**ABSTRACT**

Enteroliths are concretions of minerals that cause partial or total obstruction of the intestinal lumen, resulting in recurrent and chronic colic in horses. This pilot study aimed to evaluate the *in vitro* solvent effect of carbonated beverages (Coca-Cola® and Coca-Cola® Zero), and papain and cellulase enzymes (Robinson Pharma®, Santa Ana, CA, USA) on enteroliths obtained from horses. Six 51-grams-samples of six enteroliths were assigned to six treatments of immersion solutions: T1, Coca-Cola®; T2: Coca-Cola® Zero; T3: distilled water + papain (90 mg) and cellulase (120 mg); T4: Coca-Cola® + papain and cellulase; T5: Coca-Cola® Zero + papain and cellulase; and, CT: distilled water (control). The volume for immersion in the assigned solution was 150 mL, at a pH of 7.1, using an incubation shaker (Heidolph®, Germany) at 37°C and 25 rpm, for 72 h. The evaluation periods of the dissolution percentage (difference between the initial weight and final weight of the samples) were 0, 3, 12, 24, 36, 48, 60, and 72 h. After 72 h of immersion, solutions T4, T5, and T1 presented 47, 38.8, and 14.9% of dissolution, respectively. The other solutions did not have major differences with CT (control). Under the *in vitro* conditions of this pilot study, papain and cellulase enzymes potentiated the dissolving effect of the carbonated solutions on the enteroliths obtained from horses. Further studies are suggested since the existing literature is on the dissolution of phytobezoars and not of enteroliths.

**Keywords:** Horse. Colic. Enterolithiasis. Intestine. Obstruction.

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**RESUMO**

Enterólitos são concreções de minerais que causam obstrução parcial ou total do lume intestinal, resultando em cólica crônica e recorrente nos cavalos. Este estudo piloto teve como objetivo avaliar *in vitro* o efeito dissolvente sobre os enterólitos das bebidas carbonatadas (Coca-Cola® e Coca-Cola® Zero) e a solução à base das enzimas papaína e celulase (Robinson Pharma®, Santa Ana, CA, USA). Seis (6) amostras de seis (6) enterólitos de 51 gramas de peso foram distribuídas em seis tratamentos de imersão: T1: Coca-Cola®; T2: Coca-Cola® Zero; T3: água destilada + papain (90 mg) e celulase (120 mg); T4: Coca-Cola® + papain e celulase; T5: Coca-Cola® Zero + papain e celulase; e, CT: água destilada (controle). O volume das soluções de imersão foi de 150 mL, com pH de 7.1, usando um *shaker* de incubação (Heidolph®, Germany) com 37°C e 25 rpm, durante 72 horas. A avaliação dos períodos da porcentagem de dissolução (diferenças entre o peso inicial e o peso final das amostras) foram 0, 3, 12, 24, 36, 48, 60 e 72 h. Depois de 72 h de imersão, as soluções T4, T5 e T1 apresentaram 47, 38,8 e 14,9% de dissolução, respectivamente. As outras soluções não tiveram diferenças com relação ao CT (controle). Nas condições *in vitro* deste estudo piloto, as enzimas papain e celulase potenciaram o efeito dissolvente das bebidas carbonatadas sobre os enterólitos obtidos de cavalos. Mais estudos são sugeridos, uma vez que só existe literatura sobre a dissolução de fitobezoares e não de enterólitos.

Enteroliths are concretions derived from mineral precipitation, around a nucleus or nest of organic or inorganic material, located in the equine gastrointestinal tract (Hassel, 2002; Perez et al., 2006; Turek et al., 2019). These foreign bodies have various shapes, with the most common being the spherical or tetrahedral, and irregular ones, with different sizes and weights (Cohen et al., 2000; Pierce, 2009). The presence of these foreign bodies in horses is estimated to be present in up to 15.1% of the patients with colic. Similarly, 27% of the patients undergoing exploratory laparotomy were diagnosed with enterolithiasis (Pierce, 2009). However, it has also been reported that only a third of horses with enterolithiasis have presented colic episodes and that 13% of them have a history of elimination of one or more enteroliths in their feces (Hassel, 2002).

Enteroliths formation involves several factors related to the environment, diet, breed, intestinal pH, and presence of a nucleus (nest) in the gastrointestinal tract. However, the pathogenesis is not yet fully understood (Hassel, 2002; Hassel et al., 2004, 2008, 2009). Enteroliths cause partial or complete obstruction of the equine gastrointestinal tract, specifically in the intestinal segments with the smallest luminal diameter, such as the right dorsal colon, transverse colon, and minor colon (Turek et al., 2019). This clinical condition can produce enteric disorders, from recurrent colic to devastating consequences such as necrosis, ulcerations, and intestinal rupture with the development of peritonitis and possible death of the animal, if it is not detected and treated in time (Hassel, 2002; Pratt et al., 2003).

The clinical signs and the intermittent and chronic course of the abdominal crisis orient the diagnosis of enterolithiasis. Transrectal palpation has shown to be 58% sensitive, being difficult to perform with the presence of high colonic distention. Diagnostic aids such as abdominal digital and computerized radiography have shown to be 77-87% sensitive detecting enterolithiasis in the greater colon (Kelleher et al., 2014; Maher et al., 2011; Turek et al., 2019), and 42-54% in the lesser colon by conventional radiography (Hassel, 2002; Pierce, 2009). Recently, computed tomography has been used to accurately identify the location of enteroliths (Nakamae et al., 2018). However, the required equipment is sophisticated and of high power, becoming a limitation for its use in the clinical routine. In the case of ultrasonography, the observation of compatible-to-enteroliths findings depends on whether the affected segment is close or not to the ventral part of the abdominal cavity. However, despite the availability of diagnostic aids, the definitive diagnosis and therapy are achieved through laparotomy and/or exploratory celiotomy and enterotomy, respectively.

Once the presence of enteroliths is diagnosed, surgical treatment is indicated to allow its extraction, evaluate the integrity of the intestinal wall, and exclude the presence of more foreign bodies. In this sense, there is no less invasive treatment or effective preventive measures to reduce the impact of complications that can arise from abdominal surgical procedures in horses (Pierce et al., 2010). Also, due to the nature of recurrent and chronic colic due to enteroliths, the patient is referred to a high degree of organic decompensation, which can increase anesthetic and surgical risks.

Several preventive strategies have been reported in the control of enterolithiasis in horses. Nevertheless, the multifactorial nature of its development makes it difficult to define an alternative to surgery once foreign bodies have been formed. Such alternatives have not been described for enteroliths different from phytobezoars, especially diospyrobezoar. In case reports in humans and horses, the use of solvent solutions such as Coca-Cola® has been explored (Banse et al., 2011; Iwamuro et al., 2014; Ladas et al., 2013; Lee et al., 2009; Martinez de Juan et al., 2006). Consequently, the present pilot study evaluated the in vitro dissolving effect of two carbonated beverages (Coca-Cola®, Coca-Cola® Zero) and enzyme-based solutions (papain and cellulose) for enteroliths obtained from equines.

**Materials and Methods**

**Enterolith samples**

Enteroliths were obtained from horses undergoing celiotomy at various veterinary clinics. Once photographed, the enteroliths were weighed and classified according to...
their appearance, shape, and size. Then, a fragment was extracted using an endless electric saw. Six (6) fragments of the most compact surface of the six (6) enteroliths were used (Figure 1A).

**In vitro disintegration and solvent analysis**

For the analysis and verification of the dissolving effect of the selected solutions, 51-gram-samples of similar texture enteroliths were assigned to six (6) different solvent solutions: T1: Coca-Cola®; T2: Coca-Cola® Zero; T3: distilled water + papain (90 mg) and cellulase (120 mg); T4: Coca-Cola® + papain and cellulase; T5: Coca-Cola® Zero + papain and cellulase; and, CT: distilled water (control). The volume for immersion in the assigned solution was 150 mL, at a pH of 7.1, using an incubation shaker (Heidolph®, Germany) at 37°C and 25 rpm, for 72 h. The evaluation periods were 0, 3, 12, 24, 36, 48, 60, and 72 h post-immersion, used as a reference for the determination of the degradation time. The percentage of lysis or degradation was obtained by the difference in percentage between the weight of each fragment determined at time 0 (just before the immersion) and the weight in each evaluation period, up to the maximum test time (72 h). The weight of each post-immersion fragment was determined after a 30 min drying period on absorbent paper, to eliminate excess water and humidity.

**Statistical analysis**

The weight (in grams) of each enterolith fragment at the stipulated times were systematized and tabulated in Excel spreadsheets (Microsoft Corp., Redmond, WA, USA), and then processed for descriptive statistics. All data were analyzed using the R Project software, version 3.6.3, considering each solving treatment and time as explanatory variables, and the weight (in grams) of each enterolith sample as the response. Additionally, an analysis of variance (ANOVA) was performed. Subsequently, through Post-Hoc analysis using the Tukey technique, each of the factors of the explanatory variables was compared in pairs. A significance level of $p \leq 0.05$ was considered for all the statistical analyses.

**Results**

The appearance, shape, and size of treated enteroliths are described in Figures 1B-1D including the different predominant formats observed (i.e. spherical, polyhedral, irregular with smooth, rough, and porous surfaces). The average weight of the analyzed enteroliths was 664.14 ± 385.01 grams, where the largest one weighed 1.157 grams and the smallest 127 grams.

The weight (in grams) of each sample at each evaluation period, the percentage of disaggregation at 72 h, and the difference between treatments are shown in Table 1. In Figure 2, the performance of each treatment during the period evaluated is presented. In the initial period (0 to 3h), all the samples increased in weight (24.6% on average), although the progressive increase was reflected after the 12 h immersion in all treatments except CT. Contrasting CT´s results, all the treatments showed a maximum percentage of disaggregation at 72 h, with T4, T5, and T1 showing the highest percentage, in descending order, respectively; however, only T5 showed a statistically significant difference. Additionally, dissolution began to be observed in all treatments after 24 h, although in a lower percentage.
Discussion

The evaluation of the dissolving effect of a solution on foreign bodies found in the intestinal tract under in-vitro conditions cannot consider all the factors involved in such environment, nor the possible causes and consequences of a clinical case derived from enterolithiasis, just to mention an example. Therefore, this study was not intended to cover and/or simulate a real scenario. Clinical trials are probably required to evaluate the effect of the solutions on the horse patient showing an enterolith condition, aiming to define the sufficient volume of solution and the effective administration time. Such information may be complex to achieve, due to the clinical decompensation and gastroenteric location of the enterolith. Additionally, more studies are necessary, since existing studies have been carried out in phytobezoars.

The use of carbonated Coca-Cola®-type beverages in horses is an anecdotal finding in cases of gastric compaction. Similarly, kaki (persimmon) oral administration is beneficial in the management of enteric and gastric phytobezoars in several clinical cases in horses (Banse et al., 2011) and humans (Ladas et al., 2013; Lee et al., 2009; Martinez de Juan et al., 2006). However, there are few published controlled studies to evaluate its efficacy in phytobezoars and there is a lack of information on its effect on enteroliths. Both foreign bodies differ in composition since they are concretions of a variety of minerals providing greater hardness, firmness, and resistance. Therefore, this study aimed to obtain an approximation of the dissolving effect of these carbonated drinks and enzyme-composed solutions, specifically on enteroliths.
The dissolving solutions used in the present study showed different capacities in each of the enterolith samples through the evaluated periods. The non-effect of the CT (control), which, on the contrary, showed an increase in the final weight to the initial one (representing a negative percentage of dissolution), was observed. The rest of the experimental solutions showed a variable dissolving effect, ranging from 1.5 to 47.1%, after 72 h of immersion, although only T5 showed a statistical difference. Nevertheless, the evaluation in the first 12 h showed a weight increase due to the hydration level of the sample, indicating a difference in texture, even though similar fragments were macroscopically based selected at the beginning of the trial.

The weight increase found in all samples (an average of 24.6%) in the first hours was variable, possibly due to the differences in texture, which did not influence the percentage of dissolution at the end of the 72-h period. The T4 and T5 showed the highest dissolving effect, respectively, but also the lowest percentages of weight increase in the initial period, if a comparison of those showing the greater initial weight increases (T3 and T2), but with lower percentages of dissolution is made. However, this performance was not observed in T1 and CT, indicating composition differences, in addition to the texture and intrinsic factors of the samples and of the solutions used.

In this sense, carbonated drinks (T1 and T2) showed greater dissolution capacity compared to the enzyme-based solution (T3), and therefore to the control (CT). This result is similar to the work of the same nature developed by Iwamuro et al. (2014), performed on phytobezoars, where Coca-Cola® showed complete photolytic activity in a period of 12 h. However, under present study conditions, a complete dissolution of the enterolith samples was not observed at 72 h, although it was greater than the enzyme-based solution (T3). These findings suggest that Coca-Cola® can be effective to dissolve enteroliths and phytobezoars, despite the differences in composition, compaction, hardness, and resistance.

The dissolution effectiveness of Coca-Cola® in phytobezoars has been also evaluated in several clinical reports in humans (Ladas et al., 2013; Lee et al., 2009; Martínez de Juan et al., 2006) and horses (Banse et al., 2011). In these reports, the time of use of this drink ranged between two (2) and eight (8) weeks, with a total resolution of the phytobezoars, such results contrast with the 12-h in vitro study reporting a dissolving activity of less than 20%. Meanwhile, in this study with 72 h of enteroliths immersion, dissolution was found to be less than 15%. These results may indicate that the dissolving effect of Coca-Cola® depends on the exposure time and foreign body composition material.

Nevertheless, the mixture of the enzyme-based solution with Coca-Cola® (T4) and Coca-Cola® Zero (T5) presented the highest dissolution percentages (47.1 and 38.9%, respectively), indicating a synergism that potentiated their individual-level effects. This would confirm the benefits described by its uses in previously reported clinical cases (Banse et al., 2011; Ladas et al., 2013; Lee et al., 2009; Martínez de Juan et al., 2006). However, no other in vitro work has reported the combination use of these two solutions. Only in one, using each enzyme separately, the cellulase and papain solutions showed a photolytic capacity of 10.1 ± 4.5% and 9.5 ± 6.5%, respectively (Iwamuro et al., 2014). This can indicate that the enzyme compound solution potentiated the effect of Coca-Cola®-type carbonated beverages on enterolith samples.

The solvent capacity of Coca-Cola® is attributed to the low pH (2.6) induced by the phosphoric acid (Ladas et al., 2002; McCloy et al., 1984) and by the carbonic acid produced from carbon dioxide (Martínez de Juan et al., 2006). This pH is important for fiber digestion, since it resembles gastric juice. Furthermore, sodium bicarbonate (NaHCO₃) with a mucolytic effect and CO₂ bubbles contribute to the dissolving mechanism (Ladas et al., 2002). The acid effect seems to be the main dissolving factor of this carbonated drink since the use of gastric acid suppressants has been associated with the formation of phytobezoars in both humans and horses (Banse et al., 2011; Nichols, 1981). Therefore, the dissolving effect of the solutions in this study could have been greater, since the solutions were standardized at a pH of 7 before the enterolith samples were immersed, simulating the intestinal environment where enteroliths are commonly located.

The synergistic effect of enzyme-based solutions and carbonated beverages occurred in addition to what has been previously described concerning the components of Coca-Cola®, possibly from the catalytic effect of the enzymes used, since they are proteolytic and cellulite providing photolytic properties. Despite being enteroliths, these bodies contain abundant plant material in the deepest layers of their surface. This fact was verified when the physical fragmentation of enteroliths was carried out to obtain a sample for the present in vitro study.

In this context, solutions based on the enzymes papain and cellulase potentiate the dissolving effect of the carbonated solutions on enteroliths obtained from
horses. Therefore, considering the in-vitro conditions of this study, the combined use of these solutions is effective to cause disaggregation and greater softening of enterolith samples. Our findings are in vitro evidence of the positive effects also seen in clinical case series of both humans and horses. Finally, Coca-Cola was effective in breaking up enteroliths, in a similar way to what has been demonstrated with phytobezoars. For that reason, clinical studies evaluating the efficacy and possible adverse effects for its use during the indicated time and location of the foreign body in horses are required. Further studies are suggested since the existing literature is on the dissolution of phytobezoars and not of enteroliths.

Conflict of Interest
The authors declare no conflict of interest.

Ethics Statement
The study was approved by the Ethics Committee on Animal Experimentation of Universidad de Antioquia (protocol No. 1062016).

Acknowledgements
To the animal physiology laboratory of the School of Veterinary Medicine, Faculty of Agrarian Sciences, University of Antioquia, for allowing the experimental part to be carried out.

References


Financial Support: This work was financed by the Research Development Committee, the Research Center of the Faculty of Agrarian Sciences (CIAG), and the CENTAURO research group, Universidad Antioquia (Medellin, Colombia) [Sustainability Strategy, 2020].