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Technical Note: *In vitro* evaluation of the slow-release properties of two commercial hindgut buffers for equines

Foad Hosseinzadeh[1], Seyed Hadi Ebrahimi[1], Abdol Mansour Tahmasbi[1], Vahideh Heidarian Miri[2], Amin Darzi Lemraski[2]*

[1]*Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*
[2]*Science and Technology Park, Ferdowsi University of Mashhad, Mashhad, Iran*

[*]Corresponding author,
E-mail address:
shebrahimi@um.ac.ir

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ORCID
Foad Hosseinzadeh
0009-0006-0240-0288
Seyed Hadi Ebrahimi
0000-0002-0156-0646
Abdol Mansour Tahmasbi
0000-0002-6764-1668
Vahideh Heidarian Miri
0000-0002-1804-5544
Amin Darzi Lemraski
0000-0002-5610-7857

Abstract This study aimed to quantitatively evaluate the acid resistance and slow-release properties of two commercial lipid-coated sodium bicarbonate buffers (buffer I and II) designed for equine hindgut supplementation using validated *in vitro* models. The ability of buffers to resist reacting with acid was evaluated by measuring the gas produced from the incubation of buffers with an acid. Furthermore, the disappearance of the fat coat and the release of sodium bicarbonate were assessed using an *in vitro* enzymatic digestion method to simulate small intestine digestibility. The results showed that gas production from buffers I and II followed a linear manner. After 30 minutes of incubation in acid, gas production was approximately 16% of that observed in the uncoated. The lipid coating of buffer II was digested to a significantly greater extent than that of buffer I ($P<0.001$). This consequently led to a significantly greater release of sodium bicarbonate from buffer II ($P<0.001$). Accordingly, the final buffering capacity of the digesta was significantly higher in the bottle containing buffer II compared to the bottle containing buffer I ($P<0.001$). *In vivo* studies involving animals challenged with hindgut acidosis demonstrate the efficacy of equine buffers in supporting gut health.

Keywords: equine, hindgut acidosis, slow-release buffer, sodium bicarbonate, coating

Introduction

Horses are herbivorous animals and can obtain more than 60% of their energy requirements through the fermentation of fibrous materials in the hindgut (Karasu et al., 2023). Cellulolytic bacteria present in anaerobic environments need optimum growth conditions; among them, pH is one of the most important factors for maximum activity (Li et al., 2023). When horses are fed diets consisting solely of forages with low to moderate non-fibrous carbohydrates (NFC) content, a rapid decline in hindgut pH is less likely to occur (van den Berg et al., 2013). This can be attributed to sufficient saliva production during mastication, which helps buffer gastric acidity, and a reduced likelihood of undigested highly fermentable polysaccharides reaching the cecum and colon (Gordon and Prins, 2023).

The energy requirement of performance horses is greater than that supplied only by forages (Ebert and Moore-Colyer, 2020). Therefore, feeding supplemental grains is a usual practice to overcome energy shortages in horses maintained on an intensive workload. It is well known that the capacity of the equine's small intestine for the digestion and absorption of NFC is limited (Coenen and Vervuert, 2010). Consequently, some undigested starch or unabsorbed sugars may enter the hindgut and undergo rapid fermentation, which causes hindgut acidosis

(Varasteh et al., 2024).

Slow-releasing buffers in equines are designed to target the hindgut and help moderate gut conditions by preventing a drastic drop in pH associated with high starch and fructan intake (Suagee-Bedore et al., 2018). Such buffers must have two properties: 1) resisting reacting with gastric acid, and 2) releasing at the end of the small intestine or when entering the large intestine. Suagee-Bedore et al. (2018) evaluated a commercial buffer using an *in vivo* study and they found this product effective for reducing postprandial lipopolysaccharide and interleukin-1β when animals were fed rapidly fermentable nonstructural carbohydrates. There are some other commercial buffers that have been claimed to be effective in supporting hindgut function and reducing the risk of subclinical acidosis in horses, but published evidence for their efficiency is lacking.

Although it is possible to test the performance of an equine buffer using animal studies, they could be evaluated using appropriate *in vitro* techniques. Therefore, the objective of the present study was to develop an assay to assess two commercial slow-releasing buffers designed to regulate the pH of the equine's hindgut.

Materials and methods

Commercial slow-releasing buffers

Two commercial slow-releasing buffers were used in the present study: buffer I (EquiShure®, Kentucky Equine Research, Versailles, KY 40383 USA), and buffer II (Rahayesh®, Faravardaneh Ferdowsi Mashhad, Mashhad, Iran). Both formulations utilized sodium bicarbonate (SB, $NaHCO_3$) as the main active component. According to manufacturer specifications, buffer I featured a protective coating of monoglycerides and hydrogenated vegetable oil, while buffer II employed saturated fats as its coating matrix. To determine the exact amount of SB (the main ingredient in the buffers), a known quantity of samples was held in an electric muffle furnace at 550 ˚C for 5 hours. The residual ash was weighed and subjected to the amount of SB that was present in the slow-releasing buffers.

The particle size distribution of the products was measured by dry sieving of 100 g representative samples through sieves of 4000, 2000, 1000, 500, 250, 125, 63, and 45 µm for 10 minutes using a vibratory sieve shaker (Restch AS 200, Germany). The percentage of materials retained on each screen was then determined, and the geometric mean diameter (GMD) and geometry standard deviation (GSD) of the sample were calculated as described by Amerah et al. (2007).

Evaluating the acid resistance property of slow-releasing buffers

Slow-releasing buffers must resist acidic gastric conditions. To evaluate this property, one gram of SB and an equivalent of slow-release buffers that provided one gram of SB were weighed and placed into small, pre-cut of thin nylon mesh. These mesh parcels were then carefully transferred into 250 mL glass bottles containing 20 mL of 0.6 N HCl. By this method, substrates were floated on top of the acid until the start of gas measurement. The bottles were tightly sealed with modified screw caps. These plastic caps were fitted with ports to connect a sensor via plastic tubing. Once sealed, the bottles were gently placed in a water bath shaker (D-38678 Clausthal-Zellerfeld). Incubation was performed at 37 ˚C for 2 hours, and gas production was measured using an automated gas pressure recording device as described in our previous work (Varasteh et al., 2024). Immediately after connecting the bottles to the gas pressure measuring system, the bottles were manually shaken to mix the substrates with acid and start the reaction. There were four replicates for each buffer in this trial.

In vitro enzymatic small intestine disappearances

A method previously described by Ngonyamo-Majee et al. (2009) was used to simulate the enzymatic digestion of buffers in the foregut with some modifications. Samples of buffers were weighed (to provide one gram of SB) and transferred into pre-weighed 10 × 4 cm nylon bags (31 µm pores) with four replicates. The bags were then tightly sealed with a plastic strap and placed inside 250 mL glass bottles. Then, 20 mL of 0.1 N HCl solution containing 1 g/L pepsin with pH 1.9 was added to 250 mL bottles. The bottles were then incubated at 37 ˚C for one hour in a shaker incubator (D-38678 Clausthal-Zellerfeld). After an hour, 1 mL of 0.1 N sodium hydroxide was added to each bottle, and the contents were mixed. After that, 27 mL of pancreatic phosphate buffer solution (containing 5 g/L pancreatin, Sigma) was added to each bottle. In the next step, five mL of bile (collected from the gall bladder of sheep slaughtered in the slaughterhouse) was added to each experimental unit. The bottles were then kept 3 hours at 37 ˚C in shaker incubator (D-38678 Clausthal-Zellerfeld) to perform enzymatic digestion.

After completing the digestion step, the bags were removed, washed with cold water, and dried at 45 ˚C for 48 hours. The dry matter (DM) disappearance was calculated based on the weight of the samples before and after enzymatic digestion. The dried buffer residuals were placed in an electric muffle furnace at 550 ˚C for 5 hours, and the quantities of organic matter (coating agent) and ash (SB) were obtained. Following this, fat and SB were calculated from the weight of these components before and after enzymatic digestion.

The pH of bottle contents was also measured by a digital pH meter (Metrohm 691). To measure the buffering capacity, 0.1 M hydrochloric acid was gradually added to the digesta until the pH reached seven. The buffering capacity in the contents of each bottle was calculated using the following equation:

$$\beta = \frac{n}{\Delta pH}$$

where, β is the buffering capacity (which is unit-less), n is the number of moles of acid that was added to the buffer per liter of the buffer, and Δ pH is the pH difference between the initial pH and the pH after the addition of acid to the buffer.

Statistical analysis

The data were analyzed in a completely random design using the SAS software, version 9.4 (SAS Institute Inc., Cary, NC), and the following statistical model was used:

$Y_{ij} = \mu + T_j + e_{ij}$

where, μ is the overall mean, T_j is the effect of the j^{th} treatment (buffer type), and e_{ij} is the random error.

Results

Buffer components and particle size distribution

The ash content of buffer I and II was 370.8 ± 2.9 and 393.2 ± 0.8 g/kg DM, respectively. The type I buffer had GMD and GSD of 405.82 and 2.37 µm, respectively. The GMD of the buffer II was 568.39 with a GSD of 1.84 µm. Figure 1 shows the particle size distribution of two experimental buffers used in the present study. The majority of particle sizes (46.25%) of buffer II were smaller than 1000 and bigger than 500 µm, followed by 34.25% of particle sizes that were smaller than 500 µm but were retained on the 250 µm sieve. However, in the type I buffer, most of the particle sizes (34.23%) could pass a 250 µm sieve but were bigger than 125 µm. The buffer I particle sizes retained on the 250 µm sieve, which were smaller than 500 µm, were ranked second (27.07%).

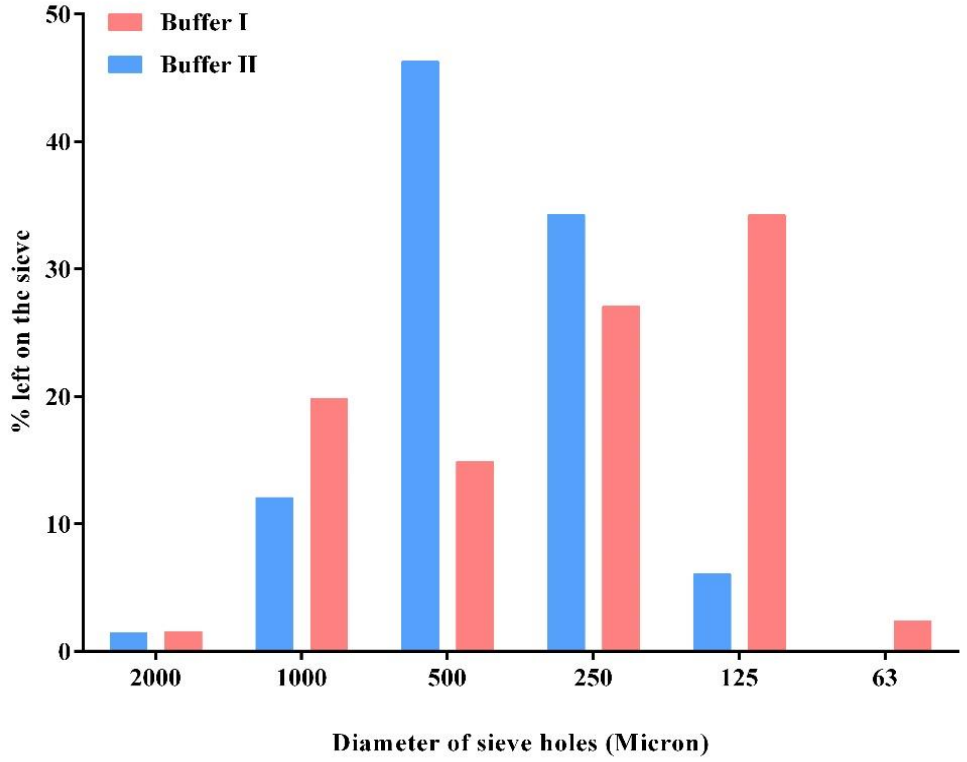

Figure 1. Particle size distribution of the two slow-releasing buffers used in the study

Gas production and final pH after in vitro gastric incubation

Figure 2 shows the 2 hours of cumulative gas production of SB, buffer I, and II after reacting with 0.6 N hydrochloric acid. As displayed, gas production of the SB exponentially increased and reached 150 mL/g after about half an hour; however, in both slow-release buffers, the amount of gas produced was around 25 mL/g of SB at 0.5 hour after starting the reaction with acid and linearly increased until the termination of incubation.

In vitro small intestine releasability of experimental buffers

In vitro small intestine disappearance of slow-releasing buffer II exhibited significantly greater lipid disappearance than buffer I (326.8 vs 296.3, P<0.001), which resulted in a greater disappearance or release of

the SB in buffer II compared to buffer I (803.0 vs 693.1, P<0.001) (Figure 3).

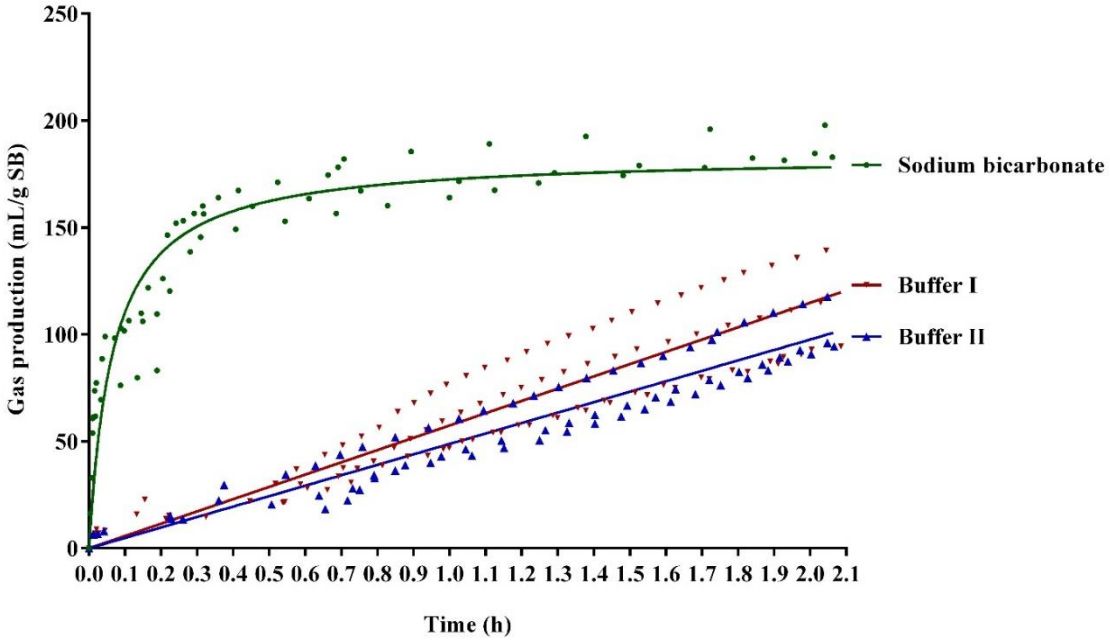

Figure 2. Cumulative gas production of sodium bicarbonate (SB), Buffers I and II, after reacting with 0.6 N hydrochloric acid

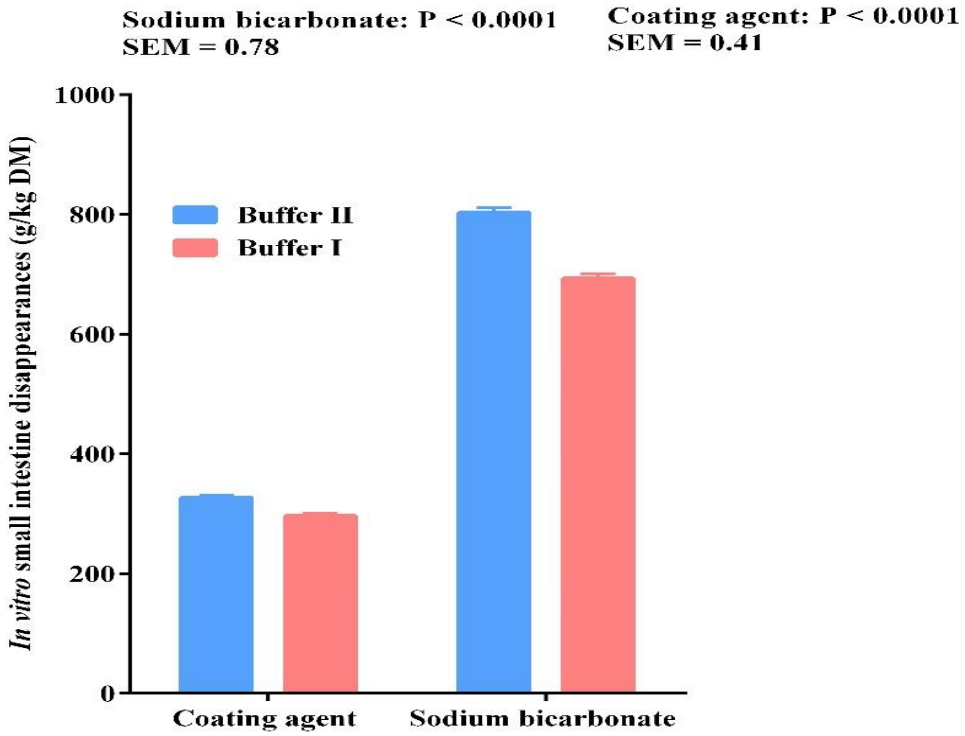

Figure 3. *In vitro* small intestine disappearance of time-releasing buffer's ingredients (coating agent and sodium bicarbonate) after three hours of enzymatic incubation

The buffering capacity of the digesta

The buffering capacity of the digesta after 3 hours of enzymatic incubation with time-releasing buffers is shown in Figure 4. Buffering capacity of the digesta in the bottle containing buffer II was greater than buffer I (0.075 vs 0.060, P<0.001).

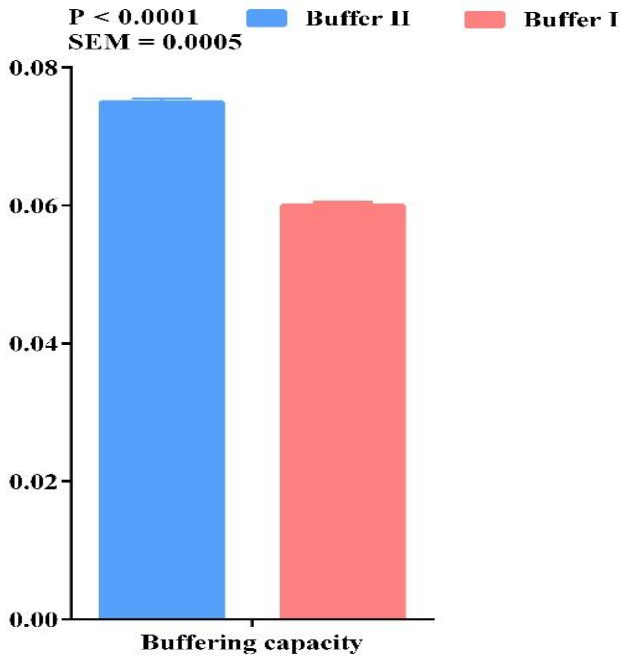

Figure 4. Buffering capacity of digesta after three hours of enzymatic incub*ation of time-releasing buffers*

Discussion

Both buffers studied in the present study were made by coating SB. When SB reacts with an acid, the following reaction occurs:

$NaHCO_3 + H^+ \rightarrow Na^+ + CO_2 + H_2O$

When SB is coated with any substances, it prevents the above reaction, and we would expect very low gas production if the coated buffer is incubated together with an acid (Sanaie-Moghadam et al., 2017). As explained above, both buffers resisted reacting with acid because the rate and extent of gas production were lower than SB when they were incubated with HCl (Figure 2). These results suggest that the lipid coating was effective in delaying the reaction of SB with gastric acid. It was found that the small particle retention time in the equine gastric is about half an hour (Clemens and Stevens, 1979). Therefore, during this period, a very low quantity of buffers could react with gastric acid.

It was supposed that after the enzymatic digestion of the lipid used for coating SB, the lipid layer would be removed. The *in vitro* enzymatic small intestine digestion indicated that in both equine buffers, the disappearance of lipid caused the release and disappearance of SB from the bags. Due to the greater release of SB in buffer II, the buffering capacity of the digesta post-incubation was higher compared to buffer I.

Conclusions

In conclusion, acid resistance property and lipid digestibility of slow-release buffers that are made with the help of saturated vegetable oil could be assessed using *in vitro* gas production and *in vitro* small intestine digestion techniques; however, *in vivo* studies using animals with hindgut acidosis challenge will confirm the capability of equine buffers to aid gut health.

Statement of ethics

All the experimental procedures and animal manipulations were approved by the Animal Care and Use Committee of Ferdowsi University of Mashhad (Approval no: 1401/149).

Conflict of interests

No potential conflict of interest was reported by the author(s).

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References

Amerah, A.M., Ravindran, V., Lentle, R.G., Thomas, D.G., 2007. Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal* 63, 439-455.

Clemens, E.T., Stevens, C.E., 1979. Sites of organic acid production and patterns of digesta movement in the gastro-intestinal tract of the raccoon. *The Journal of Nutrition* 109, 1110-1116.

Coenen, M., Vervuert, I., 2010. A minimum of roughage and a maximum of starch necessary benchmarks for equine diets. *Pferdeheilkunde Equine Medicine* 26, 147-151.

Ebert, M., Moore-Colyer, M.J.S., 2020. The energy requirements of performance horses in training. *Translational Animal Science* 4, 569-588.

Gordon, I.J., Prins, H.H.T., 2023. Forage consumption and digestion in the modern equids. In: Prins, H.H.T., Gordon, I.J. (eds.), The Equids. Fascinating Life Sciences, Springer, Cham, pp. 143-170.

Karasu, G.K., Rogers, C.W., Gee, E.K., 2023. Dietary transitions toward sustainable horse feeding. *Journal of Equine Veterinary Science* 128, 104880.

Li, H., Zhang, M., Zhang, Y., Xu, X., Zhao, Y., Jiang, X., Zhang, R., Gui, Z., 2023. Characterization of cellulose-

degrading bacteria isolated from silkworm excrement and optimization of their cellulase production. *Polymers* 15, 4142.

Ngonyamo-Majee, D., Shaver, R.D., Coors, J.G., Sapienza, D., Lauer, J.G., 2009. Influence of single-gene mutations, harvest maturity, and sample processing on ruminal in situ and post-ruminal in vitro dry matter and starch degradability of corn grain by ruminants. *Animal Feed Science and Technology* 151, 240-250.

Sanaie-Moghadam, M., Hormozi, F., Jahangiri, M., 2017. Use of experimental design to investigate the coating process of sodium bicarbonate in a conical fluidized bed. *Powder Technology* 319, 210-220.

Suagee-Bedore, J.K., Wagner, A.L., Girard, I.D., 2018. Feeding digesta well buffer to horses alters the effects of starch intake on blood pH, lipopolysaccharide, and Interleukin-1β. *Journal of Equine Veterinary Science* 61, 36-45.

Van den Berg, M., Hoskin, S.O., Rogers, C.W., Grinberg, A., 2013. Fecal pH and microbial populations in thoroughbred horses during transition from pasture to concentrate feeding. *Journal of Equine Veterinary Science* 33, 215-222.

Varasteh, F., Ebrahimi, S.H., Naserian, A.A., Zerehdaran, S., Heidarian Miri, V., 2024. Effect of micronization and meal size of corn grain on glycemic response and in vitro hindgut acidosis potential in horses. *Journal of Equine Veterinary Science* 132, 104982.