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# Pharmacokinetic and pharmacodynamic effects of 2 registered omeprazole preparations and varying dose rates in horses

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## Abstract

**Background:** Omeprazole preparations vary in bioavailability in horses.

**Hypothesis/Objectives:** To characterize the pharmacokinetics and pharmacodynamics of an existing enteric-coated oral omeprazole paste (REF) and a novel, in-feed, enteric-coated dry granule preparation (NOV).

**Animals:** Twelve Standardbred/Thoroughbred mares free from clinical disease.

**Methods:** A prospective, blinded randomized interventional study was trial, conducted in 3 parts: (a) bioavailability study, (b) dose titration study, and (c) comparative clinical pharmacodynamic study, each using a blocked crossover design.

**Results:** Consistent with the larger dose administered, Cmax (median, 1032 ng/mL; range, 576-1766) and AUC0-24 (median, 63.9 μg/mL*min; range, 42.4-152.4) were greater after single oral administration of NOV than REF (282.7 ng/mL; range, 94.8-390.2, and 319 23.8 μg/mL*min; range, 8.2-42.3, respectively; both $P$ = .004). No differences were observed between products for absolute oral bioavailability (NOV 55% range, 15-88; REF 17% range, 10-77; $P$ = .25). Treatment with both preparations was associated with reduced gastric squamous ulcer scores and increased pH of gastric fluid. Bioequivalence was demonstrated for pharmacodynamic measures with the exception of % time pH <4, despite differences in dose rate and subsequent plasma omeprazole concentrations.

**Conclusions and Clinical Importance:** The findings of this study indicate that the NOV product would be a suitable alternative to the reference product, and confirm that plasma concentrations of omeprazole and omeprazole dose do not predict drug pharmacodynamics in horses.

KEYWORDS

bioavailability, enteric-coated omeprazole, gastric ulcer healing, gastric ulcers

**Abbreviations:** $AUC_{0\text{-}t}$, area under the curve; $AUMC_{0\text{-}\infty}$, area under the first moment curve; $C_{max}$, maximum plasma concentration; EGUS, equine gastric ulcer syndrome; ESGD, equine squamous gastric disease; $F_{abs}$, absolute bioavailability; MRT, mean residence time; NGT, nasogastric tube; PD, pharmacodynamic; PK, pharmacokinetic; $t_{1/2}$, half-life; Cl, clearance; $T_{max}$, time to $C_{max}$; UPLC, ultra-high performance liquid chromatography; $V_z$, terminal volume of distribution; $V_d$, initial volume of distribution; $V_{ss}$, steady state volume of distribution.

## 1 | INTRODUCTION

Equine gastric ulcer syndrome (EGUS) is the most common abnormality of the equine stomach[1] and has been associated with colic, decreased appetite, failure to thrive, and poor performance.[2-5] Equine squamous gastric disease (ESGD) is the most recognized form of EGUS. Gastric ulcers in this portion of the stomach are likely caused by exposure to organic acids, including hydrochloric acid, volatile fatty acids, and bile acids.[6-8] Treatment of ESGD is affected primarily by sustained increase in the pH of gastric fluid, thereby limiting the exposure of the squamous mucosa to acidic contents.[7,9-12]

Omeprazole is a substituted benzimidazole that is effective for the suppression of gastric acid secretion in horses by inhibition of the $H^+/K^+$ ATPase enzyme system at the secretory surface of gastric parietal cells.[13,14] This effect of omeprazole is dose related and leads to inhibition of both basal and stimulated acid secretion.[13,14]

Plasma concentrations of omeprazole can vary considerably and be unpredictable after oral administration.[13,15] Although the drug has a short elimination half-life, absorption after oral administration might be protracted, and absorption, rather than clearance (Cl), is the major determinant of drug concentration.[16] Omeprazole is acid labile and bioavailability of the drug is improved by administration of buffered formulations or by enteric coating,[16] and by withholding feed before administration.[13,17] To date, enteric-coated preparation of omeprazole for administration to horses are suspensions of granules administered in a paste. Although omeprazole is cleared rapidly from plasma, secretion of gastric acid is reduced until resynthesis of $H^+$ pumps in the parietal cell membranes occurs. Plasma omeprazole concentration, therefore, does not necessarily predict gastric pH, and further studies are required to characterize the pharmacokinetic (PK) and pharmacodynamic (PD) effects of omeprazole in horses.

The objectives of this study were to further characterize the PK and PD of omeprazole in horses by comparative studies of an existing enteric-coated oral paste preparation and a novel, in-feed, enteric-coated dry granule preparation. We hypothesized that the PD effects of omeprazole, administered PO, would be dose-dependent, and that bioavailability of the drug would be reduced by in-feed administration of the novel product, impacting the bioequivalence of the 2 omeprazole products. We further hypothesized that plasma concentrations of omeprazole, and the effect of treatment on gastric pH, would be greater after repeated administration, as described for other oral omeprazole preparations.[18,19]

## 2 | METHODS AND MATERIALS

### 2.1 | Subjects

Horses used in this study were Standardbred and Thoroughbred mares (Supplementary Item 1) from the Charles Sturt University teaching herd. All horses were free from clinical disease, based on physical, hematological, and blood biochemical examinations, and had not been administered any medications within 4 weeks of the start of the study. Throughout the study, feed was withheld from horses for 10 hours before the administration of the assigned product, and medication was dosed according to body weight. Horses were weighed within 24 hours of the first day of treatment in each part of the study, and every 7 days when stabled, using calibrated scales. Horses were accommodated in stables during each study. Physical examination was performed daily, and fecal output, food, and water consumption were monitored twice daily for all horses when stabled. Horses were maintained at pasture when not stabled for withholding of feed, acclimatization/washout or treatment.

### 2.2 | Administration of medication

Three omeprazole products were used in the study; an omeprazole preparation for IV administration (Omeprazole Sandoz IV 40 mg; Sandoz Pty Ltd Macquarie Park, Australia), an existing enteric-coated oral paste reference (REF) preparation (Gastrozol Daily Oral Paste for Horses; 50 mg/mL: Virbac Australia Pty Ltd, Milperra, Australia; APVMA product number 58757) and a novel (NOV), in-feed, enteric-coated dry granule preparation (Equestra Omeprazole Granules: 250 mg/g: Equestra, Wodonga, Victoria, Australia; APVMA product number 87882).

Omeprazole was administered IV into the jugular vein using a 21G, 38 mm needle to ensure that the full dose was administered; blood samples after IV administration of drug were collected from catheters placed in the contralateral jugular vein. The commercial omeprazole paste (REF) was administered by placing the dosing syringe into the mouth through the lateral commissure of the lips, ensuring the entire dose was delivered and retained within the mouth. The assigned dose of the novel omeprazole product (NOV) was offered for free choice consumption in 20.0 g of bran. The calculated dose of omeprazole granules, and bran feed, were weighed using scientific scales, and omeprazole granules were mixed by hand to ensure even distribution of granules. Consumption of the entire dose was documented by the research assistant administering the medication, and the time taken to consume the entire dose was recorded.

### 2.3 | Study design

The study was conducted as a prospective, randomized, interventional study in 3 parts (Supplementary Item 1): (a) a bioavailability study comparing oral absorption of both products, (b) a dose titration study after administration of NOV at 2 dose rates, and (c) a comparative pharmacodynamics study. All parts of this study had approval from the animal care and ethics committee at “masked for review”: Part 1, Approval number A17074; Part 2, Approval number A17043; Part 3, Approval number A17076).

#### 2.3.1 | Part 1: Bioavailability study

The bioavailability study included 9 horses and was conducted as a 3 sequence, 3 period Latin square design, with horses randomly

assigned to each sequence. Three horses were randomly allocated (by ballot) to each of the 3 treatment sequences, before period 1. Plasma concentrations of omeprazole were determined after administration of a single oral dose of REF (1 mg/kg) or NOV (2 mg/kg), in comparison with the IV administration of omeprazole (0.5 mg/kg). Venous blood samples were collected from jugular catheters into heparinized vacutainers for determination of plasma omeprazole concentrations, before treatment and at 1, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, and 150 minutes and 3, 4, 5, 6, 8, 12, and 24 hours after administration of omeprazole. Samples were placed on ice, and the plasma was separated within 2 hours of collection and stored at −20°C before analysis. There was at least a 5 day washout between each period.

#### 2.3.2 | Part 2: Dose titration study

The dose titration study included 8 horses and was conducted as a blinded, randomized, blocked, 2 treatment (NOV 2 mg/kg or 4 mg/kg, in 20 g of bran), 2 period cross-over study. Initial gastroscopy and 24 hours gastric pH determination were performed in untreated horses. Horses were blocked into 2 groups of 4, with 2 horses in each block randomly assigned (by ballot) to receive NOV 2 mg/kg in 20 g of bran, and 2 horses to receive NOV 4 mg/kg in 20 g of bran. Horses were then housed in a paddock for 8 to 14 days before commencement of treatment period 1. After being returned to the paddock for a 13-day washout, each horse received the alternative dose in treatment period 2. Gastroscopy and 24-hour gastric pH determination were performed after administration of the assigned single oral omeprazole dose in treatment periods 1 and 2.

#### 2.3.3 | Part 3: Comparative clinical PD study

Comparative pharmacodynamics of REF and NOV were assessed in 12 horses in a blinded, randomized, 2 treatment (REF 1 mg/kg PO q24h for 7 days, NOV 2 mg/kg PO q24h for 7 days), 2 period, 2 sequence trial with sequential cross over. Horses were blocked into groups of 4, with 2 horses in each block randomly allocated (by ballot) to NOV treatment, and 2 horses allocated to REF treatment.

All horses underwent a 14 day acclimatization period before administration of medication and a 14 day washout period between treatment periods. Gastroscopy and 24 hour measurement of intragastric pH was performed in untreated horses on day 7 of the acclimatization and washout periods. Pharmacokinetic data were assessed by determination of plasma concentrations of omeprazole from samples collected before treatment and 15, 30, 45, 60, 75, 90, and 120 minutes and 3, 4, 5, 6, 7, 8, 12, and 24 hours on day 0, after administration of the first allocated treatment, and on day 5, after administration of the penultimate treatment. Pharmacodynamic data and treatment efficacy were assessed by determination of gastric squamous ulcer scores and 24 hour gastric pH on treatment days 1 and 6, after administration of treatment. Sampling for PK and PD analysis was performed on separate days because gastroscopy and nasogastric tube (NGT) placement required sedation, and this was considered likely to affect gastric emptying or drug metabolism. Horses were treated as previously described, except that horses receiving REF were also fed 20.0 g of bran immediately after treatment, to ensure consistency between the treatment of horses with the 2 omeprazole preparations.

### 2.4 | Gastroscopy and gastric fluid pH determination

Horses underwent gastroscopy before the administration of treatment in parts 2 and 3. Before the procedure, horses were stabled and feed was withheld for a minimum of 10 hours and were sedated by IV administration of 200 mg xylazine (Xylazil-100, TROY Laboratories Pty Ltd, Glendenning, New South Wales, Australia) and 10 mg acepromazine (Acepril-10, TROY Laboratories Pty Ltd). When gastroscopy was performed before the administration of omeprazole, medication was administered 60 to 120 minutes after sedation. Endoscopy was performed using a 3 m endoscope (Olympus Medical Systems Corporation, Tokyo, Japan). Visualization of the mucosa was optimized by insufflation with air until gastric rugae were absent. Mucosa was rinsed of adherent food material with water flushed through the endoscope biopsy channel. Squamous ulceration was assessed using a published grading scale[20,21] by an observer blinded to treatment (SLR). Gastroscopic examinations were identified using a unique 4 digit random number, and each examination was video recorded for blinded, de-identified analysis, if required.

Continuous gastric pH determination was performed for 24 hours as previously described[20] using a modified antimony pH electrode (Synectics customized multi-use 1 channel antimony pH electrode, external reference; diameter 2.1 mm, length 300 cm; product number MMS810100; MD Solutions Pty Ltd, Williamstown North, Victoria, Australia) coupled to a data logger (Orion II Ambulatory pH Recorder, Medial Measurement Systems [MMS], MD Solutions Pty Ltd). The probe was placed within an indwelling NGT (Mila Veterinary Enteral Feeding Tube, 18 French x 250 cm; product number NG18100, Mila International, Erlanger, Kentucky) which was placed via endoscopic guidance, as previously described.[20] Probe placement within the gastric fluid was confirmed before endoscope removal, and the tube was sutured to the external nares. Gastric pH was recorded by the data logger at 1 second intervals. The NGT and pH probes were removed after 24 hours of recording time and data were imported from the data logger onto a personal computer using commercially available software (Medical Management Systems database software; Version 9.5, February 23, 2017; http://ww.mmsinternational.com/) and graphs of pH over time, including periods of pH <4 were created to visually assess pH profiles. The mean pH and % time pH <4 were calculated using Microsoft Excel (Microsoft Office Professional Plus 2013, 15.0.5215.1000) after excluding pH readings <0.8 or >10 from analysis, as these results corresponded to loss of contact with gastric fluid on inspection of traces. Probes damaged or displaced within 12 hours of placement were replaced. Unless damaged during

recording or removal, probe accuracy was verified at the time of removal by placing the probe in calibration fluids of pH 7, 4, and 1 and results were discarded if not within 0.5 units of the pH standard or verified by ex vivo determination of pH of aspirated fluid, as described below.

Gastric fluid was aspirated endoscopically before placement of the pH electrode and upon removal of the electrode. When possible, gastric fluid was aspirated via the indwelling NGT every 60 to 120 minutes for 16 hours after NGT placement. The pH of the aspirated fluid was determined by a bench top pH meter (220 Portable pH/mV Meter; Instrument Choice, trading as Synotronics Pty Ltd, Regency Park, South Australia, Australia) within 2 hours of collection. The pH meter was calibrated and verified against standard solution of pH 7, 4, and 1 before sample analysis, with verification repeated after every 5 to 6 samples to ensure pH readings were within ±0.1 of expected pH for each standard solution. Assessment of gastric pH was based on data from both the probe and the aspirated gastric fluid sampled, with the lowest recorded value used for analysis.

## 2.5 | Plasma omeprazole analysis

Omeprazole concentrations in plasma were determined by ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) based on assays previously described.[20] In brief, analysis was performed using a Waters Acquity H-class UPLC system (Waters Corporation, Milford, Massachusetts) coupled to a Waters Xevo triple quadrupole mass spectrometer (Waters

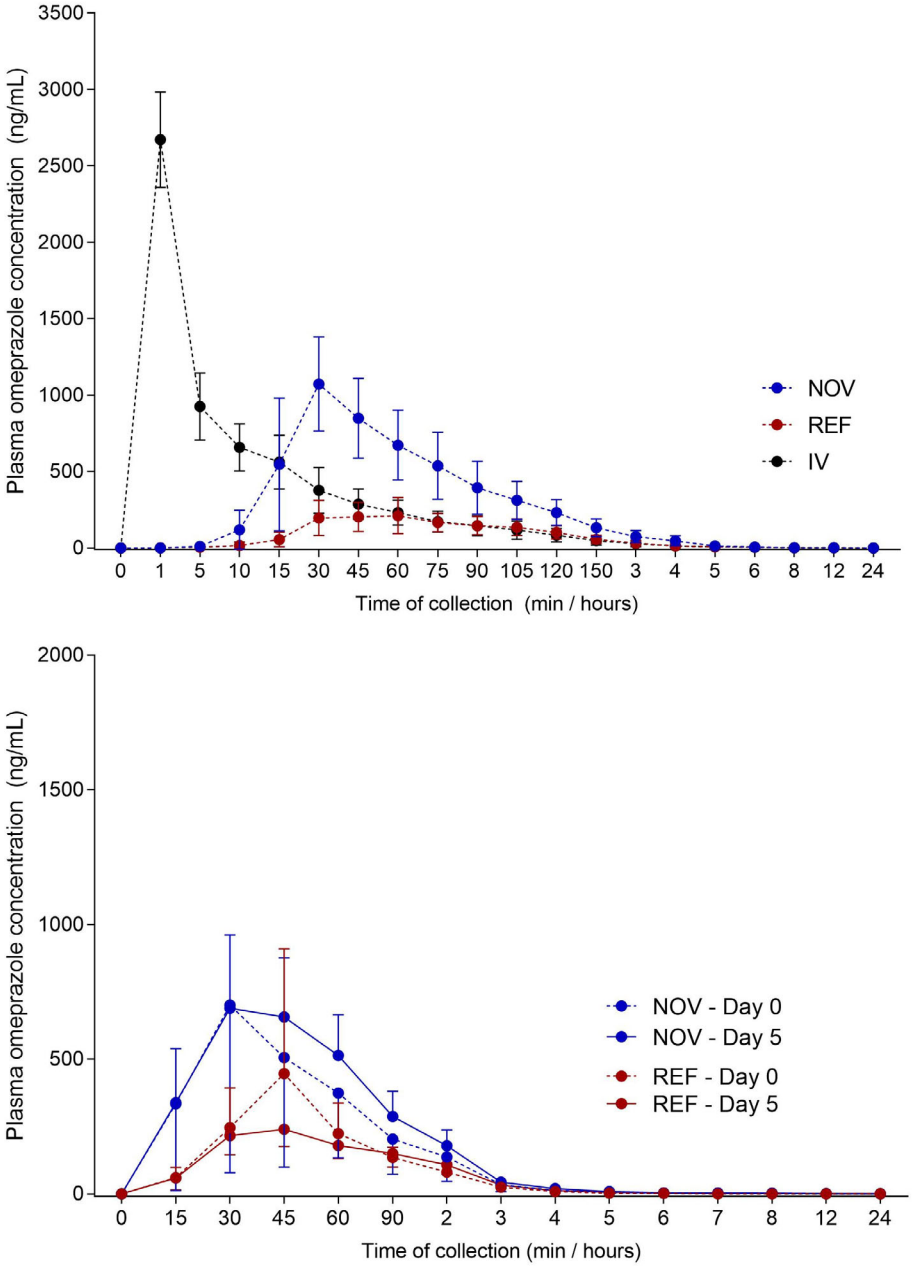


**FIGURE 1** Plasma omeprazole concentration after IV administration of omeprazole (0.5 mg/kg) and oral administration of a commercially available omeprazole product (REF, 1 mg/kg) and a novel in-feed omeprazole product (NOV, 2 mg/kg), after single (day 0) (top) and repeated (day 5) administration of NOV and REF (bottom). Results are shown as mean and 95% confidence interval

Corporation), and Aquity BEH C18 UPLC column (Waters Corporation; 2.1 × 100 mm × 1.7 μm). The UPLC was operated with a mobile phase consisting of 0.1% (v/v) formic acid (Solvent A) and acetonitrile (Solvent B). Analyses were undertaken using multiple reaction monitoring in positive electrospray ionization mode. Assay performance was assessed by determining accuracy and precision using standards (each n = 6) at low (10 ng/mL), moderate (300 ng/mL), and high 1500 (ng/mL) concentrations. Linearity was estimated in triplicate using calibration standards containing 0, 2, 10, 50, 200, 1000, and 5000 ng/mL omeprazole in equine plasma with 20 ng D3-omeprazole added to a 100 μL aliquot of each standard. Standards were processed and analyzed, as described above, on each of 3 different days. Lower limit of quantification (LLoQ) and method detection limit (MDL) were determined by the signal to noise (S/N) ratio of a 0.2 ng/mL standard with LLoQ determined at S/N = 10 and MDL at S/N = 3. Matrix suppression was determined using drug-free equine plasma which was precipitated with 4 volumes of methanol/acetone (1:1). In a separate set of vials, omeprazole standard at moderate (n = 4) and high (n = 4) concentrations was added and the solvent was evaporated. These omeprazole standards were reconstituted with either 60% acetonitrile or with plasma-derived matrix in 60% acetonitrile before UHPLC-MS/MS analysis to determine the level of ion suppression produced by the plasma matrix by comparison ion counts. Dilution integrity was determined using a standard sample at the upper limit of quantitation (5000 ng/mL) in plasma diluted by a factor of 2 using drug-free plasma (n = 6) and also by a factor of 4 (n = 6). The diluted samples were analyzed for omeprazole, and accuracy and precision were determined to assess dilution integrity.

The absence of analyte response in drug-free plasma (F Selectivity) was assessed using drug-free plasma obtained from 6 different equine subjects, extracted and analyzed for peak area corresponding to omeprazole and D3-omeprazole. Carry over was determined after an initial injection of 5000 ng/mL omeprazole, followed by 2 blank injections (methanol alone).

## 2.6 | Pharmacokinetics

Absolute bioavailability ($F_{abs}$) was calculated according to the formula

$$F_{abs} = \frac{AUC_{PO} \cdot D_{IV}}{AUC_{IV} \cdot D_{PO}} \times 100$$

where area under the curve ($AUC_{0\text{-}t}$) is the area under the plasma drug concentration-time (0-24 hours) curve and $D$ is the dose administered. The relative bioavailability of both oral omeprazole products was calculated on results from bioavailability and bioequivalence studies, as previously described,[21] normalized for the different doses (REF 1 mg/kg, NOV 2 mg/kg) and assuming that drug Cl was equal for both preparations. Maximum plasma omeprazole concentration ($C_{max}$) and time to $C_{max}$ ($T_{max}$) were determined directly from data. Area under the curve ($AUC_{0\text{-}t}$) and area under the first moment curve ($AUMC_{0\text{-}\infty}$) were calculated using the linear trapezoidal rule, and the terminal elimination rate constant ($\lambda_z$) was calculated from the terminal phase of the log-linear drug-concentration time curve using PK Solver[22] ensuring at least 5 data points were included. Remaining PK parameters, including terminal volume of distribution ($V_z$), initial volume of distribution ($V_d$), steady state volume of distribution ($V_{ss}$), half-life ($t_{1/2}$), Cl and mean residence time (MRT) were calculated using accepted methods (Supplementary Item 2).

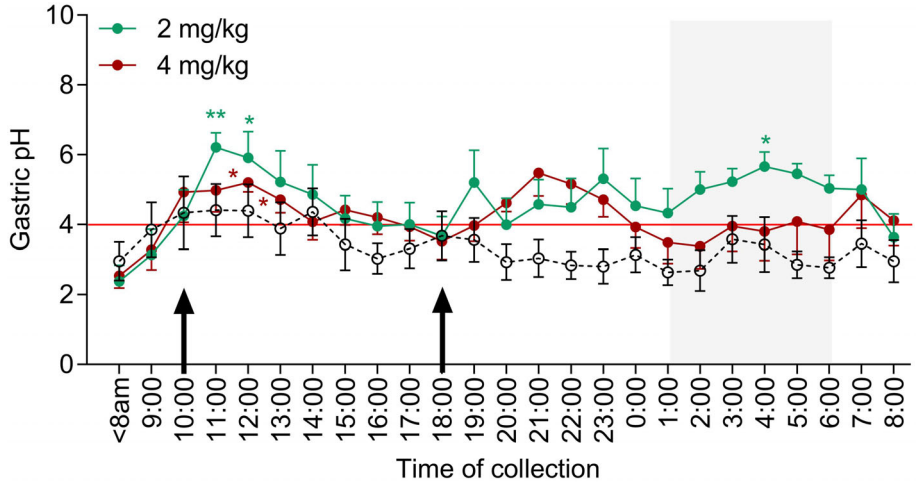


**FIGURE 2** Gastric pH in untreated horses (baseline, dashed line), and after administration of omeprazole granules (NOV) at 2 mg/kg (green) and 4 mg/kg (red). Data are shown as mean and SEM. Time had an effect on gastric pH ($P$ = .003), whereas there was no difference between treatments ($P$ = .12) or an interaction between time and treatment ($P$ = .46). Within each treatment, differences in comparison to pretreatment values are shown (**$P$ = .005; *$P$ < .05). Results from untreated horses were not different at any time point. Values obtained between 0100 and 0600 hours (shaded gray) were derived from gastric pH probe only (not compared with results from aspirated gastric fluid), and were excluded from statistical comparisons. Horses were treated at 0800 hours and fed at 1000 and 1800 hours (arrows)

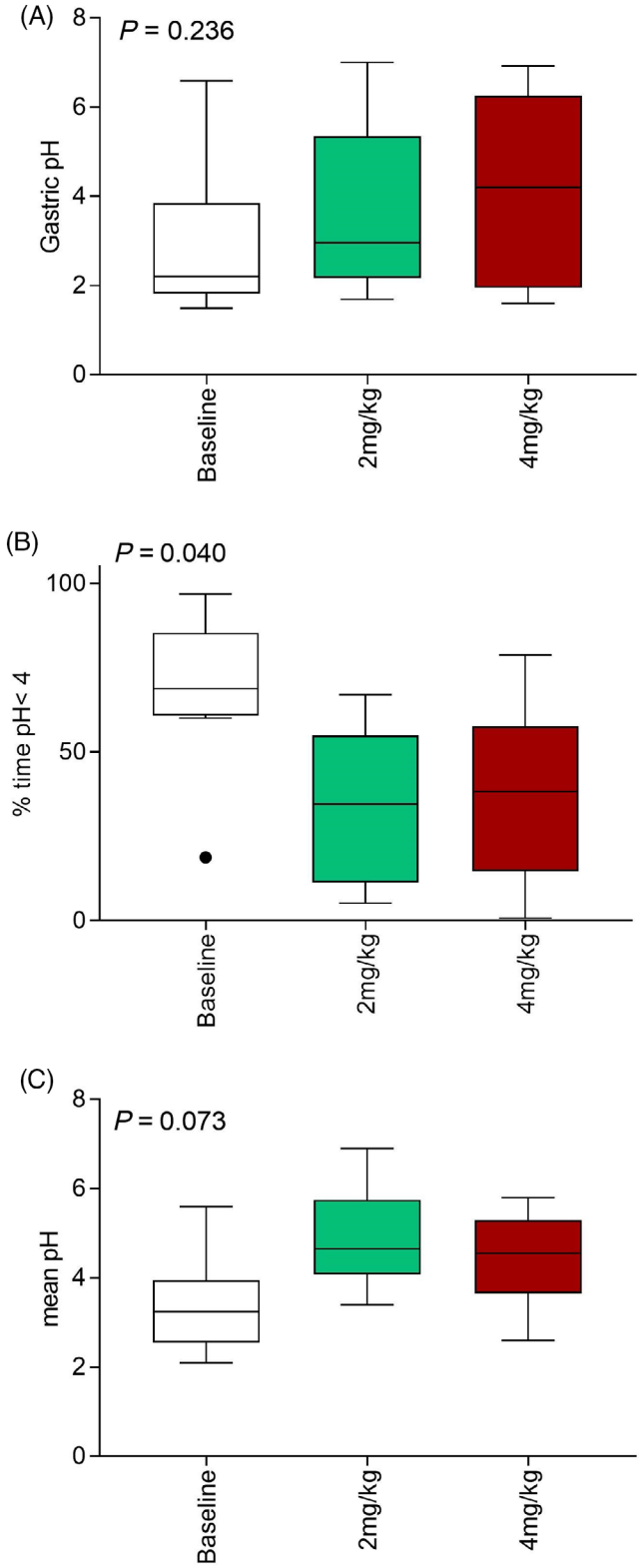


**FIGURE 3** Effects of treatment with omeprazole granules (NOV) at 2 mg/kg (green) and 4 mg/kg (red), compared with results from untreated horses (baseline, white) on gastric pH at 0800 (24 hours after treatment) (A), percentage of time pH <4 (B), and mean pH of gastric fluid (C). Results of Kruskal-Wallis test are shown; differences in post hoc comparisons were not significant. Data are shown as median (horizontal line) and quartile (box) with whiskers determined by the Tukey method

### 2.7 | Statistical analyses

Sample size calculations, based on previous results,[20] indicated that 8 horses would discriminate a difference in gastric pH of 1.4 with power of 0.8 and $\alpha$ of 0.05, assuming a standard deviation of 1. Data were tested for normality using the Shapiro-Wilk test and for outliers using Grubb's method. Outliers were retained in reporting descriptive data and in comparative analyses. Pharmacokinetic parameters were compared by Wilcoxon matched-pairs signed rank test. Log gastric pH was assessed by restricted maximum likelihood mixed-effects model with time and treatment as fixed factors, and horse as a random factor, with post hoc testing by Tukey method. Model assumptions (checked before analyses) were that residuals were normally distributed, had constant variance and were independent. Summary statistics (initial pH, % time pH <4 and AUC pH-time) were evaluated by 1-way repeated measures ANOVA, or ANOVA on ranks using the Friedman test for nonparametric data, with post hoc pairwise comparisons by Tukey or Dunn's test, respectively. Significance was set at $P \leq .05$. All analyses were performed using GraphPad Prism (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla, California, www.graphpad.com) and R statistical software (R version 3.6.0, The R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/).

The equivalence of PD effects was assessed using log-transformed results from parts 1 and 3 after first excluding the possibility of carryover effects because of period or sequence by restricted maximum likelihood linear mixed model (response ~ mean + drug + period + sequence + horse) with horse as a random term and all other terms as fixed factors after testing model assumptions (normal distribution of residuals, constant variance, and independence). Equivalence of factor level variances was assessed using the Brown-Forsyth test. The confidence of the null hypothesis was calculated using the tost function in the equivalence package in R and expressed as the mean difference of the 2 drugs and the standard error of these differences for $\alpha = 0.05$ and $\varepsilon = 1$. Bioequivalence was demonstrated when the back-transformed 90% confidence interval for the ratio of the 2 treatment means was contained with the limits 0.8 to 1.25.

## 3 | RESULTS

All animals completed each phase of the trial, and no adverse effects attributable to treatment were identified. The granule preparation (NOV) was well tolerated, with the entire dose consumed within 2 to 5 minutes, except on days when horses had been sedated for gastroscopy and NGT placement. No adverse responses were evident after administration of the registered oral paste (REF) or IV administration of omeprazole.

### 3.1 | Omeprazole assay performance

The assay met acceptance criteria for performance (Supplementary Item 3), with intra- and interday precision relative standard deviation

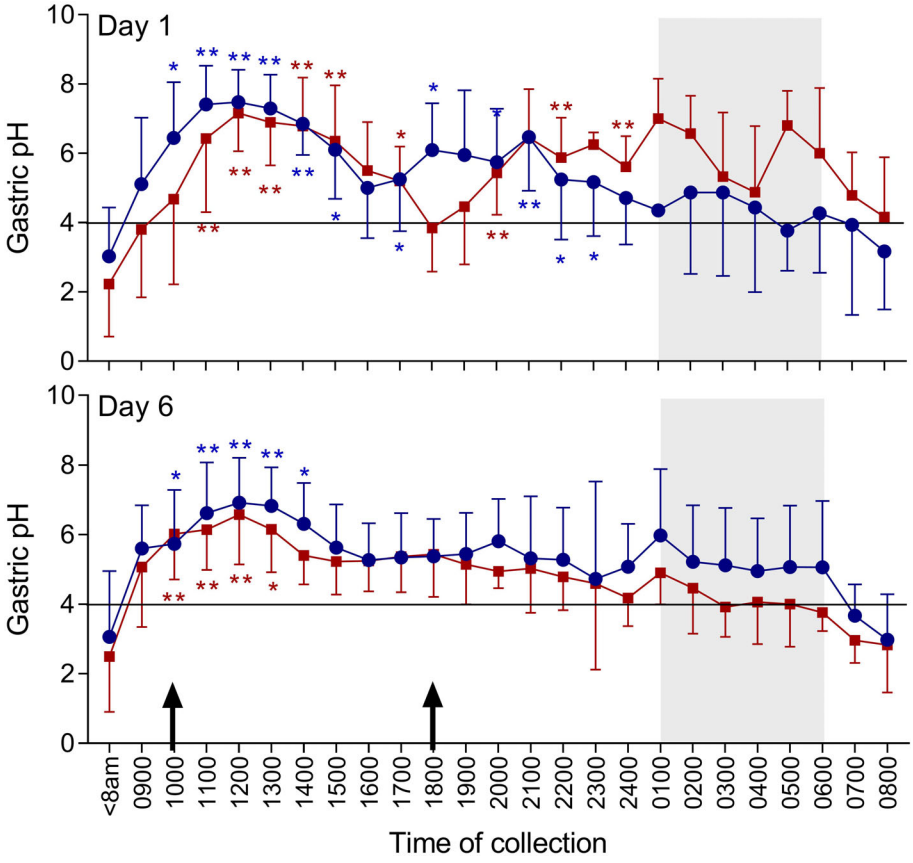


**FIGURE 4** Gastric pH in treated horses on day 1 (top) and day 6 (bottom) after daily oral administration of a novel granule formulation (NOV, 2 mg/kg, blue), and a registered oral paste formulation (REF, 1 mg/kg, red). A time effect was observed on both days ($P < .001$), with differences in comparison to the initial sample (<8 am) shown for each treatment (**$P = .005$; *$P < .05$). There was no effect of treatment ($P = .66$, day 1; $P = .22$, day 6) or interaction effect on day 6 ($P = .98$). The interaction term (time*treatment) was significant on day 1 ($P = .01$); however, differences in pairwise comparisons were not present at any time point. Data are shown as mean and SEM. Values obtained between 0100 and 0600 hours (shaded gray) were derived from gastric pH probe only (pH determination on aspirated gastric fluid did not occur during his time), and were excluded from statistical comparisons. Horses were treated at 0800 hours and fed at 1000 and 1800 hours (arrows)

(RSD) <15% for high (4.5% and 1.7%, respectively) and medium (10.5% and 0.8%, respectively) concentration standards.[23] Accuracy ranged from 92% to 95% and 92% to 95% of expected values respectively for high and medium concentration standards. Linearity ($r^2 > 0.9998$) was demonstrated over the calibration curve range from 0 to 5000 ng/mL, and LLoQ and MDL were determined at 0.04 and 0.012 ng/mL, respectively. All other assay performance measures were within acceptable limits and are presented in Supplementary Item 5.

### 3.1.1 | Part 1: Bioavailability study

Plasma concentrations of omeprazole after the single administration of NOV and REF, and after IV administration, are shown in Figure 1, and PK parameters are provided in Supplementary Item 4. Consistent with the higher dose rate administered, $C_{max}$ (median, 1032 ng/mL; range, 576-1766) and $AUC_{0\text{-}24}$ (median, 63.9 μg/mL*min; range, 42.4-152.4) were higher after single dose oral administration of NOV than REF (282.7 ng/mL; range, 94.8-390.2; and 23.8 μg/mL*min; range, 8.2-42.3, respectively; both $P = .004$). The time at which maximal plasma concentration occurred ranged from 15 to 45 minutes after administration of NOV, and from 30 to 90 minutes for REF. There was a difference in $T_{max}$ ($P = .05$) between the 2 treatments. The terminal slope was calculated based on log-transformed plasma concentrations from 180 to 480 minutes posttreatment for both oral products, and from 90 to 360 or 480 minutes after IV treatment. In comparison to REF, the elimination rate constant ($\lambda_z$) was greater ($P = .04$) for NOV and the MRT was less for NOV ($P = .01$). The median $F_{abs}$ of NOV (55%; range, 15-88) was not different to that of REF (17%; range, 10-77; $P = .25$). The median relative bioavailability ($F_{REF}/F_{NOV}$) was 81% (range, 11-146).

### 3.1.2 | Part 2: Dose titration

Treatment with NOV at both dose rates was associated with increased gastric pH in the 24 hours after a single treatment ($P < .05$) (Figure 2) and a lower % time pH <4 ($P = .04$) (Figure 3). There was no difference in gastric fluid pH between groups treated with 2 or 4 mg/kg omeprazole (Figures 2 and 3).

### 3.1.3 | Part 3: Comparative clinical and PD study

*Gastroscopy and gastric fluid pH*

In untreated horses, the mean pH of gastric fluid was <4 for the majority of the 24 hour sampling period (Supplementary Item 5). A transient, postprandial increase in gastric pH was observed after feeding.

Repeated daily oral administration of NOV (2 mg/kg) and REF (1 mg/kg) elicited similar effects on pH of gastric fluid in the 24 hours after treatment on days 1 and 6 (Figure 4), and was associated with

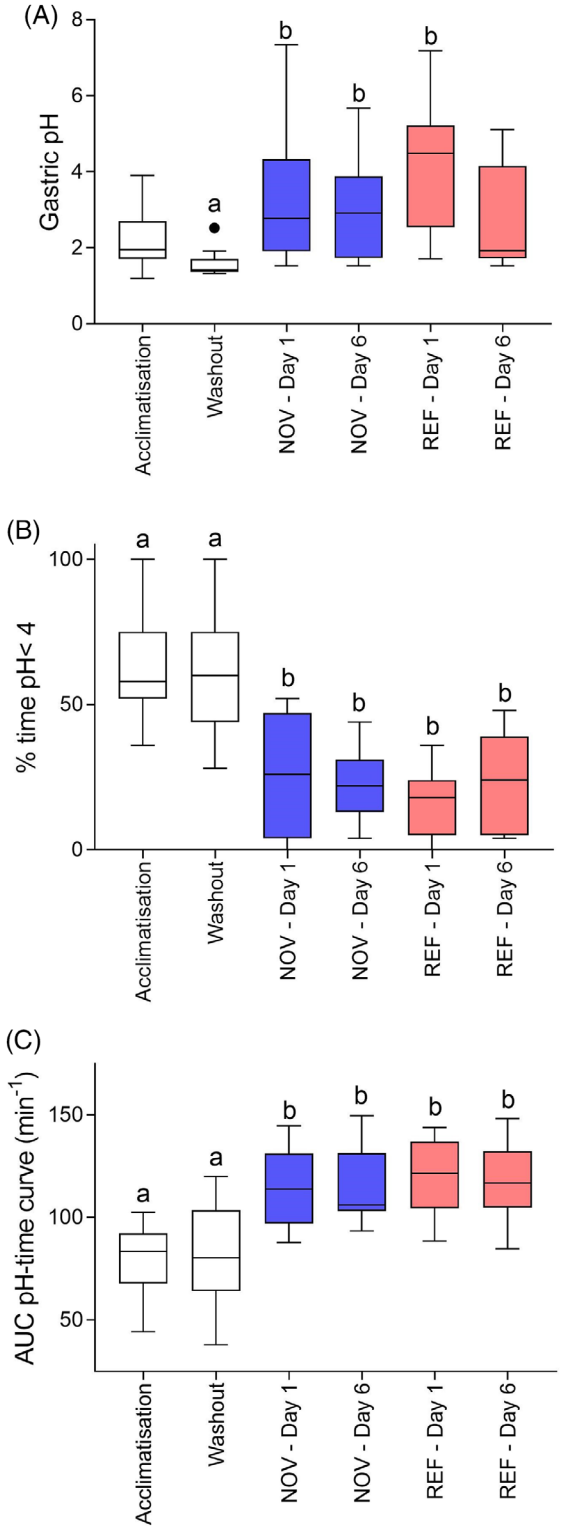


**FIGURE 5** Effects of treatment with omeprazole granules (NOV) at 2 mg/kg (blue) and omeprazole paste (REF) at 1 mg/kg (red), compared with results from untreated horses during acclimatization and washout phases on gastric pH at 08.0 (24 hours after treatment) (A), percentage of time pH <4 (B), and area under the time-pH curve (C). Treatment effects were observed for each variable; results with differing subscripts are different ($P < .05$). Data are shown as median (horizontal line) and quartile (box) with whiskers

increased area under the time-pH curve, increased pH of gastric fluid 24 hours after treatment, and decreased % time pH <4 (Figure 5). Differences were not observed between NOV and REF on day 1 (second daily treatment) or day 6 (seventh daily treatment), and there was no increase in pH of gastric fluid associated with repeated administration of either product. Bioequivalence results for PD variables are provided in Table 1. Bioequivalence was demonstrated for all PD measures with the exception of % time pH <4.

Repeated daily administration of both NOV and REF was associated with a lower gastric squamous ulcer score on day 6 when compared with results obtained on day 1 and with results from untreated horses during the acclimatization phase (Figure 6). There was no difference in scores between treatments. Squamous lesions most commonly involved the lesser curvature. Ulceration of the glandular mucosa was not assessed.

### 3.2 | Plasma concentration of omeprazole

Plasma concentrations of omeprazole after single (day 0) and repeated (day 5) daily administration of NOV and REF are shown in Figure 1, and PK parameters are provided in Supplementary Item 4. Both oral products demonstrated similar plasma concentrations in parts 1 and 3, characterized by rapid absorption and elimination. The median $C_{max}$ on day 5, after repeated daily administration of NOV, was 891.7 ng/mL (range, 88.7-1351) and was greater than that observed for REF (median, 309.1 ng/mL; range, 93.5-384.4, $P = .001$; Figure 1). There was no difference in $C_{max}$ on day 0. As in part 1, the $AUC_{0-24}$ was higher after a single administration of NOV (median, 67.5 μg/mL*min; range, 21.2-84.3) than REF (median, 25.9 μg/mL*min; range, 15.9-36.9), on day 0 ($P = .002$). Area under the plasma concentration-time curve was also higher after repeated daily oral administration of NOV (median, 62.9 μg/mL*min; range, 6-101.7) than REF (median, 24.8 μg/mL*min; range, 9.6-44.5) on day 5 ($P = .002$). The time at which maximal plasma concentration occurred ranged from 15 to 60 minutes after administration of NOV, and from 30 to 90 minutes for REF. There was no difference between treatments for $T_{max}$ on day 0 or 5.

The terminal slope was calculated based on log-transformed plasma concentrations from 180 to 480 minutes after treatment for both oral products. In comparison to NOV, the elimination rate constant ($\lambda_z$) was greater ($P = .04$) for REF on day 0, but there was no difference on day 5. There was no difference in MRT on day 0, however MRT was less for NOV ($P = .03$) on day 5. The median relative bioavailability ($F_{REF}/F_{NOV}$) was 0.19 (range, 0.08-1.71). REF had increased Cl ($P = .002$), $V_z$ ($P = .009$) and elimination half-life ($P = .04$) when compared with NOV on day 0, but not on day 5. Other PK parameters did not differ between oral products (Supplementary Item 4).

## 4 | DISCUSSION

The novel enteric-coated omeprazole in-feed preparation used in this study was readily consumed by unsedated horses, and our results

**TABLE 1** Results of bioequivalence evaluation of pharmacokinetic (PK) and pharmacodynamic (PD) outcome variables for a commercially available omeprazole product (REF, 1 mg/kg) and a novel in-feed omeprazole product (NOV, 2 mg/kg)

| Parameter | Mean difference (log transformed) | Standard error of differences | *P* value | Outcome |
|---|---|---|---|---|
| Pharmacokinetic parameters (day 5) | | | | |
| $t_{½}$ (min)* | 0.05 | 0.12 | <.001 | Bioequivalent |
| $T_{max}$ (min) | 0.1 | 0.10 | <.001 | Bioequivalent |
| $C_{max}$ (ng/mL) | 1.02 | 0.26 | .53 | NOV > REF[a] |
| $AUC_{0\text{-}t}$ (μg/mL*min) | 0.78 | 0.28 | .20 | NOV > REF[a] |
| $AUC_{0\text{-}\infty}$ (μg/mL*min) | 0.78 | 0.28 | .19 | NOV > REF[a] |
| $AUMC_{0\text{-}\infty}$ (μg/mL*min$^2$) | 0.61 | 0.25 | .06 | NOV > REF[a] |
| $MRT_{0\text{-}\infty}$ (h) | 0.17 | 0.08 | <.001 | Bioequivalent |
| $V_z$ (L/kg) | 0.13 | 0.37 | .01 | Bioequivalent |
| Cl (mL/kg/min) | 0.08 | 0.28 | .001 | Bioequivalent |
| Pharmacodynamic parameters (day 1) | | | | |
| $AUC_{pH\text{-}time}$ | 0.08 | 0.08 | <.001 | Bioequivalent |
| Initial pH (preTx) | 0.34 | 0.16 | .001 | Bioequivalent |
| Mean pH | 0.03 | 0.08 | <.001 | Bioequivalent |
| % time pH <4 (24 h) | 0.2 | 0.52 | .07 | NOV > REF |
| % time pH <4 (12 h) | 0.81 | 0.55 | .35 | REF > NOV |
| Pharmacodynamic parameters (day 6) | | | | |
| $AUC_{pH\text{-}time}$ | 0.01 | 0.08 | <.001 | Bioequivalent |
| Initial pH (preTx)[b] | 0.02 | 0.17 | <.001 | Bioequivalent |
| Mean pH[b] | 0.02 | 0.07 | <.001 | Bioequivalent |
| % time pH <4 (24 h) | 0.07 | 0.43 | .01 | Bioequivalent |
| % time pH <4 (12 h) | 0.45 | 0.26 | .06 | REF > NOV |

[a]Consistent with higher dose rate for NOV.
[b]Significant effect associated with phase.

indicate that it would be a suitable alternative to the reference product, particularly for horses refractory to the administration of oral paste. There are several conflicting reports on the impact of feeding on bioavailability and efficacy of omeprazole,[20] and some authors have recommended that the drug be administered before feeding.[13,19] The consumption of a small amount of food during drug administration did not adversely affect drug absorption in the current study, as bioavailability was similar for both NOV (administered with a small feed) and REF (administered without feed). This study did not explore the effects of larger volumes of feed on the bioavailability of omeprazole.

In the current study, the PK parameters for omeprazole were similar to results of previous studies in horses.[11,12,16,21,24] As observed in other species,[25,26] absorption after oral administration was rapid, with *T*max observed within 60 minutes of ingestion for both NOV and REF products. Plasma concentrations were greater for NOV than REF. This might reflect the higher dose of NOV administered or the bioavailability of the different products. Dose rate, product formulation, and feeding practices might affect the bioavailability of PO administered omeprazole.[11,19] A higher dose rate was selected for NOV because of concerns that the granule preparation might be difficult for horses to consume in entirety and that the small amount of feed administered concurrently might reduce bioavailability. The bioavailability of NOV was approximately 50%, and higher than REF (approximately 30%) in the majority of horses. Oral bioavailability might increase during repeated administration,[27] as inhibition of gastric acid secretion is likely to decrease drug degradation after oral administration. Although $F_{abs}$ after repeated administration was not determined in the current study, plasma concentrations of omeprazole after daily administration for 6 days (day 5) in part 3 were not increased relative to day 0, suggesting that bioavailability was similar on both occasions. These observations suggest that the enteric coating used in both products successfully protected the drug from degradation in acidic gastric fluid, but differences in drug absorption attributable to product formulation were not assessed in the current study.

In our study, omeprazole was rapidly eliminated (mean elimination half-life of approximately 60 minutes) after administration of both oral preparations, as has been described previously.[16,25,26,28,29] Omeprazole might display concentration-dependent elimination kinetics,[27] but differences were not consistently observed between NOV and REF, despite the disparate dose rates administered and the greater plasma concentrations observed for NOV. As has been previously reported after IV administration,[28] there was no evidence of drug accumulation with repeated oral administration in the current study.

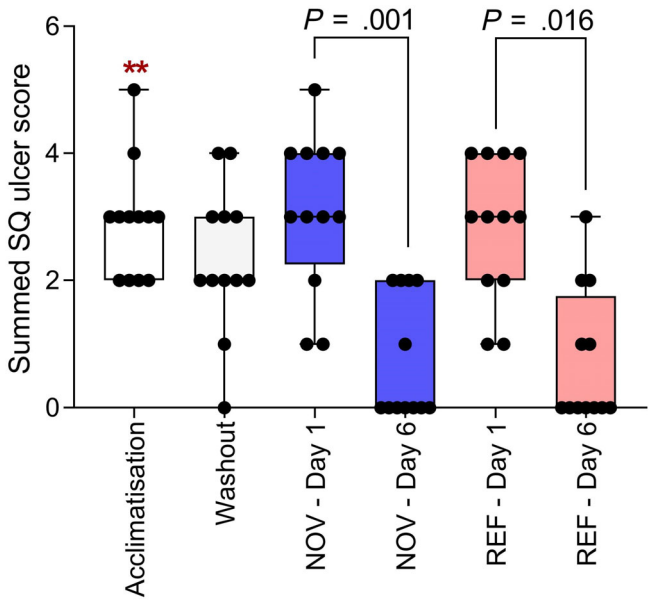


**FIGURE 6** Gastric squamous ulcer scores during treatment. Analysis of variance on ranks (Friedman test) demonstrated a treatment effect, with scores on day 6 lower than on day 1 after treatment with both granules (NOV, 2 mg/kg, $P = .001$) and paste (REF, 1 mg/kg, $P = .02$) formulations. Post hoc testing (Dunn's method) demonstrated a reduction in gastric squamous ulcer score on day 6 in comparison with values determined in week 1 (acclimatization, **) after treatment with both NOV ($P = .004$) and REF ($P = .007$). Results are shown as median (horizontal line), interquartile range (box) and range (whiskers), with all data points shown

Despite differences in dose rate, both products demonstrated similar beneficial effects on gastric pH and healing of gastric squamous ulcers. The feeding protocol used in the current study was identical to that used previously by this group,[20] and was associated with a low pH in the acclimatization and washout periods, such that pH was <4 for approximately 60% of the 24 hour period in untreated horses. Despite rapid Cl of plasma omeprazole concentrations, oral omeprazole treatment was associated with a protracted suppression of gastric acid secretion. We were unable to demonstrate a dose-response association after administration of a single oral dose of NOV in part 2. This might indicate that the observed PD responses were close to maximal. Similarly, although repeated administration of omeprazole has been associated with increased suppression of gastric acid secretion,[28,27] results on days 1 and 6 of the current study were not different. The effects of omeprazole administration on squamous ulcer score were assessed after repeated daily administration in part 3: despite the short study period, beneficial effects on gastric squamous ulcer score were observed after the repeated administration of both NOV and REF. The PD data support once daily administration of oral omeprazole to horses, as measures of gastric acid secretion, including $AUC_{0\text{-}24}$, mean pH, initial pH and percent time pH <4, demonstrated appropriate inhibition after once daily administration. There was no evidence of drug accumulation at this dose interval. In the current study, PD effects were further assessed by tests of bioequivalence to reduce chances of type 2 error. Using this approach, we were able to demonstrate that the majority of PD parameters were the same for NOV and REF.

The pH of gastric secretion has been determined previously by collection of samples from gastric cannulas,[13,30,31] aspiration of gastric fluid via endoscopy or nasogastric intubation, or indwelling nasogastric pH probes.[20,32,33] In the current study, the pH of gastric fluid was determined by both continuous measurement using indwelling antimony probes, and intermittent aspiration of gastric content, as described previously.[20] This approach avoids surgical creation of permanent access to the gastric lumen, but necessitates sedation to permit NGT placement. As sedation might have affected gastrointestinal motility and time to consume NOV, PK and PD studies were not performed concurrently, and this precluded direct comparison of plasma drug concentration and physiological effect. As both methods used in this study are subject to false readings or missing data, for example, because of partitioning of gastric content, probe breakage, reflux of duodenal content, or aspiration of saliva, the simultaneous use of both methods allowed comparison of results and minimized missing data points. In all cases, where values were available from both probe and aspirated fluid, the lower value was used, as has been recommended,[34] to ensure that treatment effects were not overestimated. As gastric fluid was not aspirated after 00.00 or before 07.00 hours, pH results between 01.00 and 06.00 hours (inclusive) were obtained only from gastric probes and, as such, were not substantiated as was possible for other data points.

A further limitation of the current study is that treatment effects of omeprazole on squamous mucosal ulcer scores were observed in a small sample of Thoroughbred and Standardbred mares with mild squamous ulceration of questionable clinical importance. Squamous ulceration scores were assigned by a single investigator blinded to treatment at the time of probe placement and removal. Glandular ulceration scores were not assessed because probe placement, feed retention, or a combination of both, after omeprazole treatment precluded full assessment of the pyloric antrum in many cases, and because oral omeprazole treatment of glandular ulceration is not as effective as for squamous lesions.[35] Horses with more severe, spontaneous gastric disease were not utilized, and a negative control group was not included in the current study. Although an effect on gastric squamous ulcer score was observed, the current study was not designed to assess clinical efficacy and a larger, controlled study of horses with spontaneous disease is required to better characterize the effect of treatment on ulcer healing.

## 5 | CONCLUSION

Administration of both omeprazole products resulted in increased gastric pH relative to untreated horses. Similarly, mean pH was increased and the percent of time that gastric pH <4 was decreased by treatment, and bioequivalence of both products was demonstrated based on these 2 parameters, indicating that PD effects were the same

regardless of dose rate for both products. The findings of this study confirm that plasma concentrations of omeprazole and omeprazole dose do not predict drug PD.

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## CONFLICT OF INTEREST DECLARATION

The funder Equestra Pty Ltd did not influence the design or conduct of the study, assessment of the data, or writing of the manuscript. The authors have no conflicts of interest.

## INSTITUTIONAL ANIMAL CARE AND ETHICS APPROVAL

Part 1, Bioavailability study Approval number A17074; Part 2, Dose Titration Study Approval number A17043; Part 3, Bioequivalence and clinical efficacy study Approval number A17076).

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Part 1, Bioavailability study Approval number A17074; Part 2, Dose Titration Study Approval number A17043; Part 3, Bioequivalence and clinical efficacy study Approval number A17076).

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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