the lesions of the squamous mucosa (ESGD) before (T0) and after (T1) 28 days of supplementation; on the left results for each animal, on the right distribution of the lesions in the population.

After supplementation 5/16 (31%, Table 3 nos.2-4-8-12-16) horses showed a score improvement for ESGD, whereas 8/16 (50%, Table 3 nos. 1-3-6-10-11-13-14-15) showed no change and 3/16 (19%, Table 3 nos.5-7-9) displayed a higher score (figure 1).

While 4/5 horses (80%, Table 3 nos. 3-8-15-16) positive for EGGD no longer showed glandular lesions at T1, 1/5 (20%, Table 3 nos.6) still tested positive. 2/11 horses (18%, Table 3 nos. 2-10) that were negative for EGGD at T0 showed lesions in the glandular mucosa after the treatment (fig. 3).

After supplementation, weight had increased in 11/16 horses (69%, Table 3 nos. 2-3-4-6-7-9-10-11-12-15-16), decreased in 2/16 (12%, Table 3 nos. 13-14) and did not change in 3/16 (19%, Table 3 nos. 1-5-8).

ESGD scores, the presence of EGGD and weight did not show statistically significant differences before and after supplementation (fig 1, 3 and 4).

All owners, however, noticed an improvement in the horse's behavior: the animal was more willing to work and displayed a relaxed attitude during the training session.

DISCUSSION

Although all ages and breeds are susceptible to EGUS, diet and management appear to be predisposing factors and play a key role in ulcers formation and maintenance [1,2,4,5,12,18,20]. Therefore, interventions acting on these factors may help to limit the risk of gastric lesions [1,2,4,12,14,17]. A recent study by Luthersson et al [40] highlighted the importance of specially formulated diets to maintain adequate gastric function and decrease the risk of relapse after treatment with omeprazole.

The most widely used drug therapies to treat ulcers aim to decrease gastric secretions by increasing the pH, in order to create a more favorable environment for the healing of mucosal lesions [1,2,5,14,17,20]. The drug recommended is omeprazole possibly associated, in presence of EGGD, with sucralfate [1,2,4,17].

Recent interest in the use of plant-based products, nutraceuticals, oils and minerals has been increasingly growing as drugs are expensive, they can be considered as doping in certain competitions, they require a veterinary prescription and long-term therapies may lead to an excessive increase in pH with subsequent problems to digestion and absorption [1,2,4,14,17,21,22].

Given this growing trend to seek therapeutic alternatives, the aim of the study was to evaluate whether the use of a feed supplement (Gastro Horse Relax, NBF Lanes Milano) containing extracts of *Griffonia simplicifolia* and *Olea europaea* may be a beneficial support tool in the case of gastric ulcers in horses. A study by Jan in 2010 [34] assessed the effect of the administration of *Olea europaea* on the volume of gastric secretions and on the acidity in rabbits after induction of gastric secretion with carbachol, obtaining positive data on both its efficacy and its safety of use. The 13% leaf extract has been shown to have the ability to block calcium channels (Ca ++) [33,37,38]. Agents that have this action are mainly used for treatment of cardiovascular diseases as they inhibit muscle contraction. Gastric motility and secretion, however, depend on the concentration of calcium ions: the release of histamine by the peritoneal mast cells is closely linked to the extracellular concentration of Ca ++, and a decrease in its levels would therefore lead to a reduction in the effects of histamine with less production of gastric acids [34,38].Thanks to its antioxidant activity and ability to control the inflammatory process, oleuropein contained in the olive leaves can be used as a natural therapy for gastritis [33,37].

Griffonia simplicifolia seeds are very rich in 5-hydroxy-tryptophan (5-HTP), a direct precursor of serotonin [29-32]. 5-HTP is able to quickly cross the blood-brain barrier, increasing its concentration in the central nervous system. Its therapeutic potential, both as an anxiolytic-like product and as a support tool in pathologies linked to serotonin depletion, has been investigated in several studies with positive results. [29,30]. To date, the seed extract of this plant is commonly used to treat anxiety, depression, insomnia, migraines and headaches [29-32]. Tests carried out on herbal preparations or nutraceuticals to treat EGUS have yielded dissimilar and not always positive results [14,17,23-27,41]. The data revealed by the study presented in this manuscript, indicates that the supplement (Gastro Horse Relax, NBF Lanes Milano) used, does not show a statistically relevant effectiveness in preventing or healing ulcers already present. It should be noted, however, that many horses (13/16, 81%) which were administered the product showed an improvement or at least a score stability for ESGD and it was effective in the majority of horses affected by EGGD (4/5, 80%). Even individual animals which did not display a change in the ESGD score (8/16, 50%) exhibited a noticeable improvement in lesion size and depth (Fig. 2). A tendency to ulcer healing

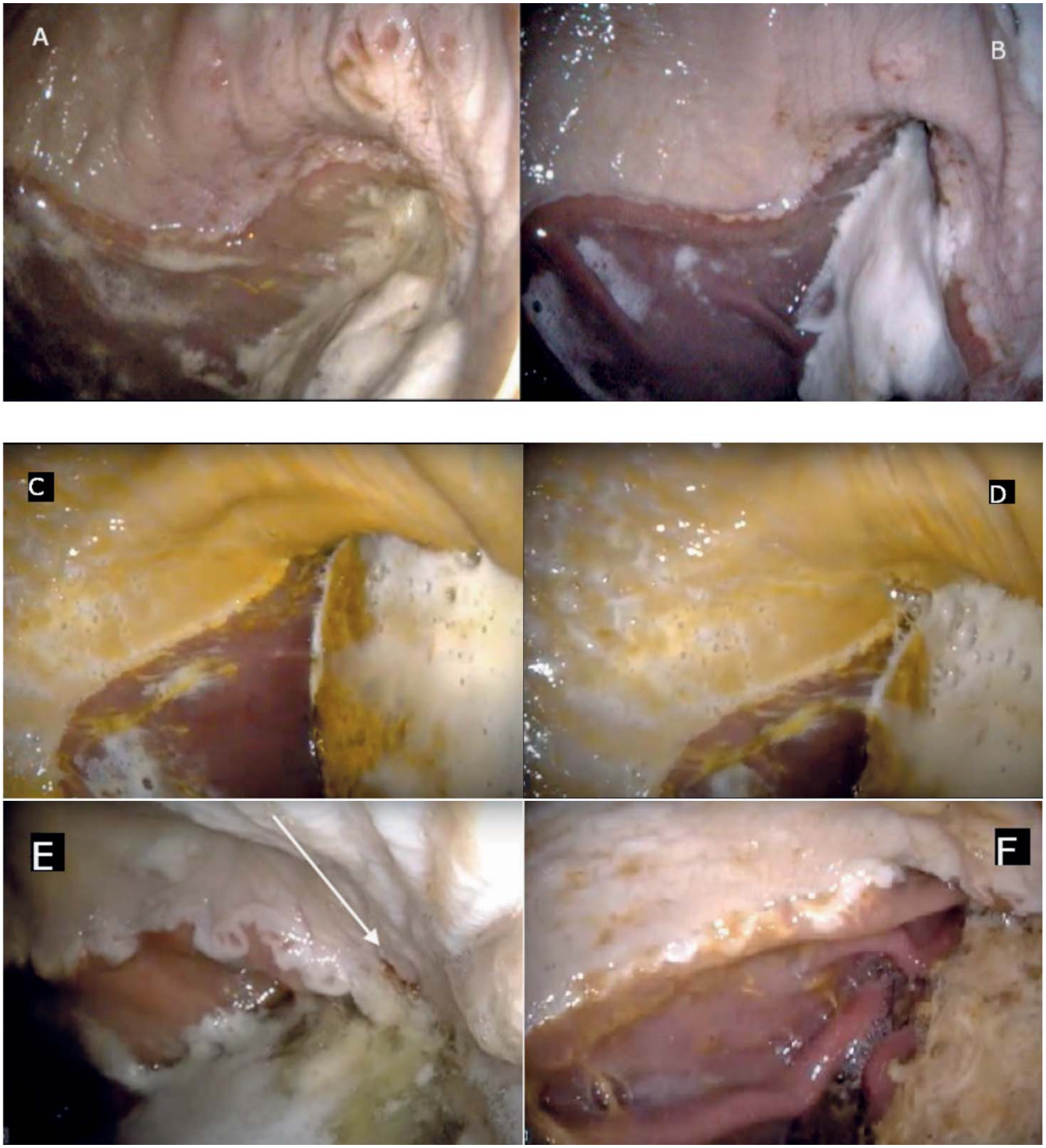

Figure 2 - Upper: Horse no. 10 A ESGD 4 at T0, presence of deep areas of ulceration on the lesser curvature, next to the cardias and along the margo plicatus, B ESGD 4 at T1, the areas of deep ulceration are still present along the margo plicatus on the lesser curvature, but the lesions are smaller in diameter and lesser in number;
Middle:Horse no. 12 C ESGD 4 at T0, presence of deep areas of ulceration along the lesser curvature, along the margo plicatus, D ESGD 1 at T1, presence of yellowing of the mucosa (hyperkeratosis), along the lesser curvature;
Lower: Horse no.16: E ESGD 4 at T0, presence of a deep area of ulceration (arrow) on the lesser curvature, F ESGD 2 at T1, presence of smaller areas of erosion along the lesser curvature, no areas of deep ulceration are visible after treatment.

was visible in many gastroscopy images and an improvement in the general appearance of the mucosa was noted (Fig. 2). In addition, the horse that had undergone a change of management (Table 3 subject No. 5) due to the move from a different stable in the month prior to the study was the only one to show a significant worsening of the ESGD score, rising from 2/4 to 4/4. This may be linked to an increase in the risk class for ulcer development: changes in management, movement and variations in the social dynamics of horses are among the major determinants of the development of gastric lesions; furthermore, the animal was subjected to a higher workload, which is also associated with an increased risk of presenting gastric ulcers [1,2,12,18]. One other horse (table 3, number 7) showed an increase in the score for ESGD, but this was not as

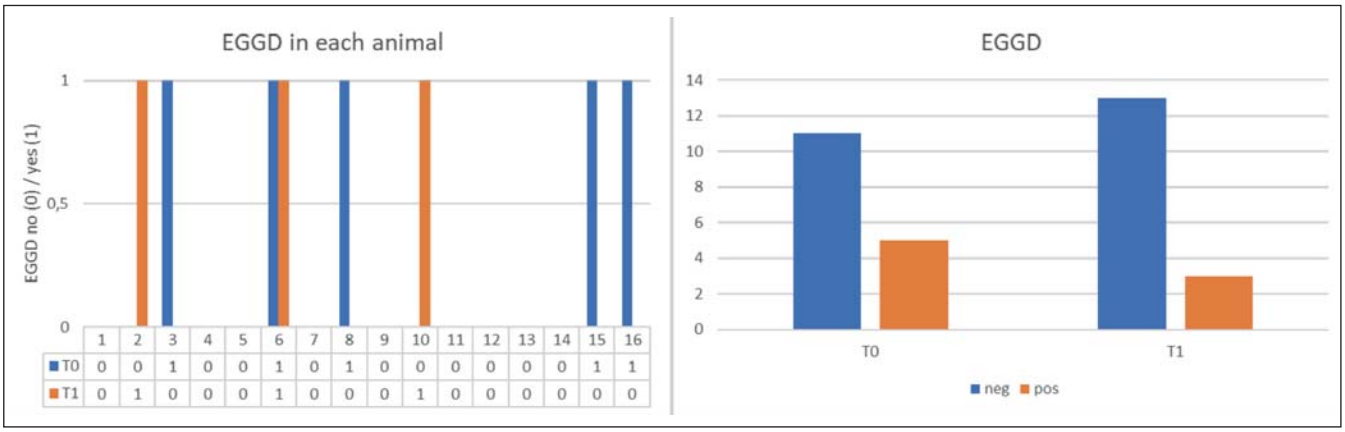

Figure 3 - Distribution of the lesions of the glandular mucosa (EGGD) before (T0) and after (T1) supplementation; 0: negative, 1: positive; on the left, results for each animal, on the right, distribution of lesions in the population.

marked, going from grade 1 to grade 2. In recent years, the clinical significance of grade 2 has been questioned, since many horses with ESGD this severe do not show clinical signs and these lesions could heal spontaneously more easily than other grades [1,2,4,5,13,18].

Many of the animals gained weight (11/16, 69%, Table 3), but the increase was not statistically significant. This could be related to a better digestion of the feed, due to an improvement in the stomach environment, secondary to the start of the healing process of the gastric lesions [1,2,5,13].

During the study, the owners reported an improvement in attitude and willingness to work, in some cases even a calmer demeanor. These improvements are consistent with the findings of various authors: reduced performance and behavioral changes may be caused by the presence of gastric diseases [1,2,4,5,14,17,20,42] and an improvement in the demeanor of the horse could be related to a decreased discomfort. Furthermore, the increase in serotonin caused by the intake of 5-HTP contained in *Griffonia simplicifolia* leaf can be responsible for the calmer attitude of the animals [29,30].

The analysis of the questionnaires completed by the owners allowed us to observe that the animals' management and diet shared many similarities, even though they were housed in two different stables. For this reason, the presence of ulcers and/or the unchanged score of some subjects may possibly be due to an individual predisposition or to a different degree/type of training.

The study shows a very high prevalence of ESGD in this population (14/16, 88%), similar to the percentages observed in sport horses [1,2,4,5,16], although higher than those identi-

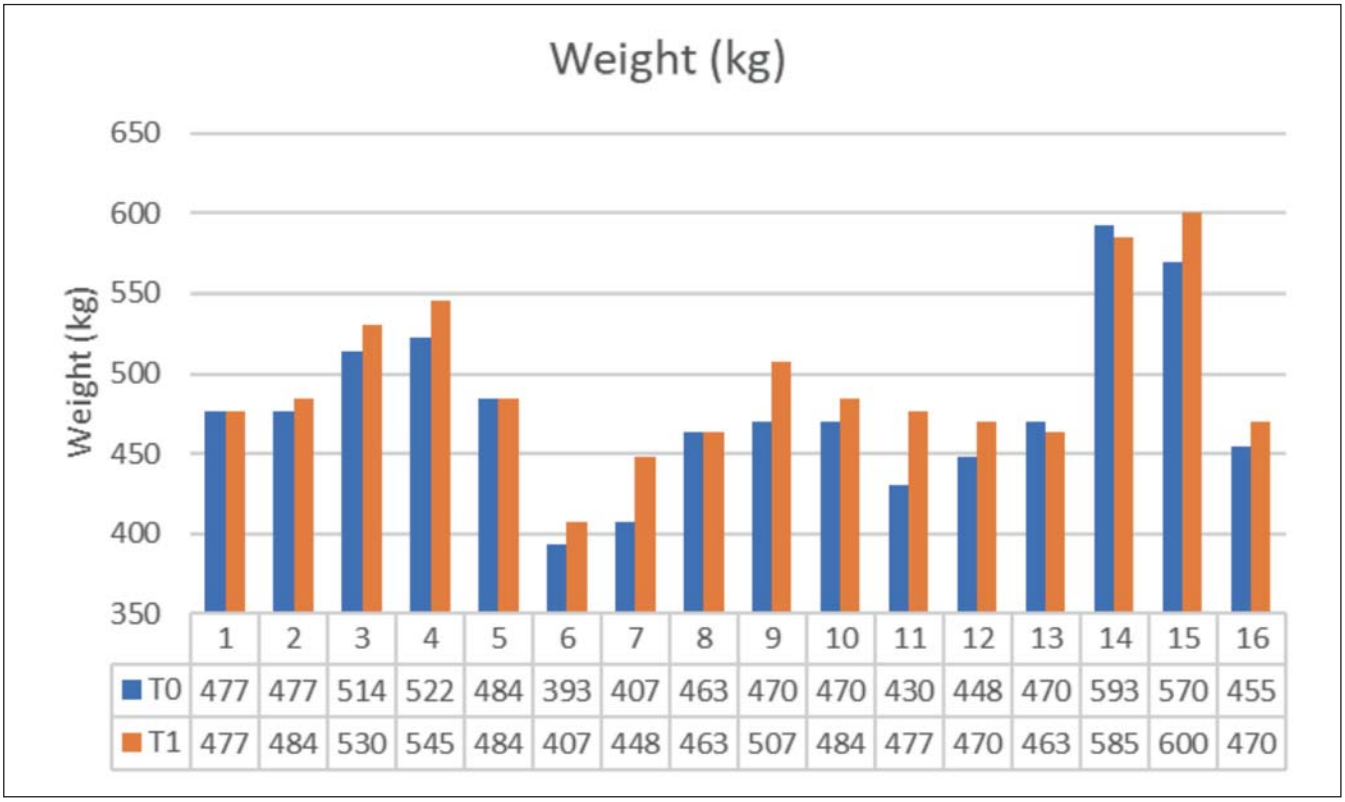

Figure 4 - Weight of the horses before (T0) and after (T1) supplementation.

fied for the Quarter Horse breed [16] which is generally used for this type of activity. This could be related to the different management of the horses in this population compared to those in the literature, but could also be caused by the different breeds included in the study.

CONCLUSIONS

The feed under study did not lead to a resolution or improvement of gastric lesions in the group of horses evaluated. However, this may be related to the small number of animals investigated and to the great variability of score at inclusion, with the presence of high-grade lesions. Nevertheless, this research has revealed a change in the appearance of mucosa with a tendency to a reduction in the number and size of lesions, even in the most severe cases.

This suggests that 28 days of therapy may not be sufficient to achieve improvement in the majority of the treated horses and that more significant results may be obtained by administering the nutraceutical for a longer period. The feed supplement enabled improved behavior of the treated individuals, which were more relaxed and willing to work. This outcome may be ascribed to the increased release of serotonin due to the presence of *Griffonia simplicifolia* seed extract in the nutraceutical. This trial is to be considered as a preliminary study that deserves further research, also in light of both the high prevalence of ESGD and EGGD in horses and the increasing request for complementary feeds to support drug treatments.

References

1. Rendle D, Bowen M, Brazil T, Conwell R, Hallowell G, Hepburn R, et al. (2018) Recommendations for the management of equine glandular gastric disease. UK-Vet Equine;2:2-11. doi:10.12968/ukve.2018.2.s1.3.
2. Sykes BW, Hewetson M, Hepburn RJ, Luthersson N, Tamzali Y. (2015) European College of Equine Internal Medicine Consensus Statement-Equine Gastric Ulcer Syndrome in Adult Horses. J Vet Intern Med;29:1288-99. doi:10.1111/JVIM.13578.
3. Sykes BW, Jokisalo JM. (2014) Rethinking equine gastric ulcer syndrome: Part 1 - Terminology, clinical signs and diagnosis. Equine Vet Educ;26:543-7. doi:10.1111/EVE.12236.
4. van den Boom R (2022). Equine gastric ulcer syndrome in adult horses. Vet J;283-284:105830. doi:10.1016/J.TVJL.2022.105830.
5. Sanchez LC. (2018) Disorders of the Gastrointestinal System. In Equine Internal Medicine, Ed. Reed S.M., Bayly W.M., Sellon D.C., 4th Ed., , p. 709-842, Elsevier, St. Louis, Missouri. doi:10.1016/B978-0-323-44329-6.00012-7.
6. Begg LM, O'Sullivan CB. (2003) The prevalence and distribution of gastric ulceration in 345 racehorses. Aust Vet J;81:199-201. doi:10.1111/j.1751-0813.2003.tb11469.x.
7. Bell RJW, Kingston JK, Mogg TD, Perkins NR. (2007) The prevalence of gastric ulceration in racehorses in new zealand. N Z Vet J;55:13-8. doi:10.1080/00480169.2007.36729.
8. Nied wied A, Kubiak K, Nicpo J. (2013) Endoscopic findings of the stomach in pleasure horses in Poland. Acta Vet Scand;55:45. doi:10.1186/1751-0147-55-45.
9. le Jeune SS, Nieto JE, Dechant JE, Snyder JR. (2009) Prevalence of gastric ulcers in Thoroughbred broodmares in pasture: A preliminary report. Vet J;181:251-5. doi:10.1016/J.TVJL.2008.03.020.
10. Tamzali Y, Marguet C, Priymenko N, Lyazrhi F. (2011) Prevalence of gastric ulcer syndrome in high-level endurance horses. Equine Vet J;43:141-4. doi:10.1111/j.2042-3306.2010.00129.x.
11. Luthersson N, Nielsen KH, Harris P, Parkin TDH. (2009) The prevalence and anatomical distribution of equine gastric ulceration syndrome (EGUS) in 201 horses in Denmark. Equine Vet J;41:619-24. doi:10.2746/042516409X441910.
12. Busechian S, Sgorbini M, Orvieto S, Pisello L, Zappulla F, Briganti A,Nocera I, Conte G, Rueca F. (2021) Evaluation of a questionnaire to detect the risk of developing ESGD or EGGD in horses. Prev Vet Med;188. doi:10.1016/j.prevetmed.2021.105285.
13. Busechian S, Turini L, Sgorbini M, Bonelli F, Pisello L, Pieramati C, Orvieto S, Rueca F.(2022)Body Condition Score Is Not Correlated to Gastric Ulcers in Non-Athlete Horses. Anim, Vol 12, Page 2637 2022;12:2637. doi:10.3390/ANI12192637.
14. Andrews FM, Larson C, Harris P. (2017) Nutritional management of gastric ulceration. Equine Vet Educ;29:45-55. doi:10.1111/EVE.12495.
15. Cate RE, Nielsen BD, Spooner HS, O'Connor-Robison CI, Schott HC. (2012) Prevalence of gastric ulcers and relationship to other parameters in Standardbred racehorses. Comp Exerc Physiol;8:47-52. doi:10.3920/CEP12009.
16. Bertone J. (2002) Prevalence of Gastric Ulcers in Elite, Heavy Use Western Performance Horses. in 48th Annu. AAEP Conv., vol. 48,, p. 256-9.
17. Zavoshti FR, Andrews FM. (2017) Therapeutics for Equine Gastric Ulcer Syndrome. Vet Clin Equine Pract;33:141-62. doi:10.1016/J.CVEQ.2016.11.004.
18. Sykes BW, Bowen M, Habershon-Butcher JL, Green M, Hallowell GD. (2019) Management factors and clinical implications of glandular and squamous gastric disease in horses. J Vet Intern Med;33:233-40. doi:10.1111/JVIM.15350.
19. Malmkvist J, Poulsen JM, Luthersson N, Palme R, Christensen JW, Søndergaard E. (2012) Behaviour and stress responses in horses with gastric ulceration. Appl Anim Behav Sci;142:160-7. doi:10.1016/J.APPLANIM.2012.10.002.
20. Videla R, Andrews FM. (2009) New perspectives in equine gastric ulcer syndrome. Vet Clin North Am Equine Pract;25:283-301. doi:10.1016/J.CVEQ.2009.04.013.
21. Sykes BW. (2019) Courses for horses: Rethinking the use of proton pump inhibitors in the treatment of equine gastric ulcer syndrome. Equine Vet Educ;31:441-6. doi:10.1111/EVE.12894.
22. Sykes BW. (2021) A free ride: Is long-term omeprazole therapy safe and effective? Equine Vet Educ;33:556-60. doi:10.1111/EVE.13458.
23. Stucchi L, Zucca E, Serra A, Stancari G, Ceriotti S, Conturba B, Ferro E, Ferrucci F (2020). Efficacy of the Administration of a Natural Feed Supplement in the Management of Equine Gastric Ulcer Syndrome in 7 Sport Horses : A Field Trial. Am J Anim Vet Sci;12:104-10. doi:10.3844/AJAVSP.2017.104.110.
24. Bonelli F, Busechian S, Meucci V, Caporrino G, Briganti A, Rueca F,Zappulla F, Ferini E, Ghiandai L, Sgorbini M. (2016) pHyloGASTRO in the Treatment of Equine Gastric Ulcer Lesions. J Equine Vet Sci;46. doi:10.1016/j.jevs.2016.06.069.
25. Sykes BW, Sykes KM, Hallowell GD. (2014) Efficacy of a Combination of Apolectol, Live Yeast (Saccharomyces cerevisiae [CNCM I-1077]), and Magnesium Hydroxide in the Management of Equine Gastric Ulcer Syndrome in Thoroughbred Racehorses: A Blinded, Randomized, Placebo-Controlled Clinical Trial. J Equine Vet Sci;34:1274-8. doi:10.1016/J.JEVS.2014.09.006.
26. Venner M, Lauffs S, Deegen E. (1999) Treatment of gastric lesions in horses with pectin-lecithin complex. Equine Vet J Suppl:91-6. doi:10.1111/J.2042-3306.1999.TB05178.X.
27. Bush J, van den Boom R, Franklin S. (2018) Comparison of aloe vera and omeprazole in the treatment of equine gastric ulcer syndrome. Equine Vet J;50:34-40. doi:10.1111/EVJ.12706.
28. Fellows LE, Bell EA. (1970) 5-hydroxy-l-tryptophan, 5-hydroxytryptamine and l-tryptophan-5-hydroxylase in *Griffonia simplicifolia*. Phytochemistry;9:2389-96. doi:10.1016/S0031-9422(00)85745-3.
29. Carnevale G, Di Viesti V, Zavatti M, Benelli A, Zanoli P. (2011) Influence of *Griffonia simplicifolia* on male sexual behavior in rats: Behavioral and neurochemical study. Phytomedicine;18:947-52. doi:10.1016/J.PHYMED.2011.02.009.
30. Carnevale G, Di Viesti V, Zavatti M, Zanoli P. (2011) Anxiolytic-like effect of *Griffonia simplicifolia* Baill. seed extract in rats. Phytomedicine;18:848-51. doi:10.1016/J.PHYMED.2011.01.016.
31. Sommer C. (2006) Is serotonin hyperalgesic or analgesic? Curr Pain Headache Rep;10:101-6. doi:10.1007/S11916-006-0020-4.
32. Esposito M, Ruberto M, Pascotto A, Carotenuto M. (2012) Nutraceutical preparations in childhood migraine prophylaxis: effects on headache outcomes including disability and behaviour. Neurol Sci;33:1365-8. doi:10.1007/S10072-012-1019-8.
33. Al-Quraishy S, Othman MS, Dkhil MA, Abdel Moneim AE. (2017) Olive (*Olea europaea*) leaf methanolic extract prevents HCl/ethanol-induced

gastritis in rats by attenuating inflammation and augmenting antioxidant enzyme activities. Biomed Pharmacother;91:338-49. doi:10.1016/J.BIOPHA.2017.04.069.

34. Jan M. (2010) Effects of "*Olea europaea*" extract on volume and acidity of carbachol induced gastric secretion, liver and kidney function in rabbits | Request PDF. J Ayub Med Coll;22:113-5.
35. Acar-Tek N, A agündüz D. (2020) Olive Leaf (*Olea europaea L. folium*): Potential Effects on Glycemia and Lipidemia. Ann Nutr Metab 2020;76:10-5. doi:10.1159/000505508.
36. González-Hedström D, García-Villalón ÁL, Amor S, de la Fuente-Fernández M, Almodóvar P, Prodanov M, Priego T, Martin AI, Inarejos-Garcia AM, Granado M. (2021) Olive leaf extract supplementation improves the vascular and metabolic alterations associated with aging in Wistar rats. Sci Rep;11:8188. doi:10.1038/S41598-021-87628-7.
37. Alirezaei M, Dezfoulian O, Neamati S, Rashidipour M, Tanideh N, Kheradmand A. (2012) Oleuropein prevents ethanol-induced gastric ulcers via elevation of antioxidant enzyme activities in rats. J Physiol Biochem;68:583-92. doi:10.1007/S13105-012-0177-8/FIGURES/6.
38. Rauwald HW, Brehm O. (1991) Screening of some medicinal plants for their possible calcium-antagonistic activity. Planta Med;57:A59-60. doi:10.1055/S-2006-960320/BIB.
39. Ellis JM, Hollands T. (1998) Accuracy of different methods of estimating the weight of horses. Vet Rec;143:335-6. doi:10.1136/VR.143.12.335.
40. Luthersson N, Bolger C, Fores P, Barfoot C, Nelson S, Parkin T,Harris P. (2019) Effect of Changing Diet on Gastric Ulceration in Exercising Horses and Ponies After Cessation of Omeprazole Treatment. J Equine Vet Sci;83. doi:10.1016/J.JEVS.2019.05.007.
41. Munsterman AS, Dias Moreira AS, Marqués FJ. (2019) Evaluation of a Chinese herbal supplement on equine squamous gastric disease and gastric fluid pH in mares. J Vet Intern Med;33:2280-5. doi:10.1111/JVIM.15603.
42. Millares-Ramirez EM, Le Jeune SS. (2019) Girthiness: Retrospective Study of 37 Horses (2004-2016). J Equine Vet Sci;79. doi:10.1016/j.jevs.2019.05.025.