Oesophageal lumen pH in yearling horses and the effect of management and administration of omeprazole

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Summary

Background: In humans, arytenoid chondritis can be caused by chemical trauma of mucosa from gastro-oesophageal reflux. While a similar process could be involved in the aetiopathogenesis of arytenoid chondritis in horses, the oesophageal lumen pH in this species is poorly understood.

Objectives: To determine if gastro-oesophageal reflux occurs in horses by characterising oesophageal lumen pH.

Study design: Blinded, randomised, placebo-controlled, crossover, experimental study.

Methods: Luminal oesophageal pH of six yearling horses was recorded for four 24 h periods using an ambulatory pH recorder attached to a catheter with two electrodes (proximal and distal) that was inserted into the oesophagus. Recordings of pH were made during three management protocols. Initially, horses grazed in a paddock (Protocol A). Horses were then moved to stables to simulate sale preparation of Thoroughbred yearlings, and were administered either omeprazole (Protocol B) or placebo paste (Protocol C) orally once daily. Protocol A was repeated for each horse (after a 13 d washout period) between Protocols B and C. Summary statistics described pH range and frequency of pH changes. Associations with predictor variables were investigated using linear mixed effects models. Data are presented as mean ± standard deviation.

Results: Oesophageal lumen pH ranged from 4.90 to 9.70 (7.36 ± 0.27 and 7.18 ± 0.24 for proximal and distal electrodes, respectively) and varied frequently (1.2 ± 0.9 changes/min and 0.8 ± 0.8 changes/min for proximal and distal electrodes, respectively). Oesophageal lumen pH was associated with time since concentrate feeding, activity and time of day but not treatment with omeprazole.

Main Limitations: A small number of horses were used and measurement periods were limited.

Conclusions: This study indicates that gastro-oesophageal reflux occurs in clinically normal yearling horses. Although omeprazole had no detectable effect, oesophageal lumen pH recorded during this study did not fall within the therapeutic range of omeprazole.

Introduction

Arytenoid chondritis is a progressive, inflammatory condition of the arytenoid cartilages [1]. The disease has been reported in humans [2] cattle [3], sheep [4], alpacas [5] and horses [6; 7]. In horses, arytenoid chondritis is most commonly diagnosed in young animals, particularly Thoroughbred yearlings and young racehorses, and is a source of wastage and economic loss to the equine industry [6]. A wide range of mucosal and cartilage lesions, including mucosal ulceration, necrosis and granuloma formation, are associated with the condition in horses and response to treatment is variable [6].

In humans, arytenoid chondritis is often associated with chemical trauma to the laryngeal mucosa due to gastro-oesophageal reflux [8; 9] and treatment is aimed at reducing gastric acidity (pH>4) by the use of proton pump inhibitors such as omeprazole [10; 11]. While the aetiology of arytenoid chondritis in horses is unknown, stress, diet, excessive vocalisation and trauma have been proposed as risk factors for the condition [6; 12; 13]. We hypothesised that reflux of acidic gastric contents could cause mucosal trauma and predispose horses to arytenoid chondritis.

The objective of this study was to determine if changes in oesophageal lumen pH, consistent with gastro-oesophageal reflux, occur in horses. A second objective was to identify time, animal and management factors that might influence oesophageal lumen pH in horses.

Materials and methods

This study was conducted at the Charles Sturt University (CSU) Veterinary Clinical Centre. The CSU Animal Care and Ethics Committee (Reference No. 13/092) approved the study.

Horses

Five yearling Australian Stock Horses (3 colts and 2 fillies, weight range 265—301 kg) and one yearling Australian Stock Horse Thoroughbred cross (colt, weight 380 kg), were recruited for the study. The horses had not been used for previous studies and were kept on pasture prior to the commencement of the study. On physical examination, all horses were found to be healthy and in good body condition.

Study design and horse management

The study was a blinded, randomised, placebo-controlled crossover design. Oesophageal lumen pH was measured for four 24 h periods in each horse. Measurements were recorded during three different management protocols. During Protocol A, horses grazed in a paddock and were fed supplementary lucerne hay twice daily. Horses were not exercised during this protocol. Protocols B and C simulated a sale preparation environment. Horses were stabled and fed a high concentrate and low roughage ration. The concentrate ration was a commercially available product appropriate for yearlings in sale preparation (15.8% crude protein, 13.6 MJ/kg digestible energy; containing extruded grains and legumes with vitamin and mineral pellets). The amount of concentrate fed to each horse was based on the product guidelines for individual body weight, and was introduced gradually over one week. Additional lucerne hay was fed twice daily. During Protocols B and C, exercise was in-hand walking for 10—15 min twice daily. All horses were walked, fed and treated within the same time period each day. Horses had *ad libitum* access to water in all three protocols.

For acclimatisation, horses were introduced to the Protocol A environment (paddock) one week prior to the first recording period (A1). Following Protocol A1, horses were moved to individual stables for 7 days of acclimatisation for sale preparation. On day 7, the horses were randomly allocated to, and commenced, either Protocol B (administration of omeprazole 4 mg/kg *per os* once daily, before food) or Protocol C (administration of placebo which consisted of the drug carrier preparation only). The formulation of omeprazole used was an oil based product given at a dose rate of 92mg/g. The investigators and horse handlers were blinded to all treatments. On day 14, oesophageal pH of each horse was recorded. Horses were then relocated to the paddock for a 13 d wash out period. Protocol A was then repeated (A2) for each horse followed by crossed-over Protocols B or C. At the conclusion of the study, the horses were returned to the care of the CSU Equine Centre. Table S1 (supplementary material) shows the day on which oesophageal lumen pH was measured for each horse after each protocol.

Oesophageal pH

Measurements were recorded automatically every five seconds for up to 24 h using a continuous pH data recorder (Orion II, ambulatory pH recorder)[a] and a pH catheter (Greenfield™, single-use pH catheter, MMS-pH-15)[b]. The pH catheter was 1.6 m long with distal (tip) and proximal (15 cm from the catheter tip) electrodes (Figure 1). The horses were placed in stocks, restrained using a nose-twitch and sedated when required using combinations of xylazine (Ilium Xylazil-100 Analgesic, Sedative and Muscle Relaxant Injection)[c] (50—250 mg), butorphanol (Ilium Butorphanol Injection)[d] (5—25 mg) or detomidine hydrochloride (Dormosedan)[e] (2 mg) administered intravenously. The larynx was then examined endoscopically. Gastroscopy was not performed to avoid introduction of acidic stomach contents into the oesophagus. The oesophageal pH catheter was calibrated prior to insertion using distilled water and pH solutions of pH 4.00 ± 0.05[f] and 7.01 ± 0.05[g], according to the manufacturer's instructions. Once calibrated, the catheter was inserted via the right nostril into the distal third of the cervical oesophagus (80 cm from the nostril) under endoscopic guidance using biopsy forceps and a piece of masking tape attached to the catheter (Figure 1). The pH catheter was secured to the skin of the nostril using a Chinese finger-trap suture. The external portion of the catheter was taped to the halter and connected to the data recorder. The data recorder was secured to the halter in the inter-mandibular space (Figure S1). Recording of oesophageal lumen pH was initiated as soon as possible following catheter insertion, generally between 0930 and 1115 each day. If a horse broke or damaged a catheter during a measurement period, a new catheter was inserted as soon as possible.

Data analysis

Data were downloaded from the pH data recorder onto a computer using dedicated pH measurement software (MMS Investigation and Diagnostic Software)[h]. Data from both electrodes (proximal and distal) were analysed using the software package, R [14]. Raw data were prepared for analysis according to the following rules:

- Readings were removed if the catheter was not in the horse or connected to the ambulatory pH data recorder.

- Obvious artefactual recordings were removed (for example, negative readings and those outside pH range 1—14 due to catheter damage).

- Fifteen minutes of data (180 readings) were removed from the time the catheter was connected and prior to disconnection or artefactual recording. This covered the time required to return the horse to the paddock or stable, the time taken to bring the horse to the stocks to remove or replace the catheter and the time it might have taken for the horse to damage the catheter.

Initial descriptive analysis (packages epiR [15], plotrix [16]) examined the distributions of pH readings and obtained summary statistics for each horse, protocol and electrode. The pH of the electrodes were compared using a paired t-test and were found to be significantly different. Therefore, data from each electrode were not combined for analysis of the effect of predictor variables on pH measurements. The number of pH changes was counted at four randomly selected 5 min time periods for each recorded period to determine pH changes/min. A pH change was defined as the point at which the pH crossed the mean pH for the protocol. Data for continuous variables were expressed as mean ± s.d., unless stated otherwise.

Associations between the oesophageal lumen pH and predictor variables were investigated using univariable and linear mixed effects models for each electrode (packages lme4 [17] and nlme

[18]) (null hypothesis that there were no significant differences between protocols; $P<0.05$). Variables were time-related (am or pm, day or night, day number), horse-related (horse identity, sex, weight) and management-related (protocol, housing environment, treatment, type of activity, amount of concentrate, time since concentrate feeding, calibration fluid batch). The variable am or pm was defined as am: midnight to midday; pm: midday to midnight. The variable day or night was described as day: 0730 to 1930; night: 1930 h to 0730 h. Types of activity included stable, paddock turn out, hand walking and grazing in the paddock. Variables were included in the multivariable regression using a forward, stepwise selection of variables. Horse identification (horse ID) and protocol were included as nested random effects to account for heterogeneity of variance (horse ID and protocol, Fligner test, $P<0.001$). Final models for both proximal and distal electrode models included the following predictor variables: time since concentrate feeding, type of activity, time of day (am or pm and night or day) and sex (proximal electrode only). Model fit was assessed using residual plots, Akaike's Information Criterion (AIC) and R^2 [19; 20].

Results

Horses

All horses were able to eat and drink normally with the oesophageal pH catheter in place. No clinical evidence of detrimental effects due to the catheters, or adverse effects due to the administration of omeprazole were observed throughout the study.

During Protocol A horses lost weight (mean 2.3 ± 3.7 kg). However horses gained weight during both Protocol B (17.7 ± 9.0 kg) and Protocol C (14.3 ± 7.9 kg). Overall, horses gained a mean weight of 29.7 ± 6.7 kg (range 17—36 kg) throughout the 7-week study period.

Endoscopy

A hematoma (approximately 2 mm diameter) involving the mucosa of the left arytenoid cartilage of Horse 4 was observed on endoscopic examination during Protocols B and C. This lesion was not detected during endoscopic examination in either Protocol A1 or A2. A 2 mm diameter, white, raised, lesion surrounded by erythema approximately 2 mm wide, was detected on endoscopic examination on the left arytenoid cartilage of Horse 6 during Protocol C. This lesion was not visible 2 d after the horse was returned to the paddock. For both of these horses, behavioural abnormalities were not observed throughout the study, and no abnormalities were detected on clinical examination during protocols in which lesions were detected. The remaining four horses had no visible lesions on the larynges throughout the study.

Descriptive data analysis

After data were trimmed, each of the six yearling horses had oesophageal pH measurements recorded for mean duration of 13:05 ± 09:06 h (range 00:48—23:12), with mean number of recorded measurements 9318 ± 6513 (range 579—16705). Although 24 h was allocated for data collection for each protocol, some horses consistently damaged catheters, and consequently, oesophageal pH was recorded for shorter periods. The histogram in Figure S2 (supplementary material) illustrates normal distribution of oesophageal lumen pH measurements and boxplots demonstrate oesophageal lumen pH measurements for horses in each protocol for both electrodes. No significant difference was detected in mean oesophageal lumen pH measurements between electrodes and protocols (ANOVA, $P>0.05$). Overall measurements for each electrode are summarised in Figure 2. Although measurements recorded by the proximal and distal electrodes followed a similar pattern (Figure 3), readings from the proximal electrodes were significantly higher than those from the distal electrode ($P<0.001$) for all horses in each protocol.

Overall, pH varied 0—19 times in each 5 min period, with 1.2 ± 0.9 changes/min for the proximal electrode and 0.8 ± 0.8 changes/min for the distal electrode. Figure 3 demonstrates the pH changes over time and Table 1 shows pH change frequencies summarised for each electrode during each protocol.

Analysis of associations between oesophageal pH and predictor variables

The association between pH and individual predictor variables was investigated using univariable mixed models (Table 2). Regression coefficients and level of statistical significance for selected fixed-effect variables in the final models are shown in Table 3.

Time within 4—6 hours post concentrate feeding was generally associated with lower oesophageal pH. During periods of hand walking, pH measurements were significantly higher than during other activities. Correlations between the variables "night or day" and "am or pm" were not found, and interactions introduced into the models did not improve model fit. Day readings were significantly lower than night readings, and pm readings were significantly lower than am readings. Therefore, the period from 1200 to 1930 had the lowest oesophageal pH readings. Male horses had significantly lower oesophageal pH at the proximal electrode, but differences between sexes were not observed at the distal electrode. Treatment (omeprazole), amount of concentrate feed, calibration fluid, day number and weight were not found to be associated with oesophageal lumen pH.

Residual plots suggested reasonable model fit (Figure S3). However, R^2 was 0.33 and 0.29 for the proximal and distal models respectively, indicating that the models did not explain approximately two thirds of variation in pH.

Discussion

Key findings in this study were the rapid variations in oesophageal pH measurements ranging from pH 4.9 to pH 9.7, the significantly lower pH of the distal electrode and lower pH during the post-prandial and afternoon periods. Because the oesophageal epithelium has no secretory function [21] these findings suggest gastro-oesophageal reflux occurs in horses. Conversely, high pH measurements (e.g. pH 9.7 recorded by the proximal electrode) are consistent with swallowed oral secretions that are alkaline [22; 23]. The absence of pathological findings in the current study suggests that repeated gastro-oesophageal reflux is a normal physiologic event in yearling horses.

We found that the time since concentrate feeding and time of day both influenced oesophageal lumen pH. The greatest reduction in pH values were seen between 2—6 h following concentrate feed being provided. These results are consistent with a study by Murray and Schusser [24] who found that gastric pH of horses was decreased 4—6 h after feeding. Other studies have found that the gastric pH of stabled horses is lower than that of horses kept on pasture, most likely due to altered eating behaviour and diet [25; 26]. Feeding concentrates is associated with reduced salivation, the stimulation of gastrin secretion in the stomach and less buffering capacity than roughage [27]. These factors might contribute to the lower oesophageal pH observed with concentrate feeding in the current study. In addition, lower oesophageal pH was more likely in the afternoon and early evening (between 1200 and 1930). This is similar to findings from a previous study in which a decrease in gastric pH was observed in the evening [28]. While there were no interactions between the effects of time of day and time since concentrate feeding it is possible that the extended time association with lower oesophageal pH found after the evening feed in the current study was due to both these factors.

The type of activity that the horses were undertaking also influenced oesophageal pH measurements in the current study. In a previous study, Lorenzo-Figueras and Merritt [29] found that gastric contents from the ventral portion of the stomach could be forced cranially during exercise at gaits faster than a walk. Although the horses in the current study were only turned out once during measurement periods, the turn-out period, during which horses galloped around the paddock, was associated with a significant decrease in the oesophageal lumen pH. This finding may reflect oesophageal reflux of gastric contents associated with exercise. Further, higher pH values were measured during hand walking of the horses and it is possible that the high head position during hand walking might limit the extent of gastro-oesophageal reflux. In comparison to paddock and hand walked periods, lower pH measurements were associated with stabled periods. This cannot be explained by exercise, and it is possible that during stabled periods other factors, such as time since concentrate feeding or roughage intake, have a greater influence.

Overall, this study suggests that gastro-oesophageal reflux occurs in horses and is influenced by management practices. It should be noted, however, that the management factors in the models explained only one-third of the variability in pH measurements. In addition, the pH changes associated with these factors were small. This indicates that there are other sources of variation in oesophageal lumen pH including horse-associated factors (e.g. temperament), effect of drug administration (e.g. sedatives were administered to horses during this study), amount of food and water consumed, or other management factors that were not included in the analysis. The effects of temperament and sedative drugs were not assessed. All horses were sedated within an hour prior to insertion of the catheters and dosage was subjective because of variation between veterinarians' assessments of horse behaviour. It is possible that sedation has an effect on cardiac sphincter tone and could influence gastro-oesophageal reflux. It might be expected that low oesophageal pH would be seen post-sedation (lower head carriage and potential effect on sphincter tone). This was not observed in this study. Due to practical constraints, the amounts of feed and water consumed were not recorded; for example, some horses spilled large amounts of feed into the sawdust bedding during sale preparation protocols, and a communal, self-filling water trough was used in the paddock protocols. Investigation of the effects of sedation, the amount of feed consumed as well as other factors that might influence oesophageal lumen pH are worth pursuing in further studies.

Apart from two small, transient, laryngeal lesions observed in two horses during sale preparation protocols, pathological changes were not found in this study. Due to the small number of lesions and horses, it was not possible to infer causation between potential risk factors and these lesions. However, it remains possible that arytenoid chondritis in horses is associated with laryngeal mucosal trauma following retrograde movement of low pH gastric contents in excess (both in reflux distance and lower pH) of that observed in the current study. A case-report in which oesophageal reflux was associated with pathology in a horses has been published; Heidmann *et al.* [30] reported a yearling filly that presented with severe oesophageal ulceration as a result of gastro-oesophageal reflux. It is possible that the larynx is sensitive to acidic trauma like the oesophagus, and that oesophageal reflux that reaches the larynx may cause inflammation and predispose to arytenoid chondritis. This would be consistent with the aetiopathogenesis of arytenoid chondritis in humans [2; 31; 32]. We suggest measurement of both oesophageal lumen pH closer to the larynx and gastric pH during sale preparation in both normal horses and horses with arytenoid chondritis to investigate correlations between gastric and oesophageal pH in horses.

In humans, omeprazole is an effective treatment of arytenoid chondritis associated with gastro-oesophageal reflux. In the current study, no association between omeprazole and oesophageal pH could be made. This is to be expected as omeprazole is active at a pH <4.0, and pH measurements this low were not observed during this study [33; 34]. If a lower oesophageal lumen pH occurs in horses with arytenoid chondritis, omeprazole might be an effective treatment.

Limitations of this study included the small number of horses and occasional short measurement periods. Although the crossover design allowed fewer animals to be used, a greater number of horses would improve the power to detect associations between management factors and pH measurements. Catheter breakage was difficult to control, especially in the paddock, because it was difficult to maintain the position of the catheter. As a result, other horses could pull the catheter or it could catch on objects such as water troughs. When this occurred, measurement periods were reduced due to replacement time. In Protocol A2, catheters were not replaced once broken due to limited resources. Measurement periods were also limited because only one data recorder was available and data were trimmed to remove artefact, which will have resulted in removal of some normal data. Increased measurement time would improve the precision of our estimates.

Other important limitations of this study were that the sale preparation protocol was only simulated and the horses were not Thoroughbreds. Although Australian Stock Horses are a similar breed [35], this might affect the generalisability of our results to the Thoroughbred population. The duration of commercial sale preparation of Thoroughbred yearlings ranges between 6—20 weeks [36], and horses in this study were under sale preparation conditions for only two weeks at a time. Also, exercise in our sale preparation was not as intense as a commercial sale preparation [36; 37]. However, given that the pH variations observed in this study were during a shorter, less intense sale preparation protocol, we believe that these changes are likely to occur, and might be greater, in commercial sale preparations.

The findings of this study challenge current belief that the cardiac sphincter prevents gastro-oesophageal reflux in normal horses, and further build the hypothesis that arytenoid chondritis might be caused by acid trauma of gastro-oesophageal reflux to the laryngeal mucosa. This study demonstrates that oesophageal lumen pH varies rapidly and over a wide range in yearling horses and is influenced by management and environmental factors consistent with the occurrence of gastro-oesophageal reflux in normal horses. To the authors' knowledge, this is the first study to document oesophageal lumen pH in horses and investigate associations between oesophageal pH and management practices, environmental variables and treatment with omeprazole. This study provides a useful baseline for future studies.

Manufacturers' details

[a]Ambulatory pH Recorder, Medical management Systems b.v. Enschede, The Netherlands

[b]Single use pH catheter, MMS-pH-15, Medical Management Systems b.v. Enschede, The Netherlands

[c]Troy Laboratories Pty Ltd, Glendenning, NSW, Australia

[d]Troy Laboratories Pty Ltd, Glendenning, NSW, Australia

[e]Zoetis Australia Research & Manufacturing Pty Ltd, Sydney, NSW, Australia

[f]Reagecon, product code 401025P, 250 mL, Reagecon Diagnostics Limited, Shanon Free Zone, Shannon, Co. Clare, Ireland

[g] Reagecon, product code 70125025, 250 mL, Reagecon Diagnostics Limited, Shanon Free Zone, Shannon, Co. Clare, Ireland

[h]Medical Measurement Systems B.V., Enschede, The Netherlands

Figure Legends

Figure 1: A pH catheter ready for insertion into the oesophagus of a yearling horse by use of an endoscope and biopsy forceps. Biopsy forceps (A) grasped masking tape attached to the catheter (B).

Figure 2: Box plots demonstrating oesophageal lumen pH measurements recorded for six yearling horses for the proximal (P) and distal (D) pH catheter electrodes. The black centre line illustrates the median (pH 7.4 and pH 7.2 for the proximal and distal electrodes respectively. Other parameters for the overall pH measurements include: mean = 7.38, s.d. = 0.54, range = 5.1—9.5 for the proximal electrode; and mean = 7.17, s.d. = 0.54, range = 4.9—9.7 for the distal electrode. There was a statistically significant difference ($P<0.01$) between the two electrodes.

Figure 3: Line plot demonstrating the rapid changes in oesophageal lumen pH in a yearling horse (Horse 6) in a sale preparation environment, treated with placebo over a 24 h period.

Table 1: Mean ±s.d. oesophageal pH change frequencies per minute for proximal and distal electrodes during each protocol in the study.

protocol	proximal electrode changes per min	distal electrode changes per min
A	1.3±1.0	0.8±0.8
B	1.0±0.8	0.6±0.6
C	1.0±0.9	0.8±0.9

Table 2: Regression coefficients and P values for predictive variables in univariable models for proximal and distal electrode measurement of oesophageal lumen pH in yearling horses. Horse ID and protocol were included in the model as random effects.

	proximal electrode		distal electrode	
predictive variable	coefficient	P value	coefficient	P value
time since feeding concentrate	-0.67	< 0.001	-0.53	< 0.001
day or night	0.15	< 0.001	0.12	< 0.001
am or pm	-0.14	< 0.001	-0.099	< 0.001
activity	0.41	< 0.001	0.44	0.07
sex	-0.24	0.03	-0.12	0.2
amount of concentrate	-0.08	0.05	-0.07	0.08
treatment	-0.18	0.3	-0.15	0.5
weight	-0.03	0.7	0.04	0.3
calibration fluid	0.018	0.9	0.04	0.7

Table 3: Regression coefficients, P values and standard errors for fixed-effect variables in linear mixed effects models for proximal and distal electrode measurement of oesophageal lumen pH in yearling horses. Horse ID and protocol were included in the model as random effects.

		proximal electrode			distal electrode		
Variable	category	regression coefficient	P value	standard error	regression coefficient	P value	standard error
	intercept	7.472	<0.001	0.101	6.944	<0.001	0.075
time since concentrate feed	2h am day 1	intercept					
	4 h am day 1	-0.093	<0.001	0.014	0.002	0.9	0.015
	6 h am day 1	0.037	0.01	0.015	0.134	<0.001	0.015
	2 h pm day 1	-0.121	<0.001	0.014	-0.098	<0.001	0.015
	4 h pm day 1	-0.167	<0.001	0.015	-0.064	<0.001	0.015
	6 h pm day 1	-0.146	<0.001	0.015	-0.105	<0.001	0.015
	2 h am day 2	-0.492	<0.001	0.016	-0.264	<0.001	0.016
	> 6 h	0.148	<0.001	0.014	0.173	<0.001	0.014
activity	hand-walked	intercept					
	paddock	-0.021	0.9	0.108	0.141	0.1	0.09
	stable	-0.072	<0.001	0.01	0.076	<0.001	0.01
	turn-out from stable	-0.112	0.007	0.042	0.105	0.01	0.043
day/night	day	intercept					
	night	0.132	<0.001	0.003	0.113	<0.001	0.003
am/pm	am	intercept					
	pm	-0.097	<0.001	0.003	-0.055	<0.001	0.003
sex	female	intercept					
	male	-0.214	0.04	0.097			

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Supplementary Information Items

Table S1: Timeline of protocol measurements for each horse where A is a paddock protocol, B is a simulated sale preparation protocol with administration of omeprazole and C is a simulated sale preparation protocol with administration of placebo.

Figure S1: Orion II data recorder attached to the halter of a yearling. The Orion II recorded pH measurements of the oesophagus continuously for up to 24 h.

Figure S2: Histogram demonstrating Normal distribution of oesophageal pH data for six yearling horses in all management protocols for two pH electrodes, with overlap of the data shown in green. Boxplots demonstrate pH measurements for all horses in each protocol.

Figure S3: Residual plots to assess model fit in a study examining the effects of management on oesophageal pH in yearling horses.