# Evaluation of diet as a cause of gastric ulcers in horses

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**Objective**—To measure pH, volatile fatty acid (VFA) concentrations, and lactate concentrations in stomach contents and determine number and severity of gastric lesions in horses fed bromegrass hay and alfalfa hay-grain diets.

**Animals**—Six 7-year-old horses.

**Procedure**—A gastric cannula was inserted in each horse. Horses were fed each diet, using a randomized crossover design. Stomach contents were collected immediately after feeding and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after feeding on day 14. The pH and VFA and lactate concentrations were measured in gastric juice. Number and severity of gastric lesions were scored during endoscopic examinations.

**Results**—The alfalfa hay-grain diet caused significantly higher pH in gastric juice during the first 5 hours after feeding, compared with that for bromegrass hay. Concentrations of acetic, propionic, and isovaleric acid were significantly higher in gastric juice, and number and severity of nonglandular squamous gastric lesions were significantly lower in horses fed alfalfa hay-grain. Valeric acid, butyric acid, and propionic acid concentrations and pH were useful in predicting severity of nonglandular squamous gastric lesions in horses fed alfalfa hay-grain, whereas valeric acid concentrations and butyric acid were useful in predicting severity of those lesions in horses fed bromegrass hay.

**Conclusions and Clinical Relevance**—An alfalfa hay-grain diet induced significantly higher pH and VFA concentrations in gastric juice than did bromegrass hay. However, number and severity of nonglandular squamous gastric lesions were significantly lower in horses fed alfalfa hay-grain. An alfalfa hay-grain diet may buffer stomach acid in horses. (*Am J Vet Res* 2000;61: 784–790)

Gastric ulcers are highly prevalent in horses.[1,2] Gastric ulcers in horses result in decreased performance and loss of revenue.[2] An increase in factors that damage the gastric mucosa (increased acid content and decreased pH) and decrease in mucosal protective factors (mucus and bicarbonate) have been implicated as causative factors for gastric ulcers in horses.[3] Dietary factors also have been implicated in formation of gastric ulcers. In 1 study,[4] horses in race training had a high prevalence of gastric squamous mucosal ulcers, and those ulcers worsened during training. Also, horses in race training commonly are fed high-concentrate, low-roughage diets.[5]

Hay and grain are staples of diets of horses and contain variable concentrations of fermentable carbohydrates. These carbohydrates may be fermented by resident bacteria to produce **volatile fatty acids (VFA)**. Volatile fatty acids have a low p$K_a$ (4.8) and are highly lipid soluble. At a low gastric pH, VFA become nonionized and may penetrate the nonglandular squamous-mucosal barrier of the nonglandular portion (gastroesophageal region) of the stomach. Once inside squamous epithelial cells, VFA cause acidification, uncoupling of sodium transport, cellular swelling, inflammation, and, ultimately, ulcers.[6] In pigs, VFA can cause cellular injury to the gastroesophageal region of the stomach, which is lined with squamous mucosa.[6,7]

Because the mucosal lining of the proximal third of the stomach of horses is similar to that found in the gastroesophageal mucosa of pigs, the stomachs of horses may be predisposed to injury by VFA as a result of a lack of mucosal protective factors such as mucus and bicarbonate. Acid may cause cellular injury and gastric ulcers.

The purpose of the study reported here was to determine the effect of 2 diets on pH and concentrations of VFA and lactate in gastric contents and to evaluate the number and severity of gastric lesions. Elucidating the role of diet in gastric ulcers in horses may provide important information that could lead to more effective management of affected horses. Furthermore, dietary manipulation may be useful as an adjunct to anti-ulcer therapy.

## Materials and Methods

**Animals and treatments**—Six 7-year-old mixed-breed female horses (mean body weight, 411.7 kg) fitted with a long-term gastric cannula were used in the study. Each cannula had been surgically positioned in the most dependent portion of the stomach, in accordance with the method of Campbell-Thompson and Merritt.[8] Horses were 4 to 7 months old at the time of cannula implantation. During the study, horses were housed separately in box stalls. A salt block was available at all times in each stall. Horses had ad libitum access to water throughout the study, except when

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otherwise indicated. Each day, horses were allowed a few hours of exercise in a gravel lot. All procedures and treatments were approved by a university animal care and use committee.

The study used a 2-period crossover design. During the first period, 3 horses were fed a ration of bromegrass hay, and the other 3 were fed a ration of alfalfa hay and grain. Diets were fed for a period of 14 days. Prior to beginning the first diet period, horses were acclimated to the diet slowly during a 1-week period. After the end of the first period, horses were allowed a week of acclimation in which the diets were gradually reversed until they were being fed only the other diet. The alfalfa hay-grain diet was chosen because it is commonly fed to Standardbred racehorses, and the bromegrass hay diet was chosen because it is commonly fed to racehorses not in training.[9]

Horses were weighed[a] before starting each diet period and again after completion of each diet period. Horses were randomly allocated to an initial diet group by use of a random digit table.[10]

Diets were analyzed for nutrient content (**Appendix 1**), and horses were fed a ration calculated on the basis of 1.9% of body weight, as determined by use of a ration evaluation program.[b] Digestible energy for the alfalfa hay-grain diet ranged from 17.72 to 23.54 Mcal/d, whereas it ranged from 11.47 to 15.29 Mcal/d for the bromegrass hay diet. When horses were fed bromegrass hay alone, digestible energy was, on average, 1.94 Mcal/kg greater than calculated requirements. When horses were fed the alfalfa hay-grain diet, digestible energy was, on average, 18.55 Mcal/kg greater than the calculated requirements. Feed was weighed carefully, using a calibrated scale.[c] Horses were fed twice daily (approx 7:30 AM and 3 PM).

**Endoscopic examination**—Prior to the start of the study (day –7) and again after horses were acclimated to each diet (day 0), but prior to being fed strictly the assigned diet, food was withheld for 18 to 24 hours. Horses were sedated, using xylazine hydrochloride[d] (0.20 mg/kg of body weight, IV) and butorphanol tartrate[e] (0.05 mg/kg, IV), and an endoscopic examination of the stomach was performed on each horse, using a 2.75-m video endoscope.[f] To enable observation of the squamous mucosa (fundus ventriculi), margo plicatus, and glandular mucosa (corpus ventriculi), the stomach was insufflated with air, and the mucosa was rinsed with tap water flushed through the endoscope biopsy channel, using 60-ml syringes. Number and severity of gastric lesions were scored in accordance with an equine gastric lesion scoring system (**Appendix 2**)[11] by a researcher (FMA) who was unaware of the diet each horse was being fed. Following endoscopy, horses continued to be fed their respective diets in accordance with the protocol. After collection of samples on day 14 of the diet, the stomach of each horse was again examined endoscopically.

**Sample collection**—At 7 AM on day 14 of each feeding period, horses were fed the calculated amount of feed. Uneaten feed was removed from the stall at approximately 8 AM. For each sample collection, horses were restrained in stocks with ropes placed in front of and behind them. Each cannula was cleaned to ensure free flow of gastric contents prior to collection of the first sample. Three 150-ml aliquots of gastric juice were collected via the cannula by allowing the fluid to flow by the use of gravimetric force. The stylet was replaced in the cannula after each collection. Samples were stored on ice for 3 hours and then refrigerated at 4 C. Aliquots (50 ml) of gastric juice were stored frozen at –20 C for subsequent analysis of VFA concentration. Gastric juice was collected from the horses immediately after the morning feeding (8 AM) and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 hours after that feeding. Additional food was withheld from horses during the 24-hour sample collection period. Horses were allowed access to water for 30 minutes after collection of each sample, but horses were not allowed additional access to water to prevent dilution of stomach contents.

**Analysis of gastric juice**—The pH of gastric juice was measured, using a pH electrode.[g] To verify that the pH of the gastric juice was representative of the pH of the entire stomach, intragastric pH was recorded, using a portable pH electrode[h] inserted into the stomach via the cannula.

Concentrations of VFA (acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids) were measured in aliquots of gastric juice that had been stored frozen at –20 C. Values were obtained by use of a gas chromatography method described by Playne[12] and modified by Mathew et al.[13]

Samples of gastric juice that had been frozen at –20 C were thawed and analyzed for D$^-$- and L$^+$-lactate. Values were obtained by use of a commercial kit[i] in accordance with the method described by Gutmann and Wahlefeld.[14]

**Statistical analyses**—Mean ± SEM was determined, using a statistical program.[15j] A repeated-measures ANOVA was used to compare differences on the basis of diet, time, and diet × time. Change in score for number and severity of gastric ulcers was examined to ensure that results were not attributable to ulcers in the stomach prior to initiation of the study. Regression analysis was performed for all variables (pH, concentrations of all VFA, concentrations of D$^-$- and L$^+$-lactate) to explain severity of nonglandular squamous lesions. A stepwise model selection process was used to determine whether multiple variables would improve prediction of scores for nonglandular squamous lesions. Significance was defined as $P < 0.05$.

## Results

Body weight of the horses before the diet periods ranged from 316.4 to 455.6 kg. Horses gained a mean of 6.1 kg when fed the alfalfa hay-grain diet and a mean of 15.3 kg when fed the bromegrass hay diet; these values were not significantly different.

Mean pH of the gastric juice varied throughout the 24-hour collection period and ranged from 2.30 to 4.84 when horses were fed the alfalfa hay-grain diet and from 1.95 to 5.12 when horses were fed the bromegrass hay diet (**Fig 1**). Values did not differ significantly between intragastric pH recorded by placing an electrode in the stomach via the cannula and pH of the gastric juice samples. The pH of the gastric juice was significantly ($P = 0.01$) higher in samples obtained 2 through 5 hours after feeding when horses were fed alfalfa hay-grain, compared with values when horses were fed bromegrass hay. Subsequently, pH was significantly ($P = 0.01$) lower in samples obtained 12 hours after feeding when horses were fed the alfalfa hay-grain diet, compared with values when horses were fed bromegrass hay.

Acetic acid had the highest concentration of all measured VFA. Concentration of acetic acid in gastric juice was highest immediately after feeding and decreased significantly over time for horses fed both diets. Mean acetic acid concentration of the gastric juice ranged from 1.10 to 16.31 mmol/L in horses when fed the alfalfa hay-grain diet and from 0.64 to 14.36 mmol/L in horses when fed the bromegrass hay

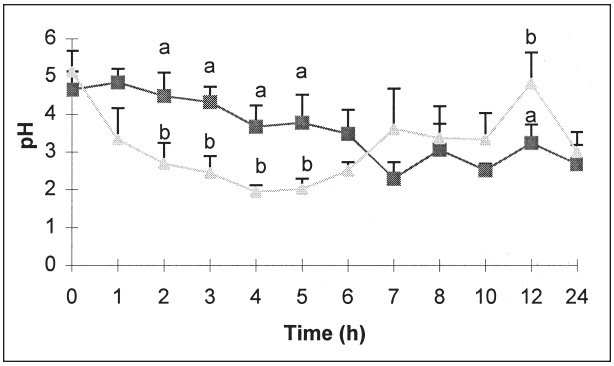


Figure 1—Mean (± SE) pH of the gastric juice when 6 horses were fed a diet of alfalfa hay-grain and when they were fed a diet of bromegrass hay. Time 0 represents sample collected immediately after feed was removed. a,b = Values with different letters differ significantly ($P \leq 0.05$). ■–■ = Alfalfa hay-grain diet. ▲–▲ = Bromegrass hay diet.

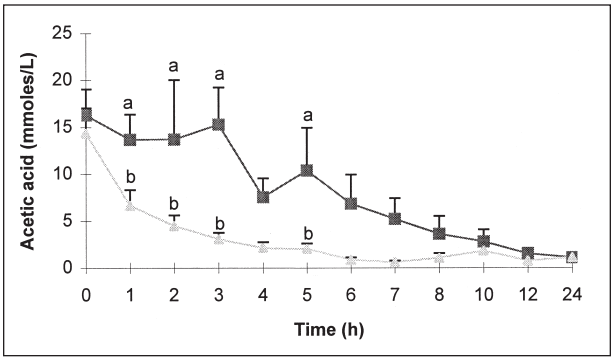


Figure 2—Mean (± SE) acetic acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

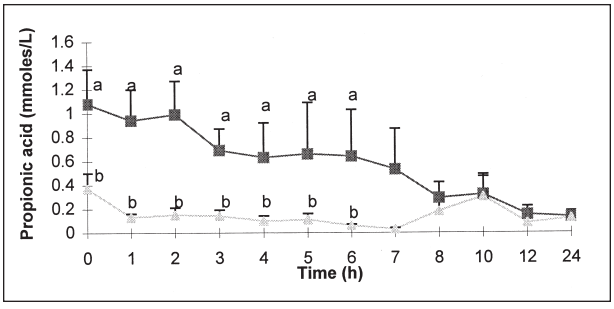


Figure 3—Mean (± SE) propionic acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

diet. Acetic acid concentrations were significantly higher ($P = 0.01$) during the first 3 hours and 5 hours after feeding when horses were fed the alfalfa hay-grain diet, compared with values when horses were fed bromegrass hay (**Fig 2**).

Mean concentration of propionic acid ranged from 0.13 to 1.08 mmol/L when horses were fed the alfalfa hay-grain diet and from 0.03 to 0.37 mmol/L when horses were fed the bromegrass hay diet. Concentration of propionic acid was significantly ($P = 0.01$) higher for 6 hours after feeding in the gastric contents of horses when fed the alfalfa hay-grain diet, compared with concentrations of horses when fed bromegrass hay (**Fig 3**).

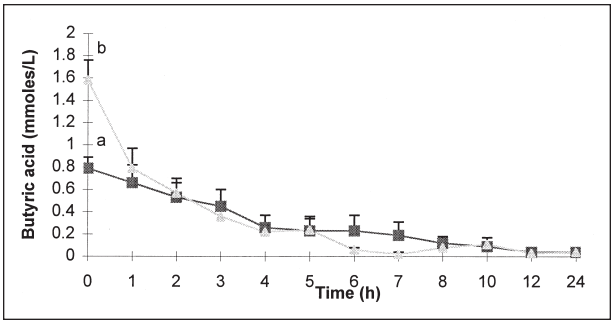


Figure 4—Mean (± SE) butyric acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

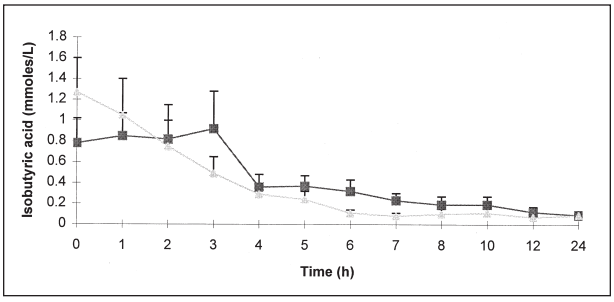


Figure 5—Mean (± SE) isobutyric acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

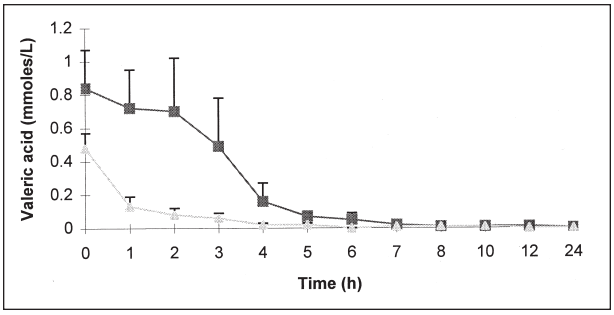


Figure 6—Mean (± SE) valeric acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

Mean concentration of butyric acid in gastric juice ranged from 0.04 to 0.79 mmol/L in horses when fed the alfalfa hay-grain diet and 0.02 to 1.58 mmol/L in horses when fed the bromegrass hay diet. Concentration of butyric acid decreased at a more rapid rate in horses when fed the bromegrass hay diet and was significantly ($P = 0.01$) higher immediately after feeding, compared with concentrations of horses when fed the alfalfa hay-grain diet (**Fig 4**).

Mean concentrations of isobutyric acid (**Fig 5**), valeric acid (**Fig 6**), and isovaleric acid (**Fig 7**) were low in gastric juice. Concentrations of isobutyric, valeric, and isovaleric acids significantly ($P = 0.01$) decreased over time during the first 4 hours after feeding in horses fed both diets.

Mean concentrations of $D^-$- and $L^+$-lactate were < 5.0 mmol/L when horses were fed the alfalfa hay-grain diet and < 3.1 mmol/L when horses were fed the bromegrass hay diet; concentrations remained low

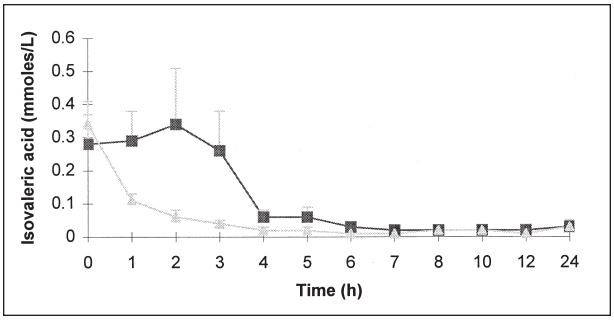


Figure 7—Mean (± SE) isovaleric acid concentration of the gastric juice of 6 horses when fed a diet of alfalfa hay-grain and when fed a diet of bromegrass hay. *See* Figure 1 for key.

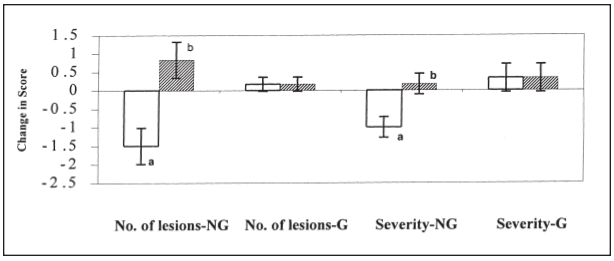


Figure 8—Mean (± SEM) change in number of lesions of the glandular (G) and nonglandular (NG) squamous mucosa and change in severity of the gastric lesions of the glandular and nonglandular squamous mucosa in 6 horses when fed a diet of alfalfa hay-grain (□) and when fed a diet of bromegrass hay (■). a,b = Values with different letters differ significantly ($P \leq 0.05$).

throughout the collection period. Concentration of L$^+$-lactate decreased significantly ($P = 0.01$) over time.

The nonglandular squamous mucosa, margo plicatus, and glandular mucosa of the stomach of each horse were examined. Lesions were primarily seen in the nonglandular squamous mucosa along the margo plicatus. Three of 6 horses had gastric lesions in the nonglandular squamous mucosa when fed the alfalfa hay-grain diet, whereas 5 of 6 horses had gastric lesions in the nonglandular mucosa when fed the bromegrass hay diet. Nonglandular squamous mucosa lesion number and severity were significantly ($P < 0.05$) lower when horses were fed the alfalfa hay-grain diet, compared with values when horses were fed the bromegrass hay diet. Mean change in the number of nonglandular squamous mucosa lesions was –1.5 (number of gastric ulcers decreased by 1.5) in horses when fed the alfalfa hay-grain diet and 0.83 (an increase of almost 1 gastric ulcer) in horses when fed the bromegrass hay diet (**Fig 8**). Mean change in score for nonglandular squamous mucosa lesion severity was –1 (severity of the gastric lesions decreased by 1) in horses when fed the alfalfa hay-grain diet, whereas it was 0.16 in horses when fed the bromegrass hay diet (virtually no change in lesion score). Nonglandular squamous mucosa lesion number and severity were significantly different ($P < 0.05$). Lesions in the glandular mucosa were seen in only 1 of the horses when they were fed both diets.

Results for a stepwise model constructed to determine those measured variables (pH, VFA concentrations, D$^-$-lactate, and L$^+$-lactate concentrations) that would best predict severity of nonglandular squamous lesion revealed that valeric acid (78.2%), pH (14.3%), butyric acid (7.3%), and propionic acid (0.2%) were useful in predicting severity of nonglandular squamous lesions for the alfalfa hay-grain diet. Valeric acid (64.1%) and butryic acid (2.1%) were useful for predicting severity of nonglandular squamous lesions when the bromegrass hay diet was fed.

## Discussion

The horses gained a mean of 6.1 kg while being fed the alfalfa hay-grain diet and a mean of 15.3 kg while being fed the bromegrass hay diet. Body weight did not differ significantly among horses prior to the study, after they were fed the alfalfa hay-grain diet or after they were fed the bromegrass hay diet, which indicates that the horses were not stressed by consumption of a diet of bromegrass hay alone.

Mean pH of gastric juice in the horses in the study reported here, regardless of the diet they were consuming, varied continuously and was similar to that in a previous report for these same horses ($3.2 \pm 2.0$)[16] and in a report of other adult horses ($2.72 \pm 1.86$, range 0.86 to 6.67).[17] In another study in horses,[18] intragastric pH was significantly higher when horses were fed hay ad libitum, compared with when hay was withheld from horses. However, until 5 hours after feeding, the alfalfa hay-grain diet resulted in a significantly higher pH of gastric contents than the bromegrass hay diet. This may have been attributable to the high calcium and protein concentrations in the alfalfa hay-grain diet, because those feedstuffs are high in these components.[19,20] In rats,[19] basal secretion of gastric acid was inhibited when a high-calcium diet was fed. The high calcium content in the alfalfa hay (14.4 mg/g of dry weight) and, to a lesser extent, the grain (3.2 mg/g of dry weight), compared with that for the bromegrass hay (7.4 mg/g of dry weight), may have inhibited gastric acid secretion and increased pH during the initial 5 hours after feeding. The effect of calcium in the alfalfa hay-grain may have resulted from an increase in absorbed calcium, similar to that in the study in rats.[19] In that study in rats, it was postulated that an increase in absorbed calcium may lead to higher concentrations of extracellular calcium, which could then lead to a higher influx of calcium into cells involved in gastric acid secretion, such as parietal and gastrin G cells. The investigators of that study also stated that a high cytosolic calcium concentration in parietal cells could reduce cAMP concentrations and thereby inhibit gastric acid secretion. In contrast, a study in ruminants[20] revealed that increasing the amount of protein in the diet increased intraruminal buffering capacity only when limestone (calcium carbonate) was not included. However, the ruminal epithelium does not secrete gastric acid. This suggests that there may be another mechanism in horses, compared with ruminants. Calcium may have a local effect even in the presence of protein in horses, although this does not appear to be the case in ruminants.

In cattle, a diet high in crude protein (14 to 17%) increased rumen buffering capacity.[20] For the diets in the study reported here, alfalfa hay-grain had high concentrations of crude protein (21% for alfalfa hay, 14%

for grain), whereas bromegrass hay had a low concentration of crude protein (8%). Buffering of gastric contents may have resulted from the high concentration of crude protein found in the alfalfa hay-grain diet. An author of another study[21] indicates that protein in food can act as a buffer against acidity and claims that the buffering power of food plays a major role in affecting ulcer formation in addition to mucosal resistance and acidity.

Seven hours after feeding, pH of gastric juice was less, but not significantly different, in gastric contents of horses when fed the alfalfa hay-grain diet, compared with that of horses when fed the bromegrass hay diet. This decrease in pH when horses were fed the alfalfa hay-grain diet continued until 12 hours after feeding, at which time values were significantly different between the diets. Diets high in calcium inhibit gastric secretion shortly after administration but subsequently may cause rebound hypersecretion of gastric acid. This rebound hypersecretion of gastric acid has been reported in other species after ingestion of calcium-containing supplements and diets and also may be evident in horses.[22,23] A decrease in pH suggests an increase in gastric acid concentration, which was evident in the horses in our study starting 7 hours after feeding of the alfalfa hay-grain diet and continuing for at least 12 hours after feeding. Because food was withheld from the horses in our study, continuous feeding or feeding at intervals of 5 or 6 hours may have helped buffer and increase the pH of stomach contents, which could be beneficial in preventing acidic injury to the squamous mucosa of the stomach of horses. Horses from which food is intermittently withheld and that have a higher prevalence of gastric ulcers have lower stomach pH when compared with fed horses, and this may be attributable to a loss in feed-buffering capacity.[18,24]

The study design did not take into consideration the differences in digestible energy and bulk between the 2 diets; thus, these factors may have played an important role in the differences between the 2 diets. Such factors, including digestible energy, addition of concentrate, and differences in bulk of the diet, may have contributed to differences in pH of stomach contents detected in these horses. In a recent study,[25] Standardbreds were fed 3 diets (unlimited hay [8 to 9 kg]; limited hay and grain [0.6 kg/100 kg of body weight and 0.2 kg/100 kg of body weight, respectively]; grain alone [0.2 kg/100 kg]). In that study, the restricted hay-grain diet resulted in an increase in plasma gastrin concentration immediately after feeding that remained high for the subsequent 4 hours. Thus, that higher plasma gastrin concentration would have resulted in an increase in gastric acid secretion and a lower pH of stomach contents. In contrast, the alfalfa hay-grain diet fed to the horses in our study resulted in a high pH immediately after feeding that remained high for 6 hours after feeding. Analysis of these data suggests that the alfalfa hay-grain diet may have had a buffering effect on stomach acid. Furthermore, in the aforementioned study,[25] Standardbreds were fed unlimited amounts of hay (a diet high in bulk), which resulted in an increase in plasma gastrin concentration that remained high for 12.5 hours after feeding. A high plasma gastrin concentration would be expected to result in an increase in gastric acid secretion and a lower pH of stomach contents. When horses were fed the bromegrass hay diet in our study, stomach contents had a lower pH. Thus, a diet high in bulk may result in increased plasma gastrin concentration, increased gastric acid secretion, and a lower pH of stomach contents.

In the study reported here, VFA concentrations were highest 2 to 6 hours after feeding, and they decreased as food moved out of the stomach. The VFA concentrations were generally < 20 mmol/L in gastric contents, which is similar to that reported in pigs.[26] However, in another report,[27] VFA concentrations ranged from 20 to 40 mmol/L, with acetic acid making up approximately 55 ± 2%. Acetic acid accounted for 78% of the VFA in the gastric contents of the horses in our study. The alfalfa hay-grain diet resulted in a significantly higher acetic acid concentration, compared with the concentration for the bromegrass hay diet. Because the fermentable carbohydrate content in the alfalfa hay-grain diet was higher then than that for the bromegrass hay diet, fermentation of carbohydrates by resident bacteria in the stomach of horses fed the alfalfa hay-grain diet may have caused the increase in acetic acid concentration. In another study,[28] VFA concentrations were insubstantial in feedstuffs fed to ruminants and nonruminants. Therefore, investigators of that study stated that VFA found in the stomach were produced in vivo. In addition, investigators in a study of 24 adult Shetland Ponies found that substantial quantities of VFA (up to 100 m*M*) were produced by microbial digestion in the stomach of the ponies.[29]

Propionic acid concentration was low in this study, ranging from 0.03 to 1.08 mmol/L. Propionic acid concentration was significantly higher in horses when fed the alfalfa hay-grain diet, compared with concentrations during consumption of the bromegrass hay diet. In pigs,[27] propionic acid concentration was believed to be reciprocal to acetic acid concentration, which may be the case with horses. The increased production of propionic acid in the stomach of horses fed the alfalfa hay-grain diet, compared with production when fed the bromegrass hay diet, may have resulted from the fermentation of highly digestible carbohydrates in the alfalfa hay-grain diet.

Butyric acid concentration was also low in this study, ranging from 0 to 1.6 mmol/L. Butyric acid concentration was significantly higher immediately after feeding when horses were fed the bromegrass hay diet, compared with concentrations when horses were fed the alfalfa hay-grain diet. The high butyric acid concentration is surprising and contradicts results of another study[30] in which horses fed grass hay did not produce butyric acid. The higher butyric acid concentration at a lower pH may contribute to acidic injury of the nonglandular squamous mucosa. In the study reported here, concentration of isobutyric acid in gastric contents was higher than the concentration of butyric acid. Isovaleric acid concentration was significantly higher in the gastric contents when horses were fed the alfalfa hay-grain diet, compared with concen-

trations when horses were fed the bromegrass hay diet. Concentrations of isobutyric and isovaleric acids were probably higher in gastric contents than their straight-chained counterparts butyric acid and valeric acid, because branch-chained VFA are not readily absorbed in the squamous mucosa.[31]

Mean concentration of D⁻- and L⁺-lactate was generally < 6 mmol/L in the horses, regardless of diet. In hay-fed cattle, the rumen pool of L⁺-lactate is small, usually approximately 0.12 µmol of lactate/ml.[32] Bacteria that produce D⁻-lactate may not have had a sufficient amount of time in the 2-week feeding period to adapt to the high-grain diet conditions to produce more D⁻-lactate.

Number and severity of nonglandular squamous mucosa lesions were significantly ($P$ = 0.01) lower when horses were fed the alfalfa hay-grain diet, compared with values when horses were fed the bromegrass hay diet. The alfalfa hay-grain diet may have resulted in fewer and less severe nonglandular squamous lesions because of the higher pH of stomach contents, when compared with the bromegrass hay diet, despite higher VFA concentrations in the gastric contents. High VFA concentrations in combination with a low stomach pH have been implicated as a cause of acidic injury and gastric ulcers in pigs.[7] The VFA are monomers in the luminal aqueous phase and can easily be absorbed by the mucosa of any segment of the digestive tract of mammals so that there is complete absorption of VFA.[33] The author of that study states that because several species such as pigs, horses, rabbits, and laboratory rodents have the orad portion of the stomach lined with nonglandular stratified squamous epithelium, such as the forestomach of ruminants, it can be expected that VFA are absorbed in the stomach. Also, the antacid potential of the high-protein, high-calcium alfalfa hay-grain diet may have protected the nonglandular squamous mucosa, even though there were high VFA concentrations. At high stomach pH, VFA do not become dissociated and are not able to penetrate squamous mucosal cells and cause cellular injury.[6]

A stepwise model was used to determine the gastric juice variable (pH, VFA, D⁻-lactate, L⁺-lactate) that would best predict severity of nonglandular squamous lesions. Results for the model revealed that valeric acid (78.2%), pH (14.3%), butyric acid (7.3%), and propionic acid (0.2%) could be used to predict severity of nonglandular squamous lesions when horses were fed the alfalfa hay-grain diet. On the other hand, results of the model revealed that valeric acid (64.1%) and butyric acid (2.1%) could be used to predict severity of nonglandular squamous lesions when horses were fed the bromegrass hay diet; however, the combined predictability for these 2 variables was only 66.2%. Although the alfalfa hay-grain diet produced less severe nonglandular squamous gastric ulcers than the bromegrass hay diet, 3 of 6 horses had gastric lesions while consuming this diet. Horses fed the alfalfa hay-grain diet that had nonglandular squamous gastric lesions had higher concentrations of valeric, butyric, and propionic acid and a lower gastric juice pH than the horses without nonglandular squamous lesions that were being fed the same diet. It must be kept in mind that statistical models are not definitive formulas for a particular problem and that they are only suggestive of possible factors. Other factors also may be important. It must be scientifically proven that variables indicated in the model are important.

Five of 6 horses had nonglandular squamous gastric lesions when consuming the bromegrass hay diet. Horses with nonglandular squamous gastric lesions in this group had higher valeric and butyric acid concentrations than the horses that had fewer or did not have nonglandular squamous gastric lesions. Increased VFA concentrations, especially butyric acid, have been implicated as a cause of gastric ulcers in horses.[30] In a study of 56 horses that were fed a complete feed or a hay diet, 14 of 31 horses that were necropsied had gastric ulcers, and all 14 horses had been fed complete feed. In that study, butyric acid concentrations reached 10% in horses fed the complete feed but were undetectable in horses fed the hay diet. Thus, the author of that study speculated that butyric acid may be a cause of gastric ulcers. In our study, butyric acid concentrations were detected when horses were fed both diets, but pH of gastric contents was higher when horses were fed the alfalfa hay-grain diet. Thus, butyric acid may cause gastric lesions when pH is low but not when pH is high. Butyric acid in an environment with a low pH may become dissociated, leading to acidic injury.[7] It appears that factors in the diets in our study were in sufficient quantity to produce nonglandular squamous gastric ulcers, and these factors may be gastric acidity and VFA concentrations. However, other factors such as duration of withholding of feed, variation in acid secretion among horses, particle size of feed, and stress may play a further role in the high prevalence of gastric ulcers in horses.[2] Additional research is needed to determine the importance of these factors in causing gastric ulcers in horses.

Although *Helicobacter pylori* are an important cause of peptic ulcers in humans, it is probably not part of the mechanism for ulcerogenesis in horses. Researchers did not find bacteria that resembled *Helicobacter* in the glandular or nonglandular stomach of race horses.[34] Parasitic organisms apparently do not play a substantial role in ulcer development, because only 9 of 169 racehorses in that study had parasitic organisms in the stomach.

[a]Allflex scale, Allflex Inc, DFW Airport, Tex.
[b]Spartan equine ration evaluator, Cooperative Extension Service, Michigan State University, East Lansing, Mich.
[c]Shor-Line SL-4 scale, Shor-Line, Kansas City, Mo.
[d]Rompun, Bayer Corp, Shawnee Mission, Kan.
[e]Torbugesic, Fort Dodge Laboratories, Fort Dodge, Iowa.
[f]Endoscope, Fujinon Inc, Wayne, NJ.
[g]General purpose combined pH electrode, Radiometer Inc, Copenhagen, Denmark.
[h]Accumet AP10 portable pH meter, Fisher Scientific, Pittsburgh, Pa.
[i]Lactate analysis kit No. 826-B, Sigma Chemical Co, St Louis, Mo.
[j]SAS, Version 6.0, SAS Institute Inc, Cary, NC.

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## Appendix 1

Analysis of the components of 2 diets fed to 6 horses

| Component | Dry matter (%) | Crude protein (%) | Digestible energy (Mcal/kg) | Acid detergent fiber (%) | Neutral detergent fiber (%) | Calcium (mg/g of feed) |
|---|---|---|---|---|---|---|
| Grain | 99.85 | 14.81 ± 0.17 | 3.76 | 7.34 | 23.78 | 7.37 |
| Alfalfa hay | 94.52 | 20.85 ± 0.24 | 2.49 | 26.36 | 42.12 | 14.10 |
| Bromegrass hay | 94.95 | 7.55 ± 0.31 | 2.13 | 39.44 | 67.80 | 3.17 |

## Appendix 2

Scoring system[11] used to evaluate gastric ulcers in 6 horses fed a diet of alfalfa hay-grain and a diet of bromegrass hay

| Score | No. of lesions | Severity of lesions |
|---|---|---|
| 0 | 0 | Normal appearance |
| 1 | 1 to 2 | Superficial (only involves mucosa) |
| 2 | 3 to 5 | Deeper structures involved (more than mucosa) |
| 3 | 6 to 10 | Multiple lesions and variable severity |
| 4 | > 10 or diffuse (extremely large) lesions | Deeper structures involved (more than mucosa) and active appearance* |
| 5 | — | Deeper structures involved (more than mucosa), active appearance,* and hemorrhage or adherent blood clot |

*Active appearance = Hyperemic, dark crater, or both.
— = No score.