Cyclooxygenase-2 mRNA expression in equine nonglandular and glandular gastric mucosal biopsy specimens obtained before and after induction of gastric ulceration via intermittent feed deprivation

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Objective—To measure the expression of cyclooxygenase-2 (COX-2) mRNA in gastric biopsy specimens serially obtained from horses before, during, and after an 8-day intermittent feed-deprivation trial and to investigate the mucosal location of COX-2.

Animals—9 mixed-breed horses for retrieval of gastric biopsy specimens and 16 additional horses for immunohistochemical analysis.

Procedures—Gastric biopsy specimens were obtained from 6 horses; 3 of these horses and 3 more participated in an intermittent feed-deprivation trial 9 weeks later. A quantitative PCR assay was used to determine the amount of COX-2 mRNA in biopsy specimens from nonulcerated and ulcerated gastric mucosa. Immunohistochemical staining of specimens by use of a polyclonal anti–COX-2 antibody was performed on full-thickness postmortem gastric biopsy specimens.

Results—COX-2 mRNA was expressed in all glandular gastric mucosal specimens but was only detectable in nonglandular mucosal specimens when ulceration was present or during ulcer healing. Positive staining for COX-2 was present in 12 of 14 nonulcerated glandular mucosal sections. Although such staining was weak or absent in nonulcerated nonglandular sections, stronger staining was evident in regenerating epithelium at the rims of erosions and ulcers.

Conclusions and Clinical Relevance—COX-2 was constitutively present in equine glandular gastric mucosa, although its contribution to mucosal protection remains unclear. Our finding of COX-2 mRNA expression in ulcer margins during healing may support a role for the products of this enzyme in mucosal repair. The potential roles of COX-2 should be considered when COX-2–selective inhibitors are prescribed for horses with gastric ulcers. (Am J Vet Res 2010;71:1312–1320)
defenses are modulated by endogenous PGs. It has consequently been proposed that, whereas nonglandular ulcers develop because of excessive exposure to aggressive agents, glandular ulcers develop following impairment of mucosal defenses. The COX enzyme pathway is one of the rate-limiting steps in the conversion of arachidonic acid to PGs. Inhibition of COX is the main mode of action of NSAIDs. Two isoforms of COX (COX-1 and -2) are present in the gastrointestinal mucosa of many species, including horses. Expressed constitutively, COX-1 is considered responsible for basal PG release that has been credited with physiologic housekeeping functions such as gastric mucosal defense. An inducible enzyme, COX-2 can increase PG release following stimulation by several agents including cytokines and growth factors and has been associated with PGs involved in inflammation. With this information in mind, COX-2-selective inhibitors were developed in an attempt to reduce potential adverse effects of NSAIDs considered to be caused by the inhibition of COX-1. However, more recently it was recognized that both COX isoforms may have roles in gastric mucosal defense, with PGs produced via the COX-2 pathway being particularly relevant during ulcer healing.

Despite the widespread use of NSAIDs in equine medicine and the development of a COX-2–selective inhibitor for horses, little is known of the expression or distribution of COX-2 in healthy or ulcerated equine gastric mucosa. In an earlier study, we showed that a selective COX-2 inhibitor could inhibit bradykinin-stimulated PGE, release from equine gastric tissues in vitro, suggesting that COX-2 is active in nonulcerated equine stomachs. The first objective of the study reported here was to measure the expression of COX-2 mRNA in transendoscopic mucosal biopsy specimens obtained from the glandular and nonglandular regions of the equine stomach during an initial experiment to establish basal levels and during and after ulcer induction through feed deprivation. The second objective was to identify COX-2 protein in postmortem gastric mucosal biopsy specimens from a different group of horses by use of immunohistochemical techniques. We hypothesized that COX-2 would be present in low amounts in both sets of tissues under basal conditions but that enzyme production would be upregulated during an intermittent feed-deprivation trial.

Materials and Methods

Animals—Nine healthy crossbred horses or ponies (7 mares, 1 gelding, and 1 stallion) aged 4 to 25 years were used in a study of the effects of intermittent feed deprivation on COX-2 expression. Three ponies and 3 horses were used for the initial experiment involving collection of transendoscopic biopsy specimens. Three of these (1 pony stallion and 2 Thoroughbred-cross mares) and 3 additional Thoroughbred-cross mares were used in an intermittent feed-deprivation trial that began 9 weeks later. All equids had been at pasture, with no enforced exercise, for at least 9 weeks before the commencement of each experiment and received routine anthelmintic treatment 4 weeks before each initial examination. The equids used were members of the teaching herd at University College Dublin.

For the second part of the study, gastric tissues were obtained from 16 Thoroughbred and Thoroughbred-cross horses that had been euthanatized (via free bullet) at a local horse abattoir for reasons other than gastrointestinal disease or systemic illness. The age and sex of 5 of these horses were not available. Of the 11 remaining horses, 4 were female and 7 were male; mean age was estimated by dentition at 8.4 years. The management history of these horses prior to arrival at the abattoir was unknown. The study design and protocols were established in accordance with the relevant Department of Health–granted experimental license and conformed to the guidelines of the University College Dublin Animal Ethics Committee.

Feed-deprivation protocol—Horses were maintained in familiar, individual 3 × 3-m stalls in an indoor barn for the duration of the experiment. Fresh water was available at all times, and horses had access to an outdoor sand arena for 1 hour each day while stabled. A published protocol for the induction of ulcers was used, although the experiment was performed over 8 rather than 7 days for logistic reasons. Briefly, horses were removed from pasture, stabled, and muzzled to prevent eating but not drinking for 24 hours and were then offered ad libitum access to grass hay for 24 hours. Horses were alternately deprived of feed for 24 hours, then provided with ad libitum access to hay for 24 hours until they had been deprived of feed for a total of 84 hours. Horses were then returned to pasture and were reexamined after an additional 7 days.

Gastroscopy and transendoscopic biopsy—Feed was withheld from the 6 equids undertaking the first part of the study for 8 to 12 hours. These equids were then returned to pasture. Nine weeks later, the 6 equids undergoing the feed-deprivation trial were examined after 8 to 12 hours of feed deprivation (day 1) and again following 32 to 36 hours (day 3), 56 to 60 hours (day 5), and 80 to 84 cumulative hours (day 8) of feed deprivation. After 7 days at pasture, the equids were housed and muzzled for 6 to 10 hours before the final examination and biopsy specimen retrieval (day 15).

For gastroscopy, each equid was restrained and sedated with detomidine hydrochloride (10 µg/kg, IV), and a 3-m-long videoendoscope was used to examine the nonglandular region and visible glandular mucosa of each stomach. Images were recorded on videotape and later digitized. After the examinations, images were reviewed in a blinded manner by one of the authors (NKM) and an independent experienced clinician. Ulcer scores were assigned to each stomach by use of a 5-point grading system, in which 0 indicated healthy tissue and 4 indicated severe and extensive ulceration. Because of technical difficulties, recordings could not be made on day 3 of the protocol. Instead, detailed descriptions of changes in each stomach were recorded and used later to assign ulcer scores.

Gastric biopsy specimens were obtained with long (3.25-m) oval cup forceps (internal dimensions, 3.77 × 2.23 mm) that were treated between each specimen retrieval with 0.1% diethyl pyrocarbonate (DEPC). Six
nonglandular and 4 glandular mucosal specimens were removed at each examination from the greater curvature of the stomach, above and below the margo plicatus, respectively. When mucosal defects were observed in the nonglandular mucosa, specimens were obtained from damaged areas and from ulcer margins. One biopsy specimen from each region was placed in neutral-buffered 10% formalin for histologic assessment. The remaining specimens were pooled together and placed in cryovials containing 1 mL of tissue storage reagent and stored at −70°C until performance of RNA isolation.

**Histologic preparation**—Collected tissues were fixed in neutral-buffered 10% formalin for at least 48 hours and then processed overnight for routine paraffin embedding. Fixed tissues were cut in 5-µm-thick sections and stained with H&E before mounting.

**RT-PCR analysis of COX-2 mRNA**—Ribonucleic acid was isolated and purified from the biopsy material by use of a commercial kit in accordance with the manufacturer’s instructions. All samples were incubated with proteinase K to break down collagen and other extracellular matrix proteins. This was followed by an on-column deoxyribonuclease step. The quality of the total RNA obtained was assessed by means of gel electrophoresis. Total RNA was quantified with a commercial kit in accordance with the manufacturer’s instructions. To compare the relative quantities of COX-2 in biopsy specimens, cDNA synthesis was carried out by use of an RT-PCR system and random hexamers with 200 to 500 ng of purified total RNA.

The equine COX-2 gene sequence (accession No. NCBI AB041771) was used to design a sequence-specific assay. Primers and the probe were designed by use of computer software and were synthesized commercially. The COX-2–specific primers and probe were as follows: forward primer, 5’–CATTGATGCTCATGGGGCTGTA–3’; reverse primer, 5’–CTTCCCCGAAGATGGGATCT–3’; and probe, 5’–FAM-CCTGCCCTTCTGGTGAAAGCCCT-TAMRA–3’. An 18S rRNA endogenous control set, labeled with 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxyfluorescein dye, was used to normalize the results.

The RT-PCR assay was conducted in a multiplex system by use of the following cycling conditions: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. All reactions were carried out in duplicate in a final volume of 10 µL. The relative standard curve method of analysis was used to determine the normalized relative quantities of COX-2 target following the guidelines of the PCR-system manufacturer. A standard curve (count vs quantity) was constructed for COX-2 and 18S rRNA and used to convert count values for each sample into relative quantities. The relative quantities for COX-2 were then normalized by use of the 18S rRNA relative quantity values.

**Immunohistochemical evaluation**—Full-thickness biopsy specimens from the nonglandular and glandular regions of gastric mucosa were obtained from 16 horses after euthanasia. Five of the 16 horses had no ulcers visible on inspection of the gastric mucosa. In these horses, full-thickness specimens were obtained from the nonglandular region (dorsal fundus in 4 horses and ≤5 cm dorsal to the margo plicatus in 3 horses) and from the glandular region at the fundus, at the pylorus, and immediately ventral to the margo plicatus at the greater curvature (the cardiac glands). The region of the cardiac glands was inspected in ≥4 of these 5 horses only. The remaining 11 horses had erosions, ulcers, or both in the nonglandular region of the stomach. Full-thickness biopsy specimens were obtained to include the base and rim of lesions.

After up to 14 hours of fixation in formalin, tissues were processed for routine paraffin embedding and were cut into 3-µm-thick sections and mounted. Two serial sections of each tissue were mounted, with 1 used as a negative control specimen. Sections were exposed to 3% hydrogen peroxide solution for 15 minutes at room temperature (approx 22°C) while maintained in a humidity chamber. Sections were incubated with a preheated pepsin solution and dissolved in 0.2N hydrochloric acid for 10 minutes at 37°C to facilitate antigen retrieval. Following a wash with PBS solution, sections were blocked with 5% NGS in PBS solution at room temperature for 1 hour. Afterward, 1 serial section from each tissue was incubated with 5% NGS while the second was incubated with the primary anti-COX-2 (H-62) rabbit polyclonal antibody diluted 1:50 in 5% NGS. All sections were incubated overnight in a humidity chamber at 4°C. After serial washes in PBS solution, sections were incubated for 1 hour at 37°C with peroxidase-conjugated anti-rabbit IgG secondary antibody (raised in goats) diluted 1:500 with 5% NGS. After 2 further washes with PBS solution, the slides were exposed to 100 µL of chromagen (3,3′-diaminobenzidine) for 10 minutes at room temperature. The sections were counterstained with hematoxylin and then routinely prepared for mounting.

**Statistical analysis**—Data for COX-2 mRNA expression is reported as mean ± SEM; ulcer scores are summarized as median and range. The 2-tailed Wilcoxon rank test was performed to compare the ulcer scores assigned by the 2 evaluators. Because the scores did not differ (ie, P > 0.05), the mean of each pair of ulcer scores was used. The normalized relative quantity of COX-2 mRNA expression in gastric tissue on day 1 of the intermittent feed-deprivation trial and at the initial examination performed 9 weeks earlier were compared via the Mann-Whitney U test. The Friedman test of ranks and 1-tailed Wilcoxon signed rank test were used to compare ulcer scores and to compare the normalized relative quantity of COX-2 mRNA at various stages during the feed-deprivation trial. The significance of results was assessed by use of the Wilcoxin T distribution critical values table. The correlation between nonglandular ulcer scores and the normalized relative quantity of COX-2 mRNA was tested, and the Spearman rank correlation coefficient (p) was calculated. All tests were performed with computer software. Values of P ≤ 0.05 were considered significant.

**Results**

**Animals**—There were no clinically detectable signs of abdominal discomfort or other abnormal behaviors
observed during the intermittent feed-deprivation protocol despite the induction of ulcers in 5 of the 6 horses examined.

Gastroscopic and histologic evaluations—At the first gastroscopic examination (day 1), there were no observable ulcers or erosions in the nonglandular or glandular mucosa in any participating equid. The intermittent feed-deprivation trial induced nonglandular mucosal defects in 5 of the 6 participating equids. Two had defects of the nonglandular mucosa after 36 cumulative hours of feed deprivation. Five of the six had visible nonglandular mucosal defects after up to 84 cumulative hours of feed deprivation. Ulcer status changed significantly (P < 0.001) with time, with higher ulcer scores evident on day 8 than on days 1 or 3 (P < 0.05; Figure 1). Defects were consistent in their location, with the first lesions observed immediately dorsal to the margo plicatus at the greater and lesser curvatures and latter lesions developing farther dorsally. No glandular lesions were detected in the stomach of any equid, at any time during the course of the experiment. One equid lacked apparent susceptibility to ulceration induced by this protocol, and although the nonglandular mucosa of this mare did appear hyperkeratotic and hyperemic after 84 cumulative hours of feed deprivation (day 8), no mucosal defects were visibly evident. Ulcers had resolved completely in 3 of the 5 affected equids after the 7-day period at grass. There was a significant (P < 0.025) reduction in nonglandular ulcer scores on day 15 relative to scores on day 8 of feed deprivation.

All biopsy specimens removed from the nonglandular region at day 1 and day 3 contained an intact epithelium, although edema was evident in some sections on day 3. Specimens obtained from ulcer margins when defects were present almost exclusively contained cells of thickened stratum corneum and stratum spinosum and were not useful in verifying the ulcer scores that were assigned by gross appearance. Glandular mucosal specimens contained glandular elements as well as surface epithelial cells. Although free RBCs were observed beneath the surface epithelium in some mucosal sections, no inflammatory exudate or cellular components were associated with the RBCs, and hence they were considered biopsy artifacts. No other abnormalities were evident in biopsy specimens obtained from the glandular region throughout the experiment.

COX-2 mRNA expression—The RNA was successfully isolated from all glandular samples of gastric mucosa; however, 2 of the 36 nonglandular samples (both collected on day 15 of the experimental protocol) had insufficient RNA for analysis and were hence considered missing values for analysis.

Cyclooxygenase-2 mRNA was not detectable in any nonglandular biopsy specimens obtained at the initial basal examination or during the first 2 examinations (days 1 and 3) of the intermittent feed-deprivation trial (Figure 2). The amount of COX-2 mRNA in the nonglandular tissues varied significantly over the deprivation

![Figure 1](image1.png)

**Figure 1**—Median (middle horizontal line) and range (top and bottom box limits) ulcer scores for biopsy specimens of nonglandular gastric tissues obtained from 6 horses at various points during an 8-day intermittent feed-deprivation trial followed by 7 days at pasture. Ulcer scores were assigned on a 5-point scale in which 0 represented nonulcerated mucosa and 4 represented severely and extensively ulcerated mucosa. *Values with the same letters differ significantly (P < 0.05) from one another (1-tailed Wilcoxon signed rank test).

![Figure 2](image2.png)

**Figure 2**—Mean ± SEM normalized relative quantities of COX-2 mRNA (unitless number normalized to 18S rRNA quantity) of nonglandular (A) and glandular (B) gastric tissues obtained from 6 horses at various points during an 8-day intermittent feed-deprivation trial followed by 7 days at pasture. Normalized relative quantities of COX-2 mRNA differed significantly (P < 0.05) over time for both mucosal types (Friedman test of ranks). See Figure 1 for remainder of key.
Figure 3—Photomicrographs of sections of equine gastric mucosa incubated with immunostain. A—Tissue stained by use of rabbit polyclonal anti–COX-2 antibody, revealing weak COX-2 staining in the stratum corneum. B—Negative control tissue stained without primary antibody. C—Healthy glandular mucosa incubated with rabbit polyclonal anti–COX-2 antibody. D—Healthy glandular mucosa processed without primary antibody (negative control specimen). E—Nonglandular mucosa with staining of epithelial elements of an erosion. F—Nonglandular mucosa with staining at the margin of an ulcer and in the stratum spinosum and strong staining in cells of the stratum basale. Arrows indicate areas with immunopositive staining. 3,3′-diaminobenzidine substrate and hematoxylin counterstain; bar = 10 µm.
period (P = 0.016), and COX-2 mRNA was expressed in nonglandular biopsy specimens from equids with visible nonglandular mucosal defects on days 5 and 8 (up to 60 and 84 cumulative hours of feed deprivation, respectively). Expression of COX-2 mRNA returned to nondetectable values in equids in which ulcers had healed on the final visit (day 15). When normalized to quantities of 18S rRNA, the amount of COX-2 mRNA in these nonglandular gastric specimens was lower than that routinely measured in biopsy specimens from the glandular mucosa. During the protocol and recovery period, nonglandular ulcer scores and COX-2 mRNA quantities in corresponding gastric biopsy specimens were positively correlated (p = 0.627; P < 0.01 for all). On the other hand, COX-2 mRNA was undetectable in all nonglandular samples obtained from the horse that did not develop visible gastric lesions during the intermittent feed-deprivation trial.

Cyclooxygenase-2 mRNA (values normalized relative to 18S RNA values) was detected in all glandular mucosal specimens obtained in the initial basal experiment (n = 6) or on day 1 (6) of the feed-deprivation trial following 8 to 12 hours of food withholding. Values did not differ between the 2 days (P = 0.132). Expression of COX-2 mRNA was detected in all biopsy specimens obtained from the glandular mucosa at all examinations during the feed-deprivation trial, including specimens obtained from the horse that did not develop visible nonglandular defects. A Friedman test revealed that COX-2 mRNA expression in glandular specimens also varied significantly over the feed-deprivation period (P = 0.05). Although a nonsignificant increase in mean ± SEM relative COX-2 mRNA expression was detected between days 1 (0.67 ± 0.11) and 3 (0.93 ± 0.17), expression was significantly lower on day 5 (0.43 ± 0.07) than on day 3 (P < 0.025). At the final examination (day 15), mean relative COX-2 mRNA expression (1.04 ± 0.26) had returned to day 1 and day 3 values and was significantly higher (P < 0.05) than values on days 5 and 8.

Immunohistochemical evaluation—Five of the 16 euthanatized horses had no evidence of mucosal defects in their stomachs. In these 5 horses, 1 of 4 samples collected from the dorsal fundus and all 3 of the samples collected immediately dorsal to the margo plicatus had COX-2 immunostaining present in the stratum corneum, although this staining was weak (Figure 3). Twelve of 14 glandular biopsy specimens obtained from the 5 horses had COX-2 immunostaining present. The distribution of this staining varied but was associated primarily with cells lining the upper and deeper glands including mucus cells.

Eight erosions and 8 ulcers were evident in the nonglandular region of the stomachs of the remaining 11 horses. Immunostaining for COX-2 was observed in all of these tissue sections, although staining was considered weak in 3 of the 8 erosions and 1 of the 8 ulcers. Immunostaining for COX-2 was also observed in sloughing cells of the stratum corneum at ulcer margins. However, the strongest COX-2 staining was observed in the epithelium at the rim of defects, primarily in the stratum spinosum and stratum basale. Although no positive immunostaining was evident in the lamina propria or in endothelial cells of nonulcerated tissue sections, COX-2 immunostained endothelial cells were detected in the lamina propria beneath 9 of the 16 mucosal defects examined.

Discussion

The study reported here involved evaluation of changes in mucosal appearance and expression of COX-2 mRNA in gastric biopsy specimens from equids that underwent an intermittent feed-deprivation trial. The deterioration in the gastroscopic appearance of the nonglandular mucosa observed during this trial was consistent with findings in other reports,1,9,31 as was the rate of spontaneous healing following the period during which equids were returned to pasture.9 Excessive exposure of nonglandular gastric mucosa to acid has been proposed as the primary pathophysiologic mechanism for ulcer development during such a trial,1 although the presence of refluxed bile acids in feedless stomachs may also contribute.32 In horses, the pH of the stomach falls rapidly when food is withheld, and the pH gradient that usually limits the exposure of the nonglandular mucosa to acid27 is lost. However, the susceptibility of the nonglandular mucosa to the ulcerative effects of a low pH may vary among animals,9 which may explain the apparent lack of ulceration in 1 study horse after feed deprivation. As suggested in other reports,1,9,31 no defects were detected in the glandular region of the stomach during intermittent feed deprivation, which probably reflects the strong defenses of the glandular mucosa against acid injury in vivo.

We were interested in investigating the basal expression of COX-2 mRNA in equine gastric mucosa in vivo. The nonglandular biopsy specimens retrieved from equids at the initial experiment and on the first day of the feed-deprivation trial had no detectable COX-2 mRNA present. Immunostaining for COX-2 was absent or weak in the epithelial layers of healthy nonglandular mucosal tissues. In morphologically similar healthy esophageal mucosa, COX-2 expression is reportedly undetectable,33 low,34 or inconsistent.35 In another study,37 we found that PGE2 release from the epithelial facet of equine nonglandular gastric mucosa was low in vitro, although there was some evidence of COX-2 enzymatic activity in the subepithelial elements in response to bradykinin. The use of immunohistochemical techniques in the present study did not confirm the location of this subepithelial COX-2 in nongulcerated nonglandular mucosal tissues.

A potentially damaging concentration of gastric acid and bile acids can accrue in the stomachs of horses after withholding of feed for 14 hours.32 Although damage was visible during gastroscopy and confirmed histologically in the present study, in some horses in response to 30 to 36 cumulative hours of feed deprivation, COX-2 mRNA could not be detected in biopsy specimens collected at that time. This is in contrast to esophageal findings in humans, in which exposure to gastric acid or bile acids can stimulate an increase in COX-2 expression even in the absence of visible mucosal defects.35-36 The role of the primary products of the COX enzymes, PGs, in the defense of squamous gastrointestinal mucosa is to date unclear. Equine nonglandular gastric mucosa would not appear to be susceptible
to injury caused by NSAIDs, suggesting that PGs are not of primary importance in the defense of this mucosa as they are in the adjacent glandular mucosa.30,41 Similarly, PGs are not considered to have a protective function in the esophageal mucosa, with NSAID administration reported to actually protect the esophagus from radiation-induced injury.62

During the intermittent feed-deprivation trial, COX-2 mRNA expression was upregulated in nonglandular biopsy specimens as the degree of peptic injury became more severe or extensive. In rats, enhanced COX-2 mRNA expression and protein concentration can exist at the margins of gastric ulcers.31,33,46,47 In the equids of this report, expression of COX-2 mRNA was undetectable in nonglandular biopsy specimens from stomachs in which healing appeared complete after 7 days at pasture, although it remained high at sites at which healing was progressing. It has been reported that ulcer healing is initiated soon after the development of ulcers during the feed-deprivation protocol used in our study, with epithelial proliferation at ulcer margins (in keratinized and nonkeratinized layers) and an increase in vascular cross-sectional area in the lamina propria.31 Using immunohistochemical techniques, we observed the strongest COX-2 immunostaining in the stratum basale and stratum spinosum at ulcer margins, with COX-2 immunostaining also associated with endothelial cells and capillaries beneath ulcer beds. The stratum basale of epithelial tissues at the ulcer margin and cells immediately adjacent to capillaries are reportedly the sites with greatest cell proliferation during such a feed-deprivation trial.45

Cyclooxygenase-2 has been associated with proliferating cells in various models of gastrointestinal disease23,39,46,47 and has an important role in angiogenesis.23,49 Successful epithelial wound healing requires an inflammatory response, including capillary congestion and neutrophil and macrophage influx as well as angiogenesis, epithelial hyperplasia, wound contracture, and re-epithelialization of the lesion.35 Although, to our knowledge, studies have not yet been conducted to assess the impact of COX-2–selective inhibitors on the healing of induced or preexisting ulcers in horses in vivo, inhibition of COX-2 has been shown to delay the healing of gastric ulcers in other animal species.22,25,48 It would be interesting to investigate whether administration of COX-2–selective inhibitors would have altered the rate of healing observed in our study once equids were returned to pasture.

Both before and on the first day of the feed-deprivation trial, COX-2 mRNA was consistently detected in all biopsy specimens (n = 9) from the glandular region. In humans, COX-2 mRNA expression is 10-fold higher in the gastric antrum than in the esophageal mucosa.30 It is possible that COX-2 may have been produced de novo in the glandular mucosa during our experiment in response to the 8-hour feed-withholding period required to facilitate clear image acquisition during gastroscopy. Maximal COX-2 mRNA upregulation can occur in gastric mucosa as little as 1 hour after acid exposure67 and, as previously mentioned, feed deprivation is associated with a rapid reduction in gastric pH in horses.6,7 Although nonglandular mucosa is not usually exposed to acid, glandular mucosa would be regularly responding to such conditions. Our finding that COX-2 mRNA was expressed in healthy glandular mucosa was interesting because it has also been detected in healthy equine jejunal tissues.47 and other authors have reported that COX-2 is also present in the healthy human13,34,47 and canine15 stomachs.

When glandular mucosal biopsy specimens obtained during postmortem processing were examined in our study, there was evidence of COX-2 immunostaining in most sections in mucus-producing cells and in cells deeper in the gastric glands. Cyclooxygenase-2 immunoreactivity has been detected in mucus-secreting and parietal cells of human stomachs.31 The role of endogenous PGs produced via the COX-2 pathway on mucus or parietal cells in equine stomachs has not been investigated to date, although it is possible that these cells may modulate mucus or acid secretion in certain physiologic conditions. Although some immunopositive endothelial cells were detected in the present study, COX-2 immunostaining was not evident in mononuclear cells of the lamina propria, although such staining has been reported elsewhere.13,47,50 Different antibodies used or other technical or species differences may account for this discrepancy. Whereas COX-1 is likely more important than COX-2 in basal PG production,21 the presence of COX-2 mRNA and COX-2 immunostaining in nonglandular biopsy specimens obtained in our study would suggest that COX-2 may also have a role in gastric mucosal protection.

As anticipated, the histologic and gross appearance of the glandular mucosa remained unchanged during the intermittent feed-deprivation trial. We had hypothesized that COX-2 expression in this mucosa would increase to provide additional PGs in response to the heightened lumenal challenge, as has been suggested for other species.21 However, COX-2 mRNA expression did not increase as predicted, and instead, COX-2 mRNA expression declined during the period of feed deprivation, returning to day 1 values following the 7 days horses were at pasture. This temporary decline in COX-2 may have been an anomaly, or day 1 values may have already been high because of a rapid induction of COX-2 similar to that reported in other gastric mucosae.69 More frequent sample collection in the early stages of feed deprivation may be required to determine the exact pattern of COX-2 mRNA expression in this mucosa in response to gastric acid and bile acids exposure.

The results of this study would suggest that COX-2 in horses may have a physiologic role in the nonglandular and glandular gastric mucosae of the stomach. Cyclooxygenase-2 may be important in the healing of nonglandular gastric ulcers. The use of selective COX-2 inhibitors may therefore potentially prolong the healing of preexisting nonglandular ulcers, which are common in performance horses. The consistent presence of COX-2 mRNA in all glandular biopsy specimens obtained in our study would suggest a potential role for COX-2 in the production of PGs required for the defense of this mucosa, although the exact mechanism for this remains unclear.
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