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The non-use of hay in food and the integrity of the keratinized epithelium in the stomach of non-athlete horses

Luiz Alberto da Silva Vasconcellos

Centro de Medicina Equina Integrado, Araçatuba, São Paulo, Brasil.
Correspondence: luizalbertovasconcellos@gmail.com.

Abstract. In order to check the gastric squamous epithelium of non-athlete horses, through video endoscopy (gastroscopy), 37 castrated, male horses were used, from the same rural property, where the horses fed only with grass (grazing), without no parasitological control, vaccination or even mineral supplementation and the aforementioned animals never fed on any form of hay (of any type of grass), we therefore concluded, based on the findings by gastric endoscopy, that grazing as a form of feeding for the horses is extremely important not only for general health but also for the integrity of the equine stomach mucosa.

Keywords: Endoscopy, gastroscopy, gastritis, equine gastric ulcer, equine low performance

A não utilização do feno na alimentação e a integridade do epitélio queratinizado no estômago de cavalos não atletas

Resumo. Com o intuito de avaliar a influência do pastoreio exclusivo na integridade do epitélio escamoso gástrico, 37 equinos machos, castrados, foram submetidos a exame endoscópico. Os animais, mantidos em uma única propriedade, não tinham histórico de alimentação com feno e não recebiam tratamento parasitário ou vacinal, exceto por suplementação mineral. A ausência de lesões no epitélio escamoso, observada em todos os animais, sugere que o pastoreio pode ser uma prática benéfica para a saúde gástrica equina.

Palavras-chave: Endoscopia, gastrite, gastroscopia, úlcera gástrica equina, baixa performance equina

Introduction

Athlete horses can have their performance reduced due to numerous causes such as respiratory problems reducing the air flow to the lungs as in the gas exchange tissue, heart problems reducing the volume of tissue oxygenated blood, locomotor problems with painful conditions causing a reduction in mobile capacity, thermoregulatory problems preventing the dissipation of heat produced by muscular activity which under environmental conditions of extreme heat and high humidity disable heat loss or even with congenital problems (anhidrosis), neurological causes such as congenital and or acquired cervical malformations altering mobility, which may have infectious etiology (EPM, Equine Herpesvirus Type-1) or degenerative (vitamin E deficiency, etc.), as well as gastrointestinal problems where the equine gastric ulcer (very important factor) may have the glandular epithelium affected, the keratinized epithelium affected and or both surfaces gastric ulcers, causing abdominal discomfort and consequently a reduction in athletic performance with increased lactic acid production, not understood causes yet (Aranzalez & Alves, 2014; Fraipont et al., 2011; Lo Feudo et al., 2022; Martin Júnior et al., 2000; May-Davis & Walker, 2015; Sykes et al., 2015, 2019; Vascocellos, 2022; 2024).

Focusing specifically on gastric problems, the incidence depends on several factors such as purpose, breed, level of training, as well as the location of the affected gastric tissue, and is therefore classified,

according to the European College of Internal Equine Medicine, in Equine Squamous Gastric Disease (ESGD) and Equine Gastric Glandular Disease (EGGD), according to the affected tissue, that is, squamous gastric tissue and glandular gastric tissue, respectively (Sykes et al., 2015). The horse's stomach is of the simple, unilocular type and with a capacity of only 5–15 liters, it is small for such a large animal. It is found in the cranial part of the abdominal cavity entirely within the projection of the rib dome (in the abdominal cope of the diaphragm), mainly to the left of the median plane. It is strongly flexed with the result that the entry and exit of the food flow is done through the cardia and pylorus (respectively), they are close to each other. When moderately filled, it is more pronounced on the left between the 9th and 14th intercostal spaces and when fully distended, the stomach projects towards the rib cage, but even full it cannot come into contact, remaining close to the abdominal roof (Houston & Radostits, 2002; Speirs, 1999). The cardia is attached quite solidly to the diaphragm in the esophageal hiatus, its cranial part is in permanent contact with the diaphragm and liver, while the caudal part faces the intestines. The lining of the stomach is divided by the *margo plicatus*; the squamous tissue presents a non-glandular mucosa close to the blind sac (region close to the lesser curvature and the cardia), and a glandular mucosa, close to the body or greater curvature, in the pyloric part. In the fresh organ, the non-glandular mucosa is light grey in color and rough to the touch (Figure 1), while the glandular part is redder, softer and smoother (Budras et al., 2003).

Figure 1. Normal appearance of the gastric non-glandular mucosa with its grayish color without the presence of squamous tissue hypertrophy.

Clinical signs of gastric disease, whether in the gastric squamous or glandular epithelium in horses, should be interpreted in the form of poor performance, episodes of recurrent colic, bruxism, weight loss and reduced appetite (Speirs, 1999; Thomassian, 2005); it occurs more in equine athletes with a prevalence of 17% to 58% and more precisely in racehorses of 80% to 100%, with Squamous Epithelial Disease (ESGD) and Glandular Epithelial Disease (EGGD) classified as Equine Gastric Ulcer Syndrome (EGUS) (Lo Feudo et al., 2022; Sykes et al., 2015).

In a group of 87 racehorses, 99% of the horses were found to have lesions in the squamous epithelium of the stomach (ESGD) and there appears to be a negative correlation between aerobic capacity and lesions in the squamous epithelium, findings that are still unexplained, as it is not yet known whether is the cause or consequence of the accumulation of lactic acid after intense exercise, but with the provision of large quantities of feed rich in non-structural carbohydrates, they are important factors in inducing damage to the squamous epithelium (Lo Feudo et al., 2022) done by gastroscopy (endoscopy or videoendoscopy). Complete evaluation of the pylorus and proximal duodenum is necessary for the diagnosis of gastric glandular disease. There is no relationship between the presence of ESGD and EGGD; therefore, the presence or absence of one is not predictive of the presence or absence of the other. A rating scale for ESGD has been established. Current classification recommendations for EGGD include descriptors of presence and or absence, anatomical location, distribution and appearance of

lesions (Sykes et al., 2015). This scale would be grade 0 where the epithelium is intact and without hyperkeratosis, grade 1 the epithelium would be intact but with areas of hyperkeratosis, grade 2 would be a single or multifocal lesion, grade 3 would be a large, single lesion or superficial lesions in large areas, and finally grade 4 would be multiple lesions with areas of deep ulceration (Sykes et al., 2015).

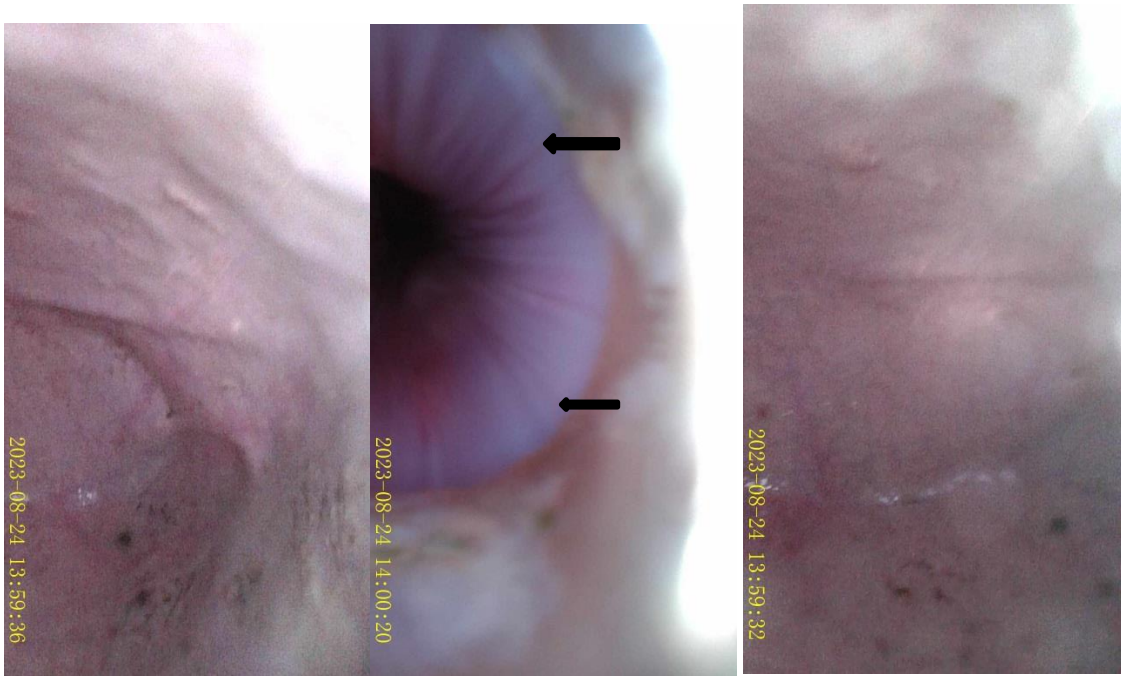
There seems to be a consensus regarding that food (associated non-structural carbohydrates, medication, high-intensity physical activity for a long time) would be the cause and at the same time treatment (use of fresh grass/grazing) would be a way of reestablishing the balance in the gastric mucosa of horses (Alves et al., 2010; Zibetti et al., 2021).

Materials and methods

In this experiment, 37 horses (from the same property) were selected, males (castrated) aged between 7 and 24 years, body weight between 380 and 420 kg, which had never ingested hay (of any type of grass), non-athlete animals, used for the management of beef cattle, without defined breed, without any dental treatment, parasitic control, vaccination control, supplementation with mineral salt specific for horses, where this group only has grass as a food source (of the most varied species) without having another source of feeding and in extreme conditions (absence of pasture depending on the time of year) they received concentrate for horses, water *ad libitum* (water came from cattle drinking troughs and or stream on the property), supplementation of the most varied brands and characteristics, i.e. animals bred in the field without due zootechnical care, as the same animals were often clipped every 4 or 6 months apart, but curiously all the animals presented a good to excellent body condition, some with shiny coats (seen more during the breeding period summer), with its normal physiological parameters (respiratory rate, heart rate, body temperature, color of the ocular and oral mucous membranes, feces with normal color and shape, without information regarding the frequency and characteristics of urine given the type of local management), apparently without no clinical changes detected during the experiment. Gastric evaluation was carried out at different times on the animals depending on their daily use on the rural property and the availability of animals, but all animals before the examination were carried out followed the same protocol, same time, and the same number of animals examined for each established stage.

For gastroscopy, the animals were fasted for 14 hours beforehand but with water which would be removed two hours before the examination, in such a way as to be able to examine the gastric mucosa without the presence of food content, avoiding making it difficult to visualize. To do this, a video endoscope (model HD Endoscope model 430) was used, 3 meters long, with a fiber diameter of 8 mm thick, where the images were stored in the form of videos and photographs, and were subsequently evaluated. To carry out the examination, the animal was restrained only mechanically with "twitch" on its upper lip, in such a way as to not use any form of sedation or chemical restraint, as the horses after gastroscopy examination were immediately released for graze and drink. It was noted upon examination in all animals in the experiment that both the squamous (non-glandular) and the smooth (glandular) epithelium were in harmony, with their grayish and vascularized color (squamous) and a pinkish color (glandular), figures 2, 3 and 4, where the *margo plicatus* region (Figure 4), (region that divides the squamous epithelium from the glandular epithelium in the gastric mucosa) was not as clear as seen in the stomachs of equine athletes. There were also no signs of parasitism found, such as the presence of *Gasterophilus* sp or *Habronema* spp, an intriguing fact given the management of the animals on this property, located in the Araçatuba-SP region, Brazil.

The 37 horses examined had their stomachs visualized by gastroscopy, being examined in groups of a maximum of 3 animals at a time, therefore using 12 days for the experiment, divided according to the availability of the animals and help from collaborators on the farm. Endoscopies were always performed at the same time, in the same place with the same protocol (Sykes et al., 2015), and the horses were not performed after examination, had no side effects, and were monitored for 1 month. Even in animals with a slightly more aggressive behavior, the examination proved to be simple and easy to carry out in the field and, more importantly, without any related complications.

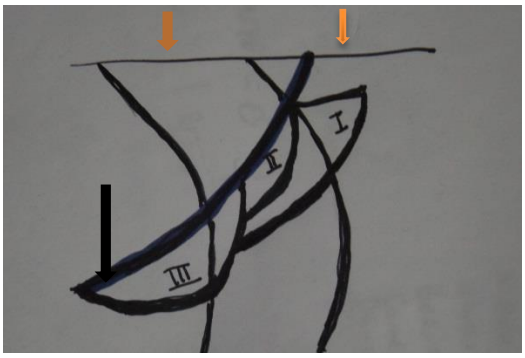


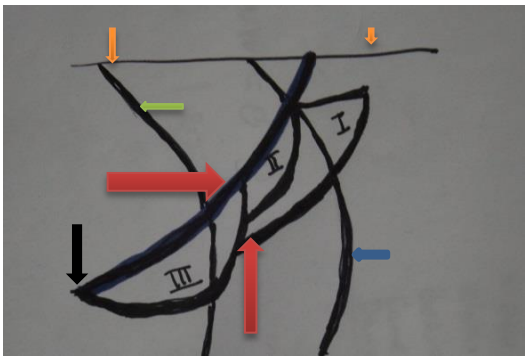
Figures 2, 3 and 4. Images 2 and 4 show the stomach inflamed with air, showing the integrity of the non-glandular keratinized tissue of the stomach, while image 3 shows the *margo plicatus* (**black arrows**) the transition region between the non-glandular and glandular mucosa (at the moment of contraction of the fundus region or great curvature.

Results and discussion

Based on the gastroscopy findings, a curious fact was the absence of gastric parasites, which we suspect was due to the farm being surrounded by sugar cane cultivation, which in a certain way would explain the absence of *Gasterophilus* sp, but not *Habronema* sp. All horses in the experiment presented some form of dental alteration, whether in the form of irregular wear or the presence of "baby teeth" still permanent in animals over 7 years of age, cavities, some with lesions in the tooth canal, traumatic stomatitis, diastema, glossitis, fistulas in the jaw (probably due to problems with infection in the tooth root) which did not reflect on the body condition, because as previously reported, the animals' body scores were between good and excellent. When we think about athletic horses, they always train and compete with their stomachs empty, an important management because high-performance physical activity could trigger problems such as gastric impaction, gastric fermentation, causing episodes of colic (Speirs, 1999; Thomassian, 2005). This period of absence of gastric contents would lead to an increase in acidity in the fundic region (region of the glandular epithelium, pylorus and anterior duodenum) (Bermejo et al., 2008; Blikslager, 2019; Pessoa et al., 2012), a fact associated with the horse's anatomy, that when you are in movement (competition, training) with your stomach empty, this acidity is distributed throughout the gastric mucosa without distinction, because structures such as the diaphragm and liver compress the stomach and intestines back (colon, more precisely the diaphragmatic flexure), it would project the fundic region of the stomach upwards, dispersing the acidity throughout the mucosa, as shown in Figures 5 and 6, which does not happen with the animals in this experiment, as their daily physical activities occur with the presence of gastric contents (chewed grass) as shown in figures 7 and 8, and that this mass in the fundal region would function as a "sponge", absorbing gastric acidity and preventing it from being dispersed across the gastric surface with the horse undergoing physical activity, therefore being restricted to fundic region, which would justify the fact that we did not find any evidence of injury in any of the animals examined, whether in the squamous mucosa or in the glandular mucosa of the stomach, according to the classification of Sykes et al. (2015).

Another observation was the large amount of liquid with grass that was found in the stomach of unprepared animals (without fasting for 14 hours), which would be capable of diluting gastric acidity (Figure 8), due to the large amount of water that the fresh grass produces, while hay, by its very nature, is a dehydrated food, rich in fiber, requiring more time for salivation, chewing and digestion in the stomach in the presence of gastric juice to begin the breakdown of the fiber.





Figures 5 and **6.** Images showing the anatomical position of the stomach on the left side, where I represents the spleen, II the stomach and III the liver; the black arrow indicates the diaphragm, the green arrow represents the 9th rib, the blue arrow the 15th rib,orange arrrow roof of the abdômen (Budras et al., 2003), the red arrows indicate the pressure that the diaphragm, liver and intestine exert on the stomach, pressing the fundal region and spreading acidity throughout the gastric mucosa, in the empty stomach, of the horse in motion.

Again using the information above and now theoretically thinking about athletic horses, there seems to be more lesions in the squamous tissue and glandular tissue, a fact that we must be aware of because factors such as the presence of acidity in the squamous tissue, given its dispersion in an empty stomach and agitated with the movement of the horse (training or competition) and the vast majority of athlete horses have hay (of the most varied types of grass) as a source of fiber, where in addition to an epithelium (squamous) that receives acidity with a coarse fiber and perhaps little chewed, with little saliva (buffering power) would be an important factor for the squamous epithelium to "defend itself" against these aggressive stimuli and we will therefore have the answer as to why the squamous epithelium is the most "attacked" in equine athletes. This fact was not found in this experiment, as all animals have their physical activity with the presence of food content (grass ingestion) and this could be the factor that would explain the absence of any type of gastric injury (Alves et al., 2010; Zibetti et al., 2021).

Figures 7 shows the gastric contents in the fundic region (functioning as a sponge and absorbing gastric acidity in non-athletic horses in movement)

Figure 8 shows a large amount of grass with amount of liquid and would serve to dilute gastric acidity.

There is also the possibility of gastric ulcers caused by *Equine Gamma Herpesvirus type-2 and type-5* (Thompson et al., 2024), and that salt chloride, in addition to being an important electrolyte replenisher due to the large amount of sweat in the equine species, would cause irritation in the stomach mucosa and could cause predisposition to ulcerations (Alshut et al., 2023). In this experiment we can verify that the horses used in this experiment did not have specific mineral supplementation for horses, however we cannot confirm the presence or absence of the virus mentioned above, but based on the endoscopy findings, once again, no gastric changes were found.

We concluded in this experiment that the permanent intake of pasture, without the use of any type of hay, is not only an important contributing factor to the health of the stomach, as well as apparently the body condition of the horses and independent of the dental condition, based on clinical findings and most importantly by the gastroscopy performed on all 37 horses.

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